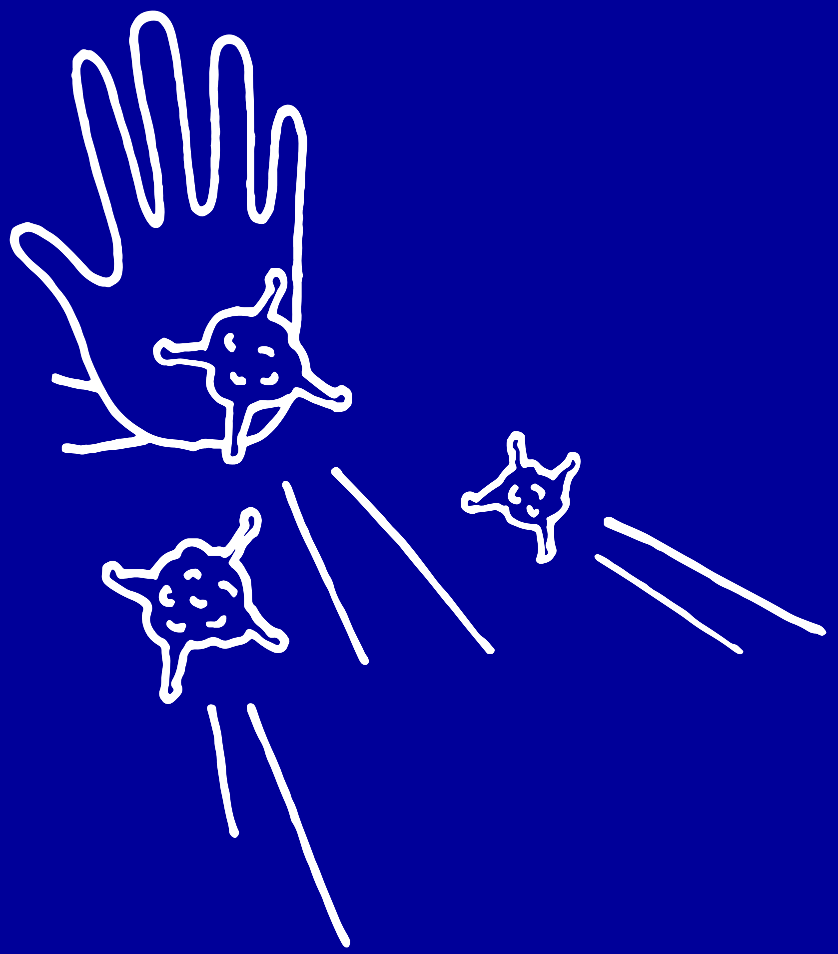
evotec

#RESEARCHNEVERSTOPS

Antiviral capabilities at Evotec



Agenda

1. Evotec Virology Overview
2. Focus on Respiratory viruses
3. Focus on Hepatic viruses (HBV and HDV)
4. NGS & Bioinformatics platforms
5. Integrated virology (Hit ID to PDC)

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1. Evotec Virology Overview
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4. NGS & Bioinformatics platforms
5. Integrated virology (Hit ID to PDC)

Foundation for antivirals: Years of experience in academia and pharma

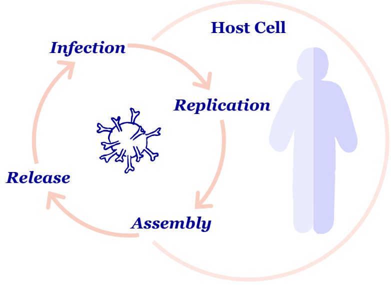
Experienced drug discovery team: Direct antiviral agents and host targeting approaches

Extensive experienced team

- Virology
- Immuno-virology
- Drug discovery

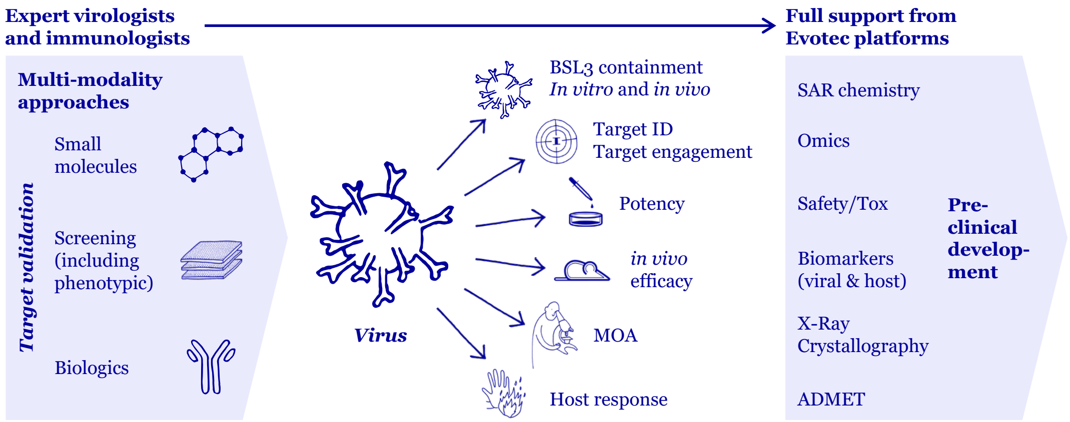
Integrated drug discovery projects

One entity and team to execute the whole project from conception to phase 1



Unique platforms and modalities to tackle viral infection from several angles

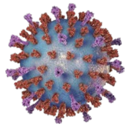
- Host targeting and Direct antivirals approaches
- Small molecules and Biotherapeutics



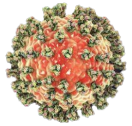
Continuous development of *in vitro* biology capability and expertise

From endemic to emerging viruses

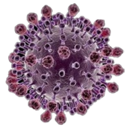
Respiratory viruses



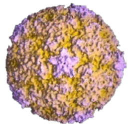
RSV (A2, Long and clinical isolates, GFP-tagged RSV-A and B)



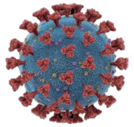
PIV (1,3 and 5, GFP-tagged HPIV-3)



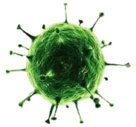
Influenza virus
FluA Brisbane/59/2007, H1N1
Flu A/Puerto Rico/8/34, H1N1



HRV (14 and 16)



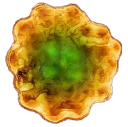
Human Coronavirus
OC43, 229E and NL63
SARS-CoV-2 all variants of concern



Adenovirus

Chronic Hepatic viruses

HBV (Genotype D)



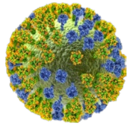
HDV (Genotype 1)

Other genotypes in development

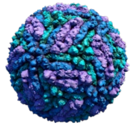
- Several standard and integrated *in vitro* and *in vivo* models
- Primary and integrated models
- Live viruses/clinical strains / recombinant viruses
- Pseudotyped viruses
- Minigenomes

Other viruses (In development)

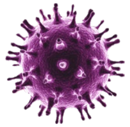
BSL3



Orthomyxovirus
Avian highly pathogenic* H5N1, H5N8

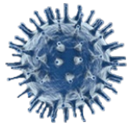


Flavivirus
Dengue*, West Nile, yellow fever, Zika

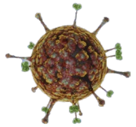


Retrovirus
Human Immunodeficiency virus (HIV)

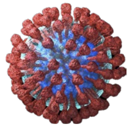
BSL2



Herpesvirus
Human simplex Virus-1 (HSV-1)
Epstein-Barr virus (EBV)



Paramyxovirus
Cedar virus
(Nipah virus surrogate)



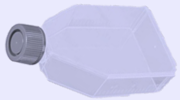
Arenavirus
Mopeia virus
(Lassa virus surrogate)

1 Authorizations acquired

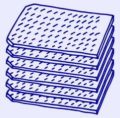
In vitro biology capability and expertise

Extensive experience in both virus- and host-targeted antiviral discovery

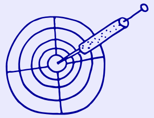
Virus production & characterisation



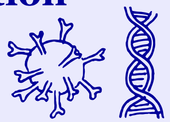
Screening



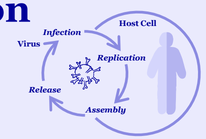
Antiviral drug profiling



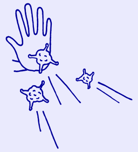
Virus detection & quantification



Mode of action evaluation

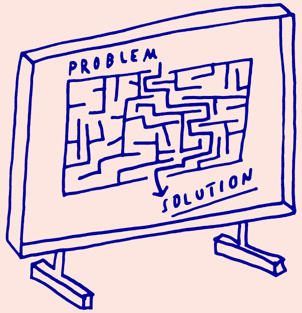


Host response



Available assays, including

- Compound testing in cell lines and primary cells
- Tailored experimental design
- Combination therapies studies
- Drug-resistance evaluation
- Target identification and validation
- Neutralization assays
- Detection and quantification of viral antigens
- Monitoring of host response



Read-out, including

- CPE-based antiviral assays
- Luminescent cell viability assays
- PFU, TCID50, FFA
- PCR, qPCR, RTqPCR of viral / cellular nucleic acids
- ELISA and ELISpot
- Hemagglutination inhibition assay
- Viral protein activity assays: endonuclease, neuraminidase, polymerase (minigenome)
- Multiparametric flow cytometry
- Immune cell isolation and characterization
- Multiplexed cytokine assays
- Live cell imaging
- Immuno and nucleic acid blotting

>15 years of screening expertise in antiviral space

Ability to screen at BSL2/BSL2⁺ & BSL3

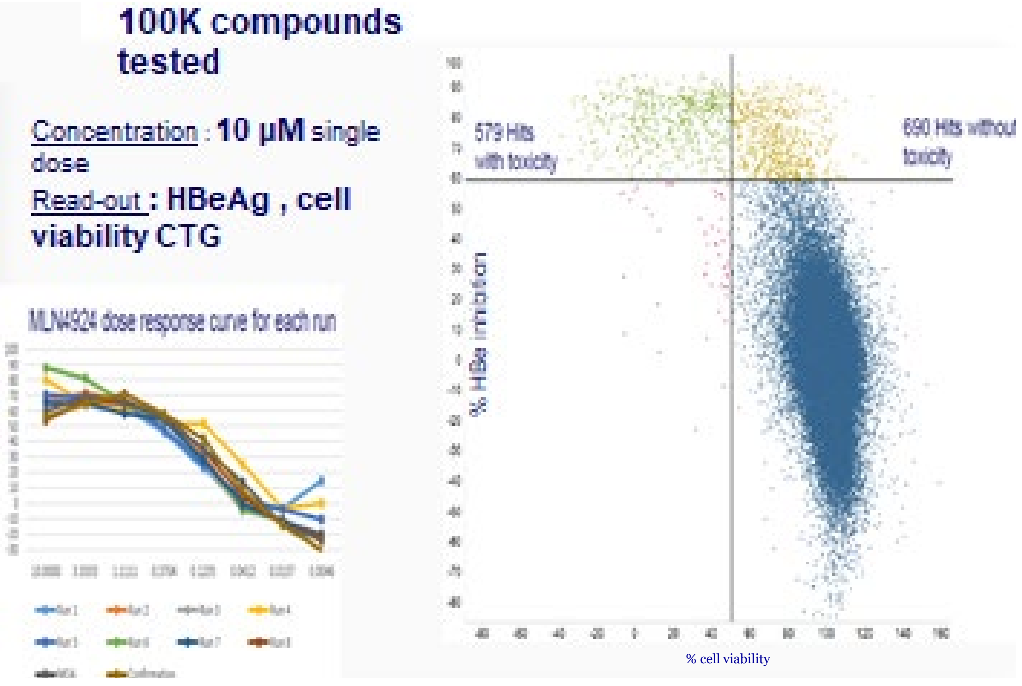
- **Screenings for several antivirals:**
  - HBV, hPIV, RSV, hMPV, rhinovirus, coronavirus, influenza
- **Medium to High Throughput**
- **BSL2/2⁺ and BSL3 facilities**
- **Access to >800,000 compounds**
- **Several readouts, including:**
  - Reporter genes
  - RT-qPCR
  - Phenotypic
  - Target-based readouts
  - High-content imaging
- Hit **expansion** and characterization

BSL2/BSL2⁺ screening capabilities for HTS/MTS

BSL3 screening capabilities for MTS

Close collaboration between scientific experts and HTS platform to setup involved screens e.g. using primary human hepatocytes

Primary screening



Continuous development of *in vivo* biology capability and expertise

Collection of validated animal models & development of bespoke models

In vivo tolerability and efficacy

PK profiling

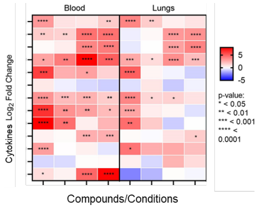
- Multiple sites/routes of drug administration
- Dose range finding/ maximum tolerated dose studies
- Blood and tissue-specific PK analysis
- Bioavailability/biodistribution assessments (ELISA, MSD)

In vivo efficacy studies

- Broad range of infecting pathogens available
- Multiple hosts: Mouse, guinea pig, hamster, cotton rat
- Multiple sites/routes of infection and drug administration
- Multiple endpoints (host response and pathogen burden)

Biomarkers assessment

- Immune cell isolation and profiling
- Multiparametric flow cytometry
- Cytokine profiling (MSD, ELISA, ELISpot, bespoke Cytokine/Chemokine profile by RT-qPCR)
- Gene expression assays (qPCR)
- Live cell imaging (Incucyte)



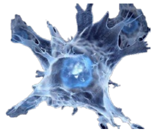
Histology and Immunohistochemistry

- Coloration, single and multiplex staining
- Validation of a biomarkers (FFPE or frozen samples):
  - IHC / multiplex IHC
  - RNA In Situ Hybridization (ISH)

Broad and deep immuno-virology capabilities and expertise

Several assays available to monitor innate and adaptive immunity

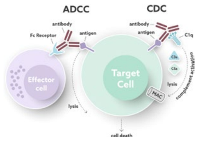
Innate immune response



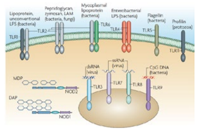
DC activation assays
(activation markers, cytokine secretion)

Macrophage activation assays (M1, M2)
(activation markers, cytokine secretion)

NK activation assays
(activation markers, proliferation, cytotoxicity, cytokine secretion)



Cytotoxicity assays with antibodies
(ADCC, CDC, ADCP)



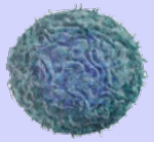
PRRs activation
Several reporter systems available
Screening for activators/inhibitors

CRS evaluation (Whole blood Cells Assay)

Adaptive immune response

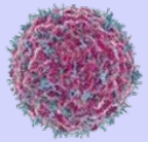
T CD4/ CD8 activation assays

- Activation markers,
- MLR « mixed lymphocyte reaction »
- Proliferation
- Cytokine secretion



B cell activation assays

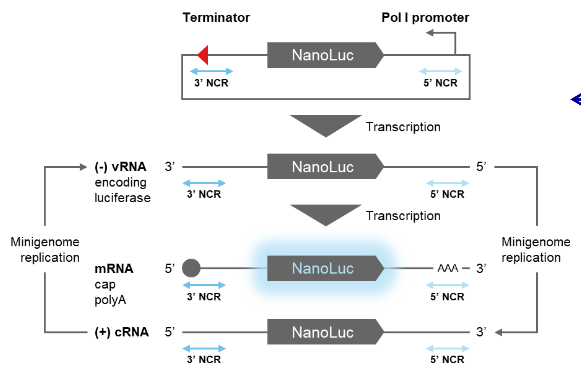
- Activation markers
- Proliferation
- Antibodies secretion

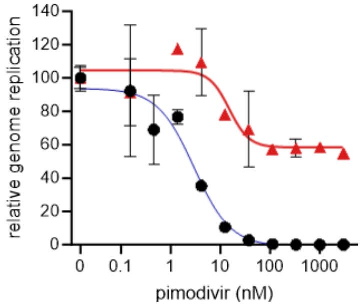


Splitting highly pathogenic viruses into pieces …

… to develop and test antivirals

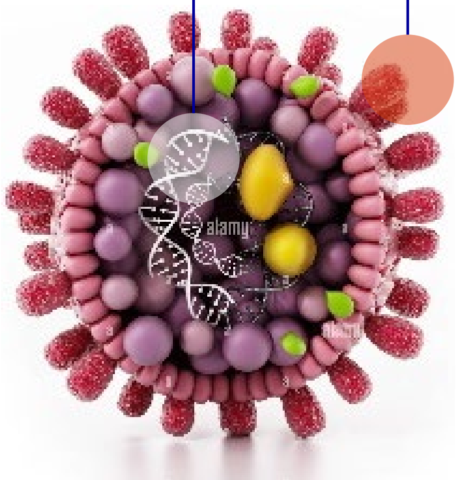
Minigenomes



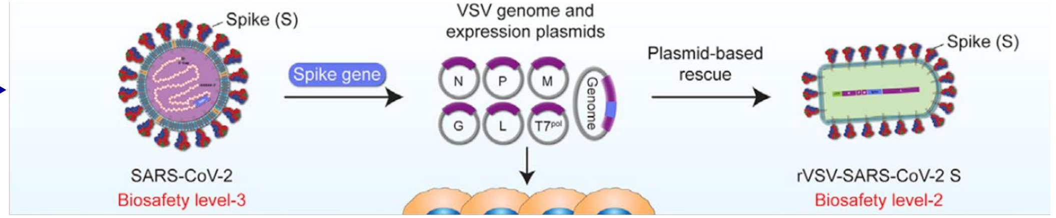


Influenza Nipah Hendra CCHF Lassa Dengue Ebola

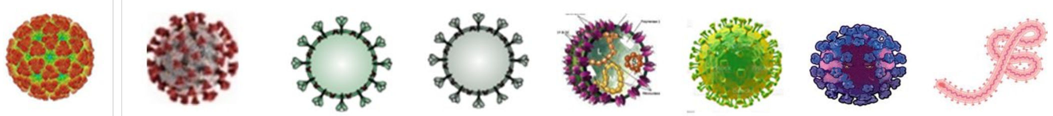
In development



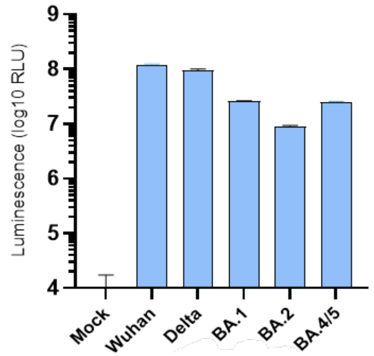
Pseudoviruses: Viral entry

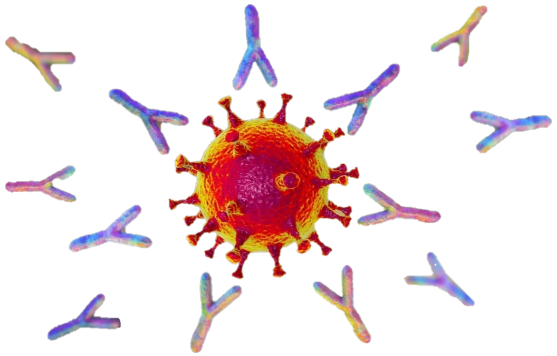


CHIKV SARS-CoV-2 SARS-1 MERS CCHF Nipah Lassa Ebola[1]



BSL3 BSL4





1 In development

A unique multi-modality & multi-targeting approach

Leveraging virus biology from all angles for therapeutic development

RNA targeting (small molecules)
Targeting key viral RNAs structures

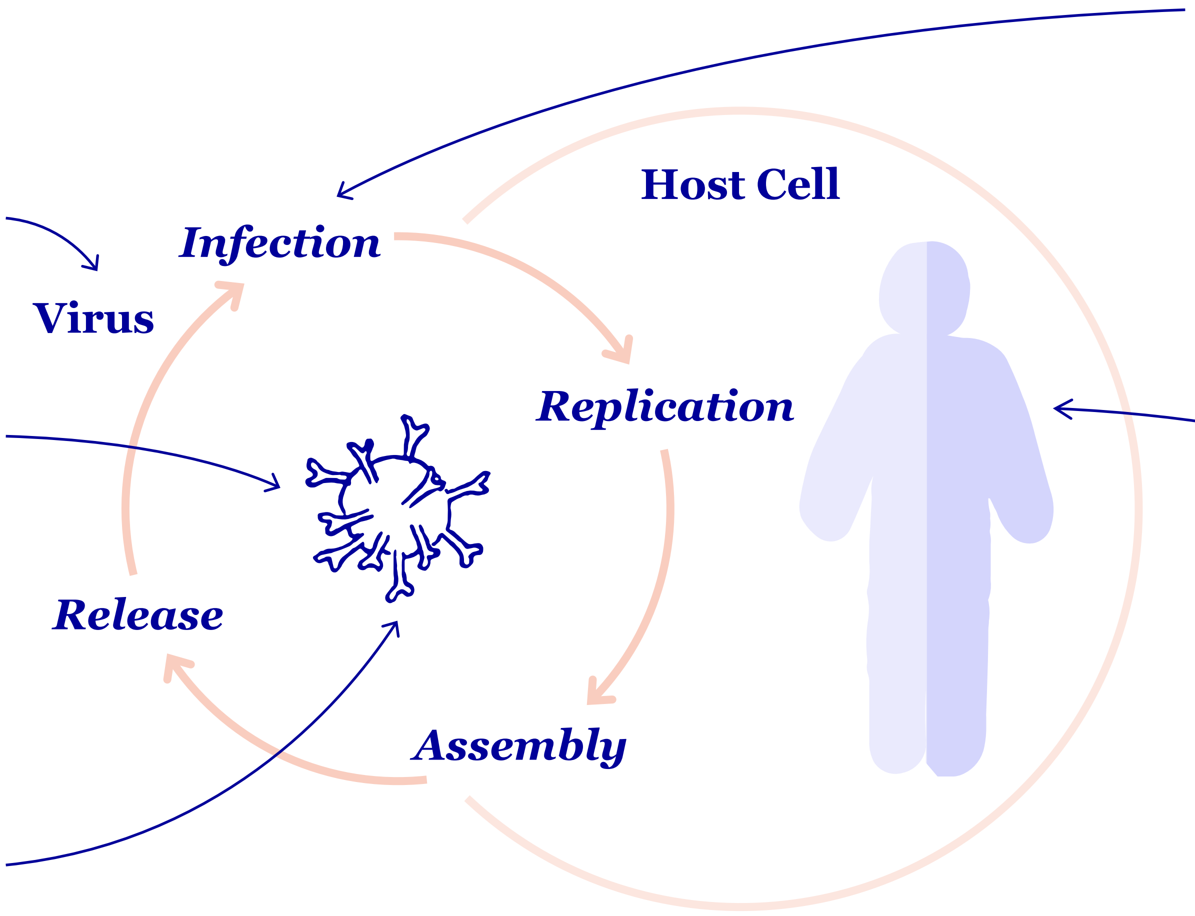
- HBV
- EBV
- Nipah
- Flavivirus (Dengue,..)

Protein Degraders
Degrading proteins not accessible to traditional therapeutics

- Influenza
- HBV
- EBV
- BKV

Enzyme Inhibition
Preventing replication and assembly

- Influenza
- Nipah
- SARS-CoV-2
- CCHF
- RSV
- Lassa



Neutralizing Abs
Preventing Viral Entry

- CHIKV
- SARS-CoV-2
- Nipah
- CCHF
- Lassa

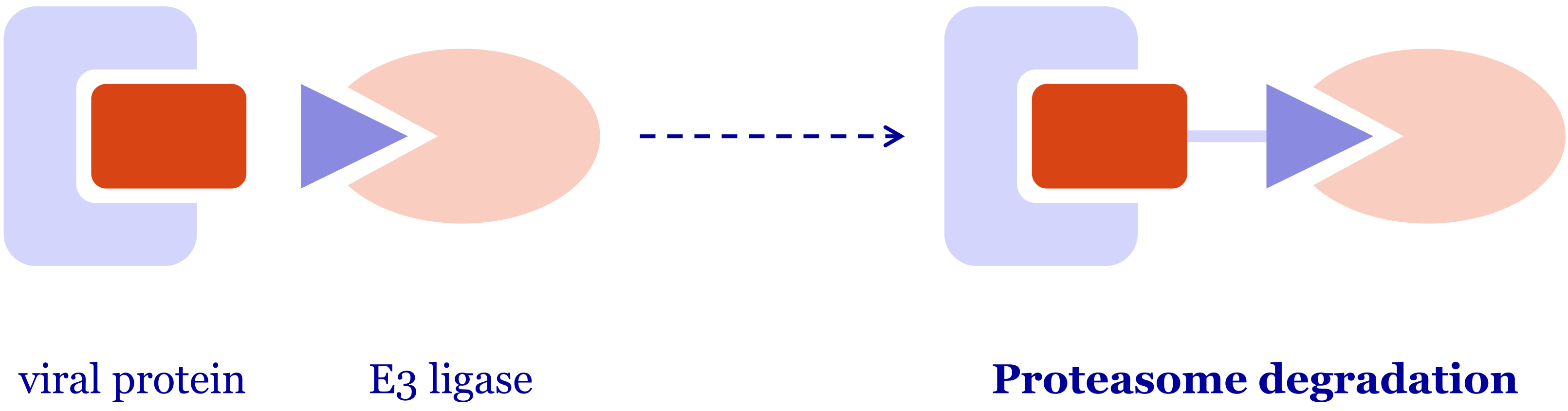
Host Targeting Immunomodulators
Boosting innate and adaptive immunity

- SARS-CoV-2
- Influenza
- RSV
- PIV
- PIV
- HBV, HDV
- Nipah
- Lassa

Targeted protein degradation to target undruggable viral proteins

Overcoming major challenges of antiviral drug discovery

- Targets “undruggable” proteins
- Applicable to any viral protein
- Resulting in complete silencing of viral activities
- Is less likely to lead to resistance
- Oral administration



- **Reporter cell lines expressing the target of interest**
  - Choice of tag (luminescent, fluorescent) & position
  - Full characterization of protein kinetics, turnover, activity
  - CRISPR knockout of E3 ligase of interest

- **High-throughput degradation assays**
  - 384-well plate format, DC_{50} and Dmax determination
- **Live degradation** kinetics (fluorescence/luminescence)

- A panel of **additional assays** to study targeted protein degradation
  - Cellular E3 ligase engagement
  - Binding affinity to viral protein (e.g. SPR, MST etc.)
  - Ternary complex formation (NanoBRET)
  - Ubiquitination (NanoBRET) / Ubiquitinomics
  - Proteomics in infectious model
  - Chemical competition / E3 ligase modulation

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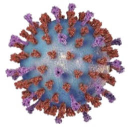
In vitro biology capability and expertise on respiratory viruses

Standard and integrated *in vitro BSL2 and BSL3* models

Respiratory viruses



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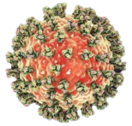

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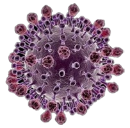
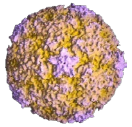


Influenza virus
FluA Brisbane/59/2007, H1N1
Flu A/Puerto Rico/8/34, H1N1



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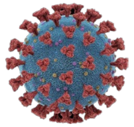

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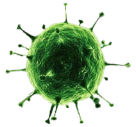
OC43, 229E and NL63
SARS-CoV-2 all variants of concern

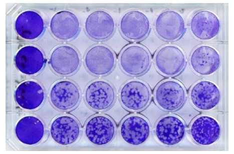


Adenovirus

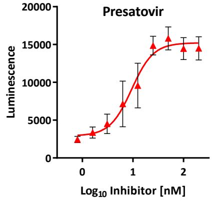
In vitro infection models

- **Various assays including:**
  - CPE-based antiviral assays
  - Cell viability (CTG, LDH)
  - PFU, TCID50, FFA
  - PCR, qPCR, RTqPCR
  - ELISA
  - Immunostaining, Live cell imaging

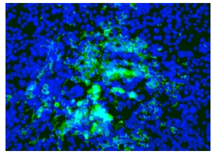
Plaque forming assay



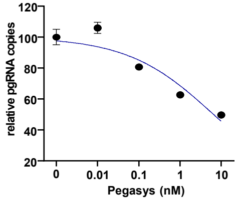
Cell viability assay



Immunostaining

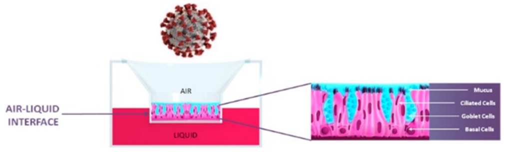


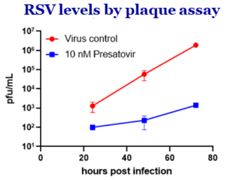
RT-PCR/ ddPCR

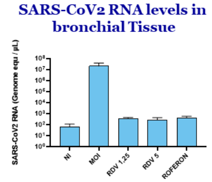


- **Various viral strains:** Live viruses, recombinant viruses[1], reference and clinical strains
- **Various cell lines**, including
  - VeroE6, MDCK, Hep-2 , A549, Calu-3, SH-SY5Y
  - THP-1, HepG2
  - Human airway epithelial cells
  - Primary cell lines

Human airway epithelial assay[2]







- **Additional Endpoints** including host biomarkers, multiplexed cytokines assay, antibody response, Multiparametric flow cytometry, immune cell isolation, …
- **Additional available assays** including enzymatic assays[3], immunoenzymatic assays, pseudotyped viruses, minigenomes, …

1 Reverse genetic for influenza in development
2 Schematic representation of MucilAirTM. (adapted from http://www.epithelix.com/products/mucilair) …
3 Includes protease, polymerase, neuraminidase, endonuclease

In vivo gold standard models in suitable animal hosts

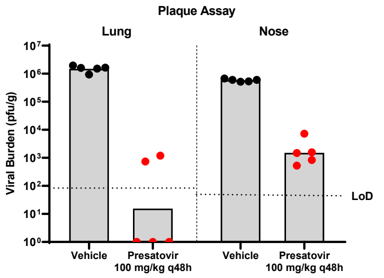
BSL2 and BSL3 facilities for respiratory viruses

Respiratory virus capabilities

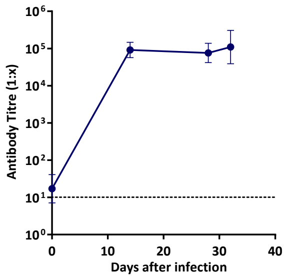
- **SARS-CoV-2** Hamster Model (intranasal Infection with SARS-CoV-2): infection model, transmission model as well as long-disease model
- **SARS-CoV-2** humanised ACE2 mouse model
- **RSV** infection model in cotton rat and mouse
- **Influenza** mouse model (survival and burden)

RSV cotton rat model

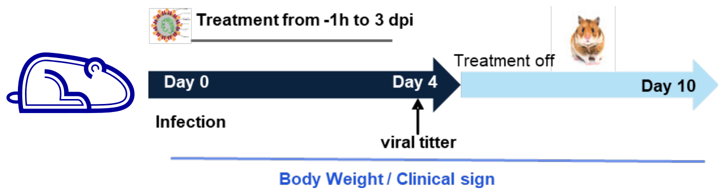
Viral burden



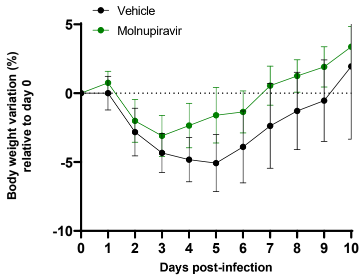
RSV specific antibodies



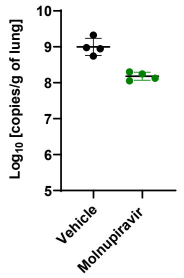
SARS-CoV-2 hamster model



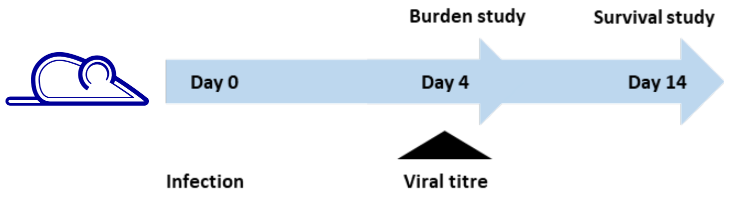
Body weights



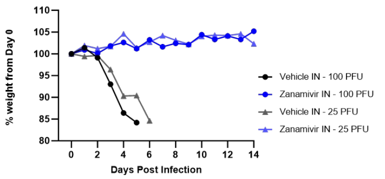
Viral burden



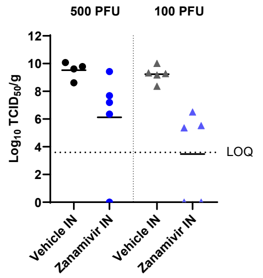
Influenza mouse model



Body weights



Viral lung burden



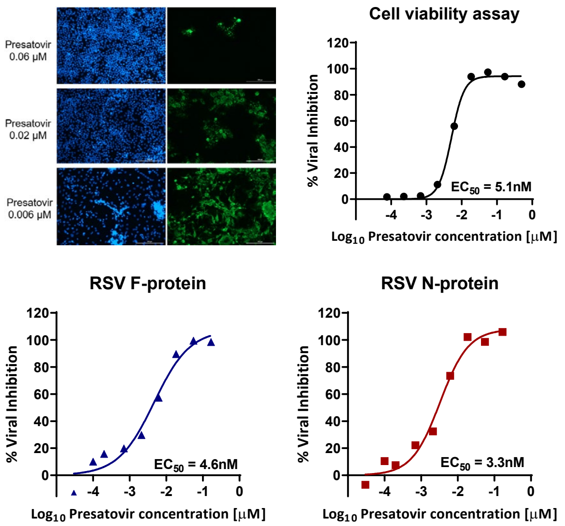
Respiratory Syncytial Virus (RSV)

Capability and expertise on respiratory viruses – example of RSV

From screening to animals

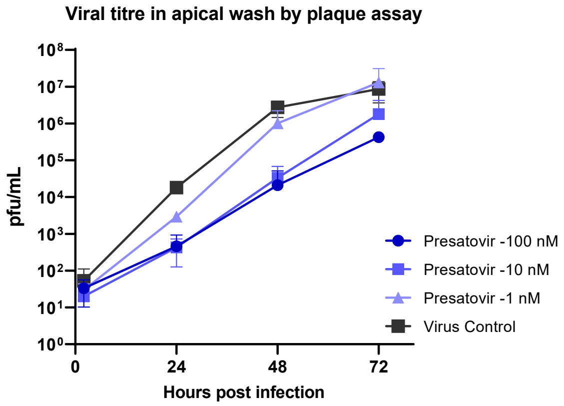
Cell based assays

- Cell viability as screening assays using Viral Toxglo™
- Direct antiviral assays for demonstration of antiviral effect and MOA studies: Plaque assay, ELISA against viral proteins, plaque staining assay



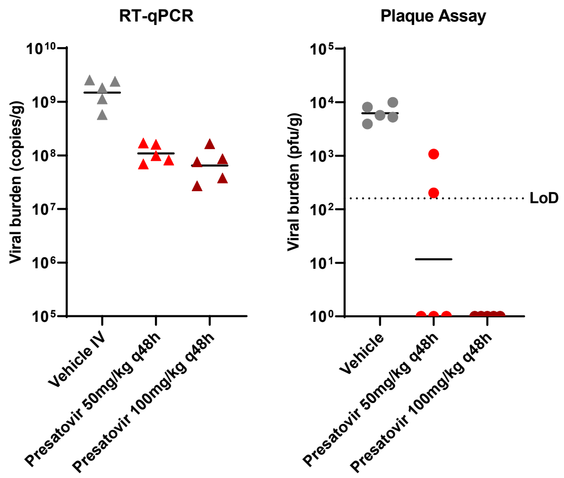
Epithelial Air Liquid Interface Assay

- RSV infection model of Mucilair™ epithelial nasal or bronchial air liquid interface tissue
- Treatment in basal medium
- Sequential sampling of apical wash
- Determination of virus load by plaque staining assay and RT-qPCR
- Determination of cytokine levels



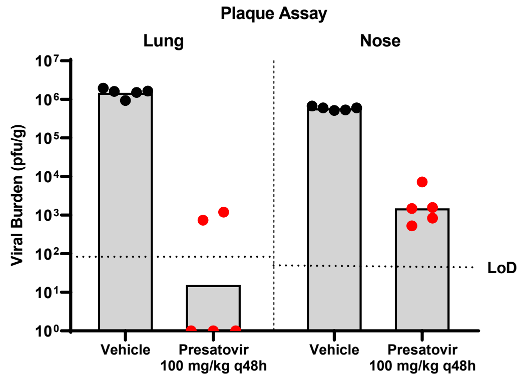
RSV mouse model

- Balb/c mouse model widely used to study RSV immunopathology
- Semi-permissive to the virus
- Intranasal infection, RSV-A2
- Viral load in lung homogenate by plaque assay and qPCR
- Cytokine levels determined by ELISA
- Viral titre peaks at day 4 and declines to undetectable at day 7



RSV cotton rat model

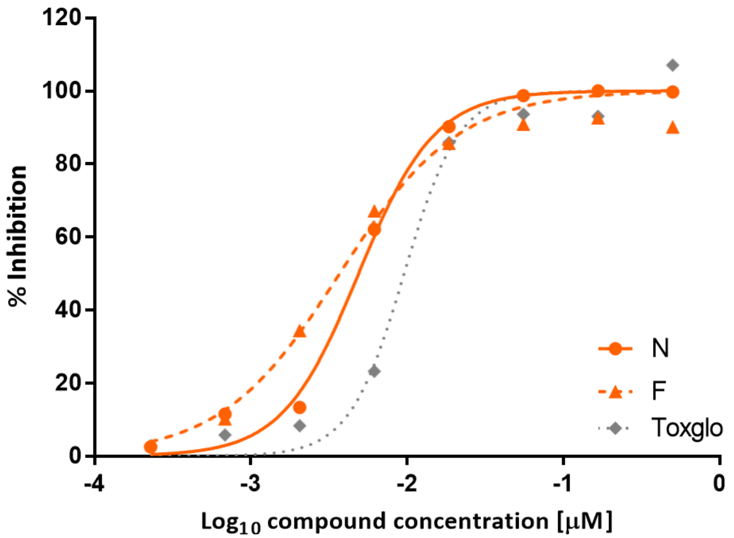
- Gold standard RSV animal model
- For vaccination and treatment studies
- Intranasal infection, RSV-A2
- Viral load in nose and lung tissue by plaque assay and RT-qPCR
- Antibody titre via ELISA
- Neutralisation assay to demonstrate presence of neutralizing antibodies
- Immunohistochemistry
- Viral titre peaks at day 4 and declines to undetectable level at day 6

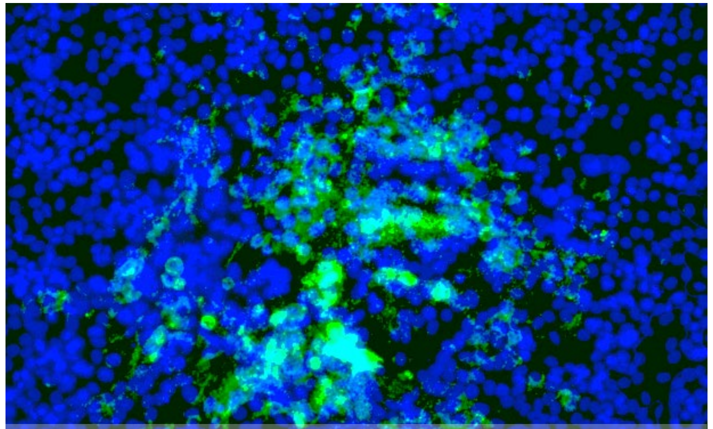


In vitro biology capability and expertise on RSV

In vitro infection models

- **Cell lines**
  - **A549:** human respiratory epithelial cells
  - **VeroE6 cells:** African green monkey kidney cell line
  - **Hep-2 cells:** immortalized cell line derived from human larynx carcinoma
  - **Human airway epithelial cells:** human upper airway epithelium cultured at the air liquid interface
- **Read outs**
  - Cell viability
  - Plaque assays
  - RT-qPCR
  - ELISA
  - Immunofluorescence





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

Various viral strains

- Human respiratory syncytial virus strain A2
- Long and clinical isolates (RSV-A) and (RSV-B strains available)
- **GFP-tagged RSV-A (strain A2), RSV-B (strain B1)**

Assay formats

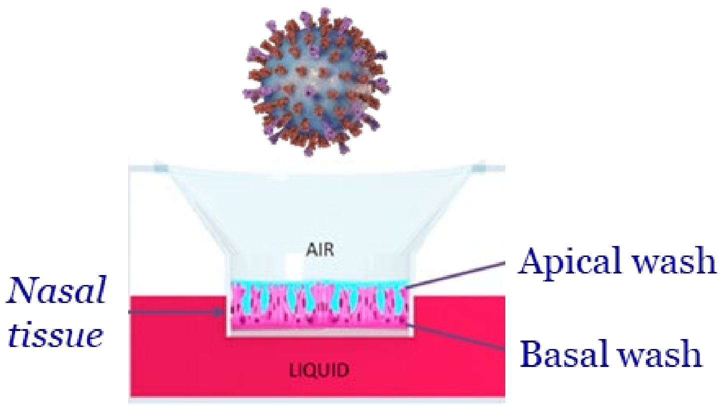
- Cell viability (Viral ToxGlo™) screening assay
  - Suitable for routine screening including HTS
  - Combination studies
  - Also suitable for cytotoxicity counter screens
- RSV plaque assay
  - Validation of viral stocks
  - Viral burden in tissue, e.g. as read-out for *in vivo* studies
  - Generation of resistant virus and mechanistic studies
- RT-qPCR
- GFP labelled virus
- RSV ELISA
  - Quantification of virus specific antibodies
  - Quantification of virus via viral proteins
- RSV microneutralization assay
  - Quantification of virus specific neutralizing antibodies

In vitro biology capability and expertise on RSV

RSV infection in human airway epithelial system

Human airway epithelial system

- MucilAir™ is a human *in vitro* model representing the upper airway epithelia containing beating cilia, goblet and basal cells
  - Single donor or pool of donors available in nasal healthy version
  - Different anatomical sites (Nasal, Tracheal or Bronchial)
- Highly relevant model to address pharmacology, toxicology and biology demands, in particular for cellular targets

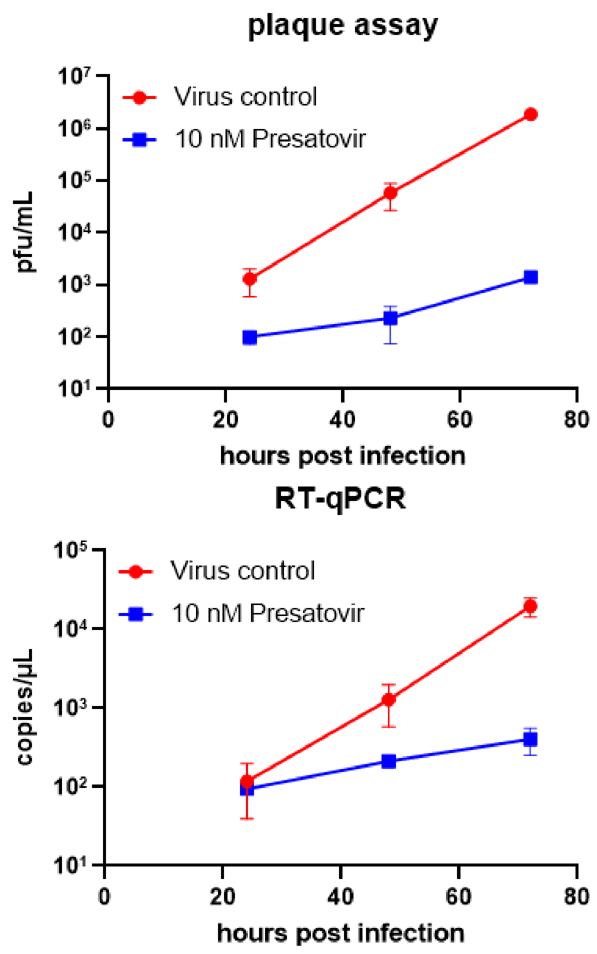


Schematic representation of MucilAir™[1]

Endpoints (in apical or basal wash)[1]

- Viral load by plaque assay or TCID50
- Viral titer by RT-qPCR
- Transepithelial electrical resistance (TEER) measurements to evaluate cell membrane integrity
- Cytotoxicity by LDH release
- Cytokine release

RSV



1 Assay has been developed for RSV, HPIV, SARS-CoV2 and influenza A virus – can be adapted for other viruses

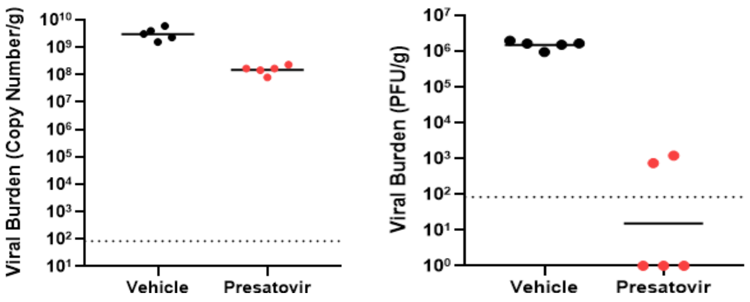
RSV *in vivo* infection models

Viral and host parameters

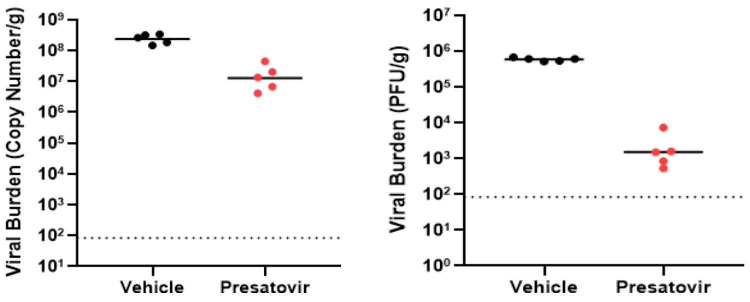
- **Animals**
  - **Cotton Rat infection model:** Gold standard for development of RSV inhibitors (vaccination and treatment studies)
  - **Mouse Balb/c mouse model** widely used to study RSV immunopathology (RSV-A2)
- **Infection**
  - Intranasal infection
- **Viral endpoints**
  - Viral burden in nose and lung tissues by plaque assay and qPCR
- **Host endpoints**
  - cytokine analysis
  - Antibody titre via ELISA
  - Neutralization / seroneutralization assays
  - immunohistochemistry
  - PK analysis

Cotton rat model

Lung

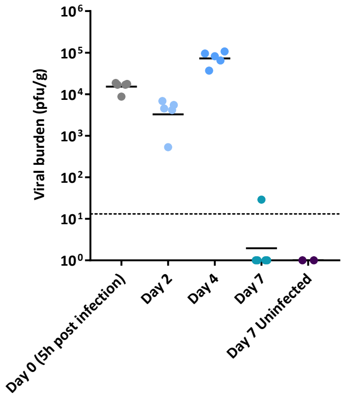


Nose



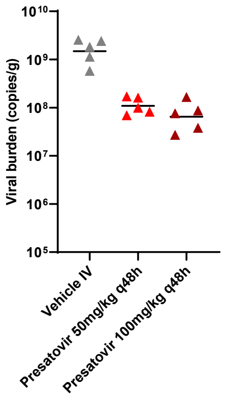
Mouse Balb/c model

Plaque assay



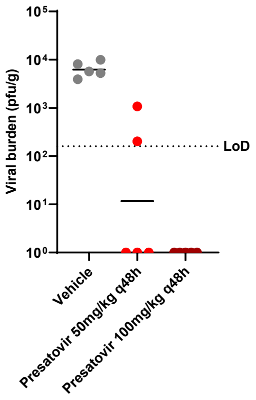
Time course of infection

RT-qPCR



Treatment study

Plaque assay



Treatment study

Influenza virus

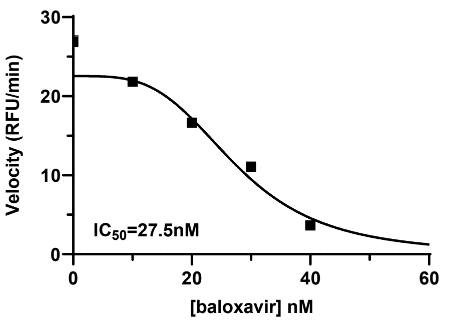
Capability and expertise on respiratory viruses – example influenza

From screening to animals

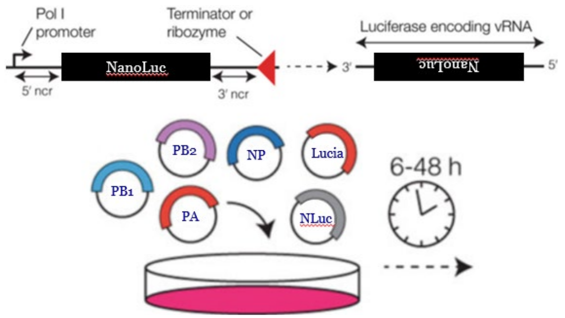
Target based assays

- Influenza virus PA endonuclease FRET assay
- Minigenome assays (polymerase)
- PA crystal structure

PA FRET assay



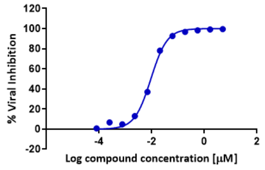
Minigenome assay



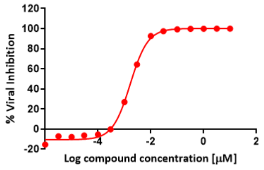
In vitro infection models

- Cellular models: MDCK, A-549, Calu-3, SH-SY5Y cells
- Cell viability as screening assays
- Direct antiviral assays for demonstration of antiviral effect and MOA studies: Plaque-forming assay, focus-forming assays, TCID50, RT-qPCR (SN, cell lysates)
- Hemagglutination assay
- Neuraminidase assay
- Immunofluorescence
- Reverse genetic

A549 cells



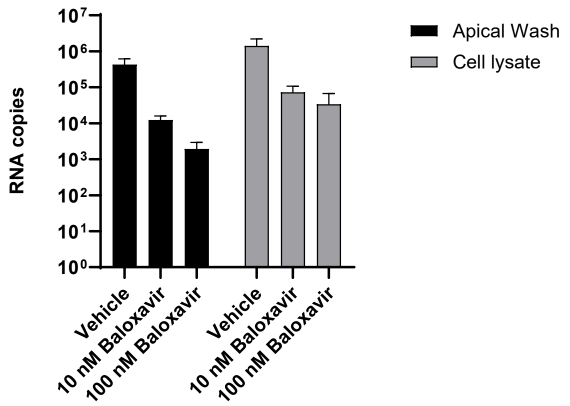
MDCK cells



Flu inhibition by Zanamivir

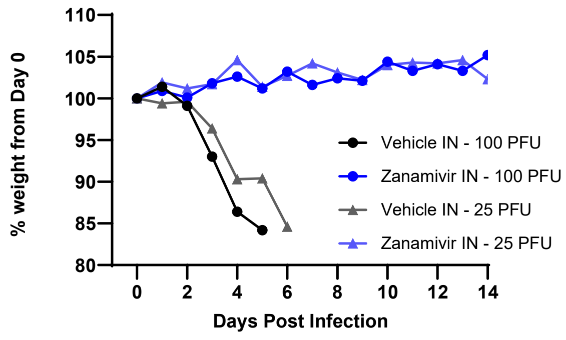
Epithelial Air Liquid Interface Assay

- Influenza infection model of Mucilair™ epithelial nasal or bronchial air liquid interface tissue
- Treatment in basal medium
- Sequential sampling of apical wash; terminal samples as cell lysate
- Determination of virus load by RT-qPCR
- Determination of cytokine levels



Influenza mouse model

- C57Bl/6 mice
- Intranasal infection with mouse adapted flu A/Puerto Rico/8/34, H1N1
- Viral load in lung homogenate by AVINA assay and qPCR
- Study length: Burden arm 4 days and Survival arm 14 days
- Endpoints: Weight loss, survival, viral lung burden determine by TCID50, NA-TCID50 (based on AVINA), RT-qPCR, WBC analysis, cytokines



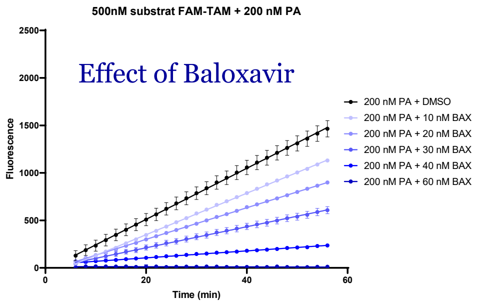
In vitro biology capability and expertise on respiratory viruses

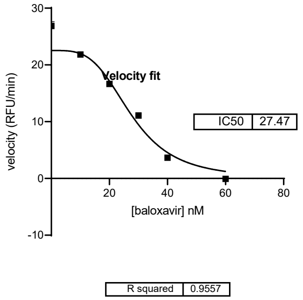
Example of influenza virus: from target-based to integrated models

Endonuclease assay

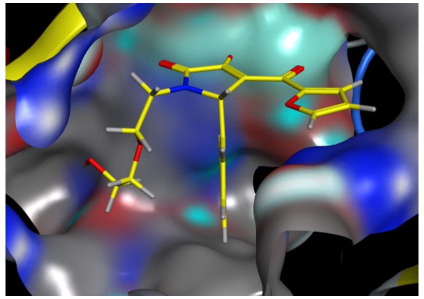
- FRET based assay for detection of PA endonuclease activity via fluorescence
- Test of WT, resistant mutants and new emerging strains
- Baloxavir as control inhibitor

PA FRET assay



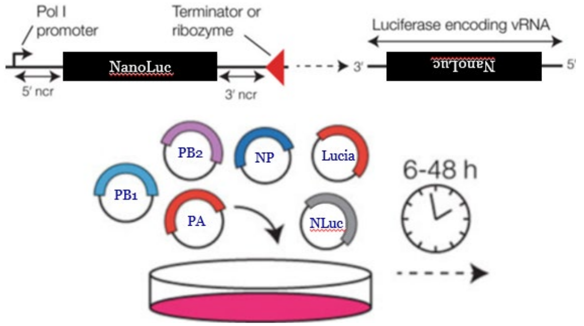


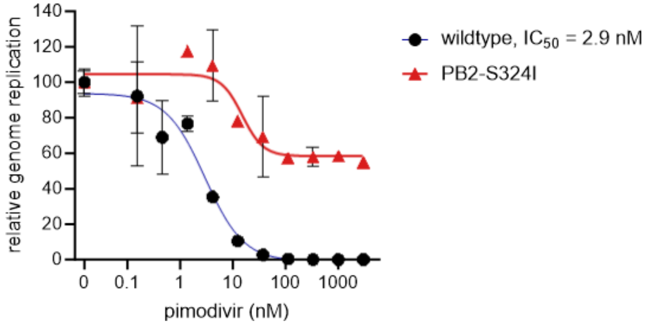
PA crystal



Minigenome assay[1]

- Genome replication measured by intracellular NanoLuciferase
- Test of WT, resistant mutants and new emerging strains
- Pimodivir/Baloxavir as control inhibitors





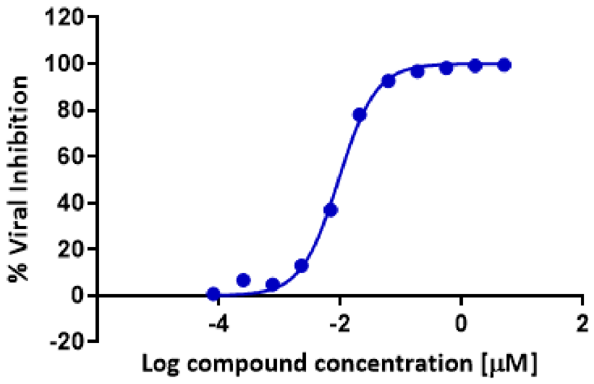
1 Robotic adaptation in development

In vitro biology capability and expertise on influenza

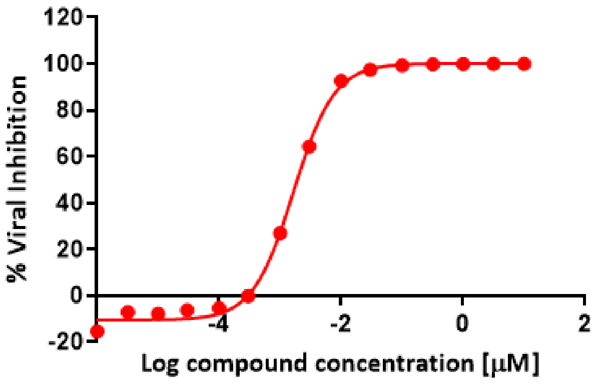
In vitro infection models

- **Cell lines**
  - **MDCK cells:** standard canine cells for influenza assays
  - **A549, Calu-3 cells:** human respiratory epithelial cells
  - **SH-SY5Y cells[1]:** neuroblastoma cells
  - **Human airway epithelial cell line:** human upper airway epithelium cultured at the air liquid interface
- **Read outs**
  - RT-qPCR
  - PFU/TCID50 methods
  - Cell viability (CPE)
  - Neuraminidase assay
  - Hemagglutination inhibition assay
  - Immunofluorescence

A549 cells



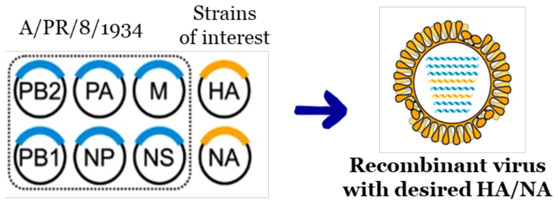
MDCK cells



Flu inhibition by Zanamivir

Various viral strains

- **Seasonal BSL2 strains:** A/Brisbane/59/2007, A/Puerto Rico/8/1934
- Highly pathogenic avian influenza **BSL3 strains** under implementation
- **Reverse Genetic technology[1]:** Generation of seasonal and pandemic strains of interest/concern



In vitro assays

- Influenza viral culture in A549 and MDCK cells
  - Virus neuraminidase activity readout (AVINA assay)
    - Medium throughput screening and dose response
    - Virus inhibition calculated relative to virus control, negative signal for inhibition
    - Also suitable for cytotoxicity counter screens
- Viral production and characterization
- Antigens quantification
- Seroneutralization assays
- Multiplexed cytokine assays

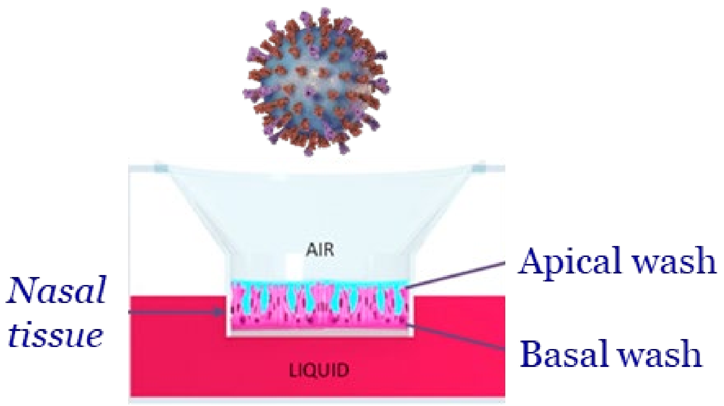
1 In development

In vitro biology capability and expertise on influenza

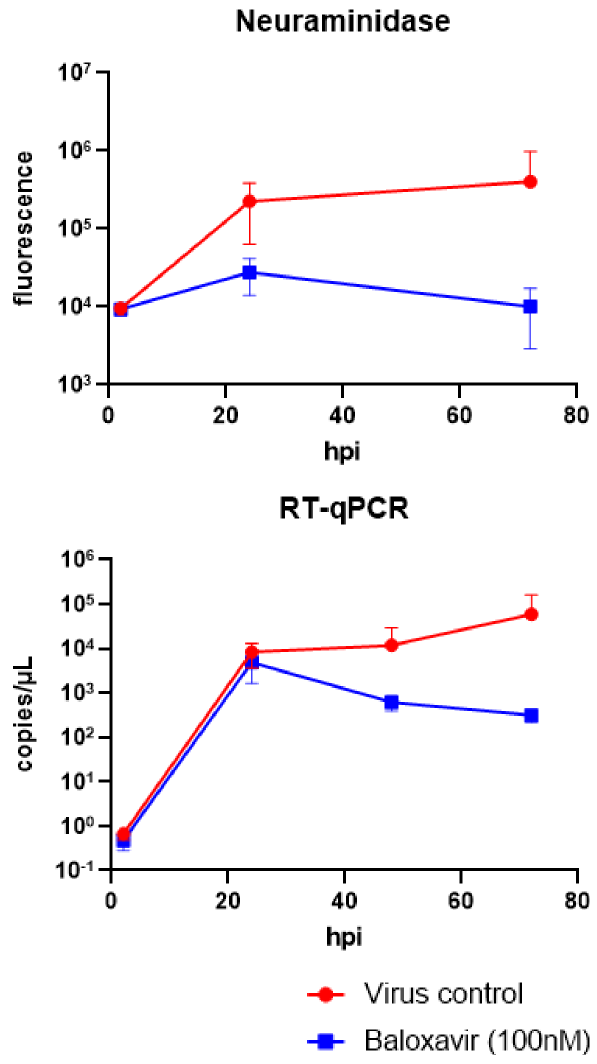
influenza infection in human airway epithelial system

Human airway epithelial system

- MucilAir^{TM} is a human *in vitro* model representing the upper airway epithelia containing beating cilia, goblet and basal cells
  - Single donor or pool of donors available in nasal healthy version
  - Different anatomical sites (Nasal, Tracheal or Bronchial)
- Highly relevant model to address pharmacology, toxicology and biology demands, in particular for cellular targets



Schematic representation of MucilAir^{TM}[1]



Endpoints (in apical or basal wash)[1]

- Viral load by plaque assay or TCID50
- Viral titer by RT-qPCR
- Transepithelial electrical resistance (TEER) measurements to evaluate cell membrane integrity
- Cytotoxicity by LDH release
- Cytokine release

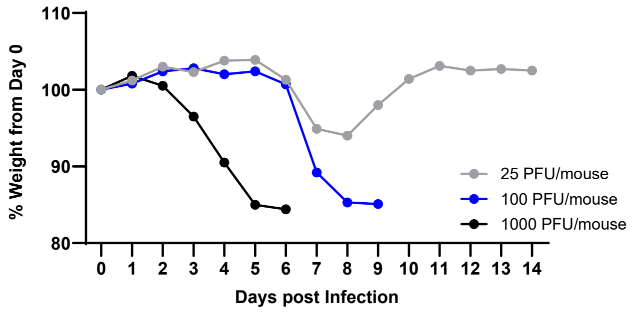
1 Assay has been developed for influenza A virus, SARS-CoV2 , RSV and HPIV – can be adapted for other viruses

Influenza *in vivo* mouse Infection model

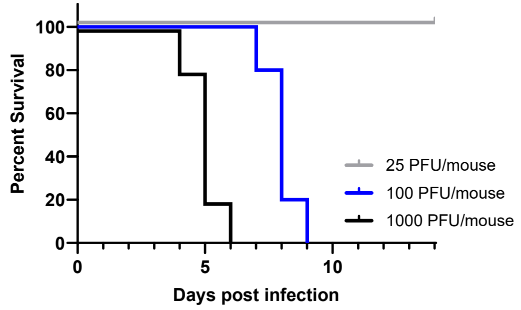
Viral and host parameters

- **Animals**
  - C57Bl/6 mice, female
  - 8 weeks of age on arrival
  - 5 mice per group
- **Infection**
  - Intranasal infection
  - Mouse Adapted-Puerto Rico 8 (MA-PR8)
- **Viral endpoints**
  - Viral lung burden: TCID50, NA-TCID50 (based on AVINA)
  - RT-qPCR
- **Host endpoints**
  - Weight loss
  - Survival (14 days)
  - WBC analysis
  - cytokine analysis
- **Model validation with ref cpds**
  - Zanamivir intranasally, 12.5mg/kg, q24h
  - 4'-FlU orally, 2mg/kg, q24h

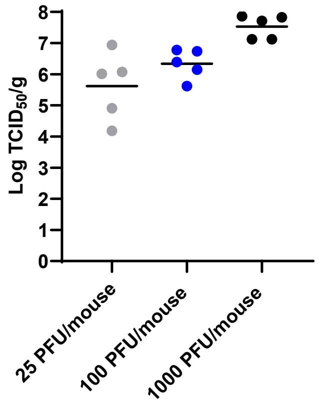
Body weights



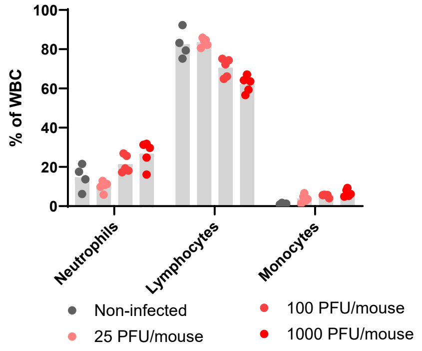
Survival



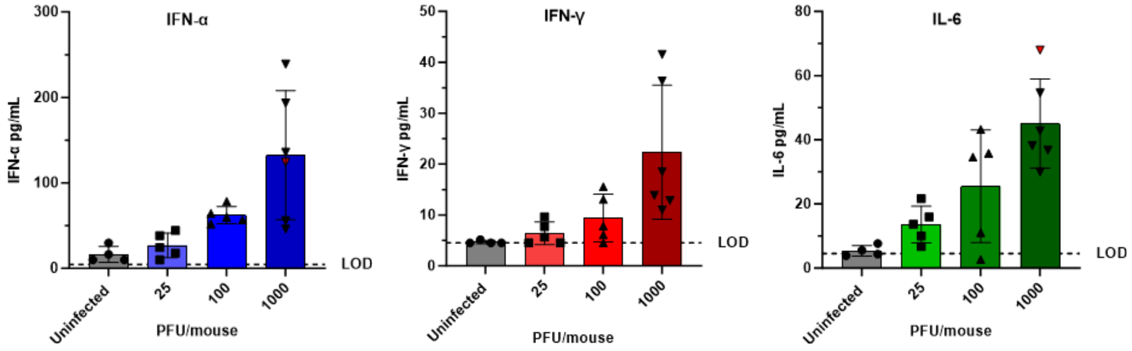
Day 4 Lung burden



White Blood Cells



Cytokines release



SARS-CoV-2 virus

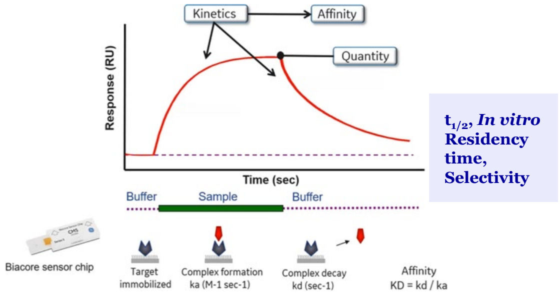
Capability and expertise on respiratory viruses – example SARS-CoV-2

From screening to animals

Target based assays

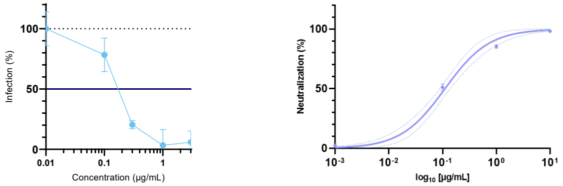
- *In vitro* pharmacology assays
  - SPR-based Assay
  - Alpha Assay technology
- SARS-CoV-2-Spike pseudotyped infection
  - VSV pseudotyped viruses available for all variants of concern

SPR-based assay



$t_{1/2}$, *In vitro* Residency time, Selectivity

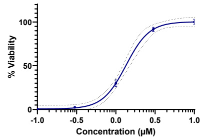
Neutralization assay of rVSV-SARS-CoV-2 Spike



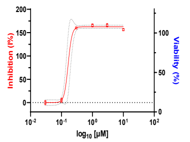
In vitro infection models

- Cellular models: VeroE6, A549-ACE2-TMPRS2, Calu3
- Cell viability as screening assays using CellTiter Glow™, XCelligence
- Direct antiviral assays for demonstration of antiviral effect and MOA studies: Plaque-forming assay, focus-forming assays, TCID50, ELISA, RT-qPCR (SN, cell lysates), synergistic assays, neutralization assays
- Generation and characterization of resistant mutants

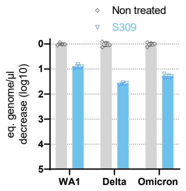
SARS-CoV-2 inhibition (Nirmaltrevir)



SARS-CoV-2 inhibition (Remdesivir)



SARS-CoV-2 neutralization assay

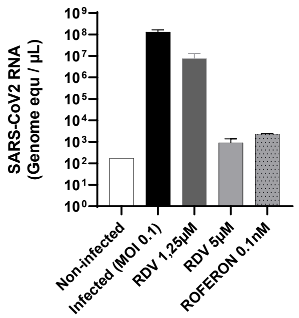


Epithelial Air Liquid Interface Assay

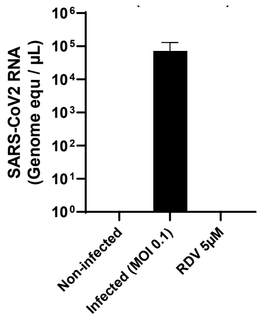
- SARS-CoV-2 infection model of Mucilair™ epithelial nasal or bronchial air liquid interface tissue
- Treatment in basal medium
- Sequential sampling of apical wash
- Determination of virus load by plaque assay and RT-qPCR
- Determination of cytokine levels

Virus titre in nasal tissue

Apical wash



Basal wash

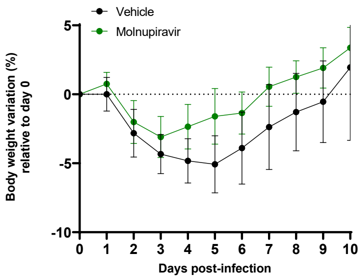


Influenza mouse model

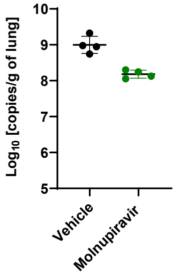
SARS-CoV-2 Hamster Model (including transmission model and long-disease model) and **SARS-CoV-2 humanised ACE2 mouse** model (vaccination and treatment studies)

- Intranasal infection
- Clinical observations
- Viral load by plaque assay and RT- qPCR
- Antibody titre via ELISA
- Neutralization assays
- Histopathology, immune responses, PK analysis

Body weights



Viral burden

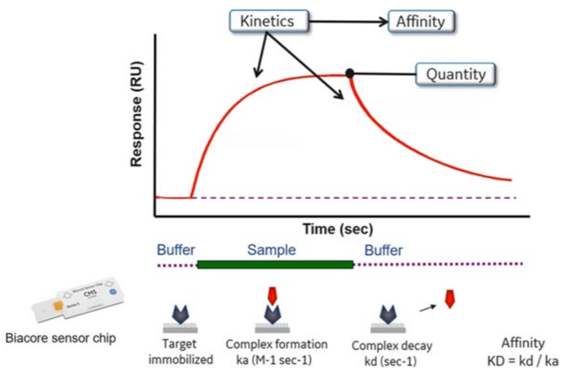


In vitro biology capability and expertise on respiratory viruses

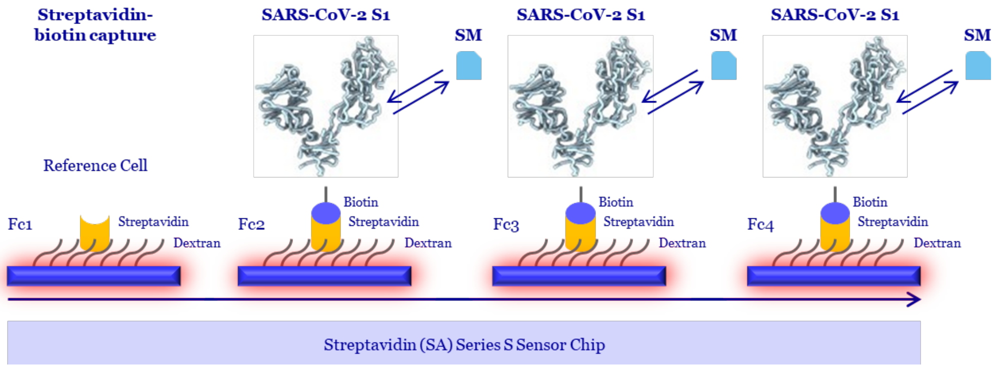
Example of SARS-CoV-2 virus: from target-based to integrated models

In vitro pharmacology assays

- 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

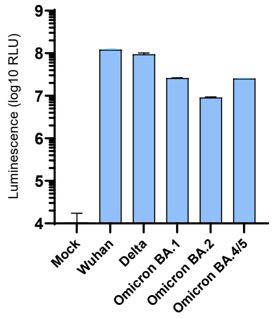


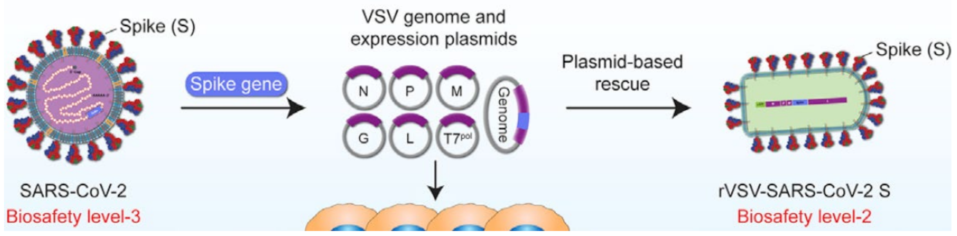
$t_{1/2}$, *In vitro* Residency time, Selectivity

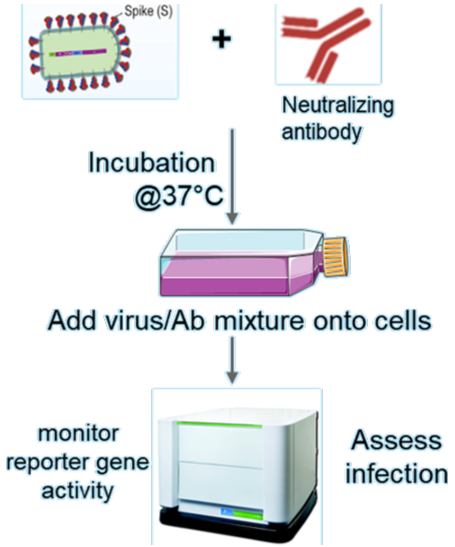


SARS-CoV-2-Spike pseudotyped infection

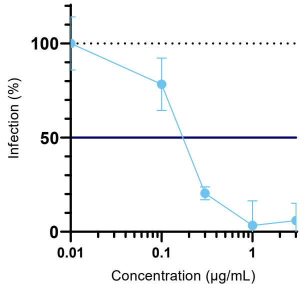
- VSV pseudotyped viruses available for all variants of concern

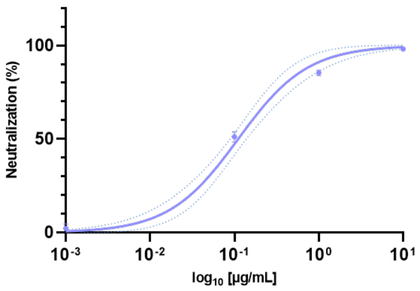






Neutralization assay of rVSV-SARS-CoV-2 Spike

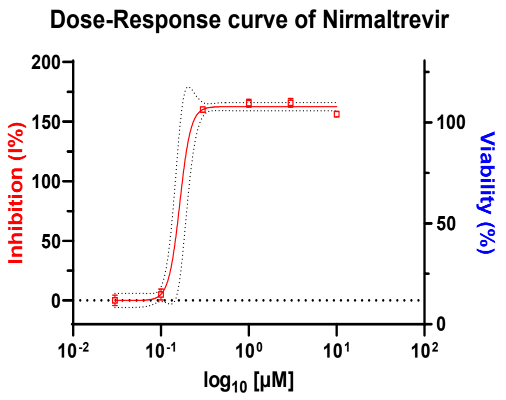




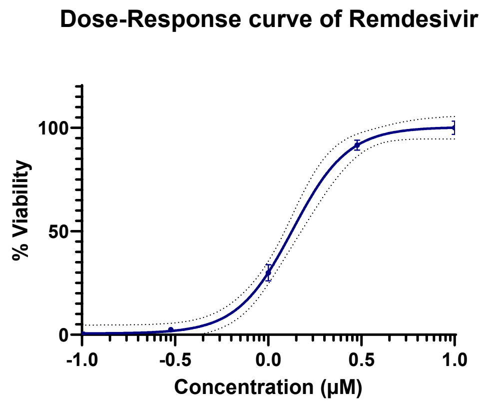
In vitro biology capability and expertise on SARS-CoV-2

In vitro infection models

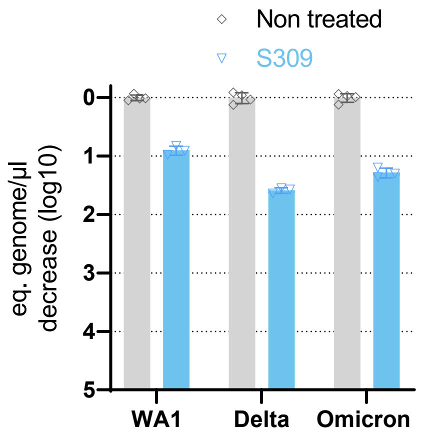
- **Cell lines**
  - **VeroE6 cells:** African green monkey kidney cells line
  - **A549-ACE2-TMPRSS2, Calu-3 cells:** human respiratory epithelial cells
  - **Human airway epithelial cell line:** human upper airway epithelium cultured at the air liquid interface
- **Read outs**
  - RT-qPCR (SN, cell lysates)
  - PFU/FFU/ TCID50 methods
  - Cell viability (CPE)
  - ELISA
  - Immunofluorescence



SARS-CoV-2 inhibition by Nirmaltrevir



SARS-CoV-2 inhibition by Remdesivir



SARS-CoV-2 neutralization assay

Various viral strains

- Numerous SARS-CoV-2 variants
  - > 45 strains incl variants Wuhan, Delta, Gamma, Omicrons, XBB, EG5.1,..

In vitro assays

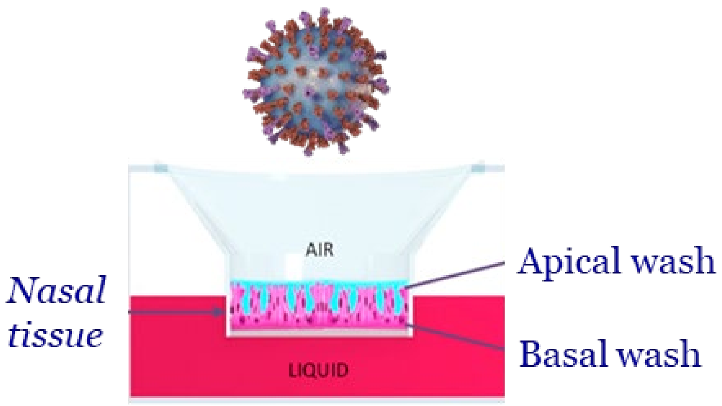
- SARS-CoV-2 viral culture in VeroE6, A549-ACE2-TMPRS2[1], Calu3
  - Cell TiterGlo™ screening assay (VeroE6)
  - MTS and dose response
  - cytotoxicity counter screens
- SARS-CoV-2 plaque assay (PFU, FFU) and TCID50
  - viral stocks and viral burden in tissue (*in vivo* studies)
  - Generation of resistant virus
  - Mechanistic studies
- SARS-CoV-2 RT-qPCR
  - MTS and dose response
  - Quantification of genomic viral RNA (cells and supernatants)
- SARS-CoV-2 ELISA: Quantification of virus and viral specific antibodies
- SARS-CoV-2 neutralization assay

In vitro biology capability and expertise on SARS-CoV-2

SARS-CoV-2 infection in human airway epithelial system

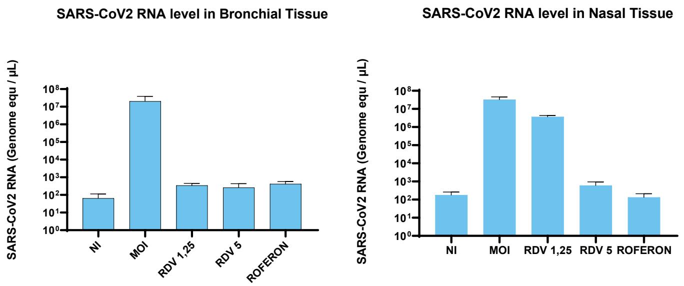
Human airway epithelial system

- MucilAir™ is a human *in vitro* model representing the upper airway epithelia containing beating cilia, goblet and basal cells
  - Single donor or pool of donors available in nasal healthy version
  - Different anatomical sites (Nasal, Tracheal or Bronchial)
- Highly relevant model to address pharmacology, toxicology and biology demands, in particular for cellular targets

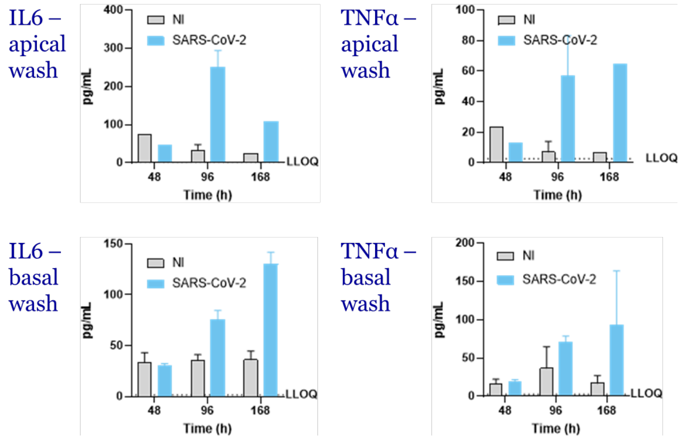


Schematic representation of MucilAir™[1]

Virus titre in bronchial and nasal tissues



Inflammatory cytokines in nasal tissues



Endpoints (in apical or basal wash)[1]

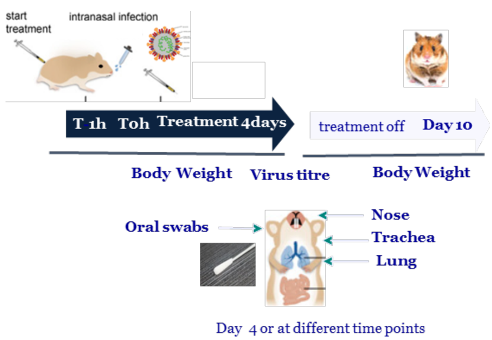
- Viral load by plaque assay, TCID50 and viral titer by RT-qPCR (intercellular and apical wash)
- Transepithelial electrical resistance (TEER) measurements to evaluate cell membrane integrity
- Cytotoxicity by LDH release
- Cytokine release

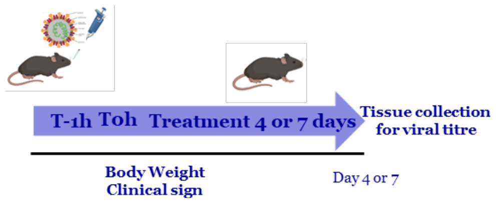
1 Assay has been developed for SARS-CoV2 , RSV, HPIV and influenza A virus – can be adapted for other viruses

SARS-COV-2 *in vivo* infection models

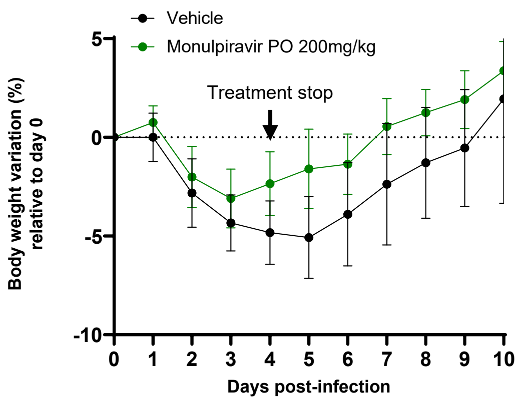
Viral and host parameters

- **Animals**
  - Golden Syrian Hamster (incl. long-disease and transmission models)
  - Humanised K18-ACE2 mouse
- **Infection**
  - Intranasal infection (Several viral strains)
- **Treatment**
  - Monotherapy or combination
  - small molecule, Antibody, Vaccine
- **Viral endpoints**
  - Viral burden in tissues and Throat swabs
- **Host endpoints**
  - Weight loss, clinical signs
  - cytokine analysis
  - Antibody titer via ELISA
  - Seroneutralization assays
  - Histopathology
  - PK analysis
- **Bespoke dosing regimen**
- **Bespoke treatment duration & Study duration**



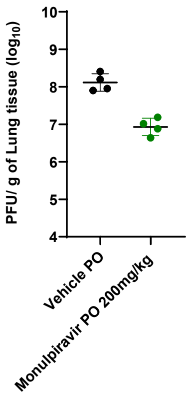


Body weight

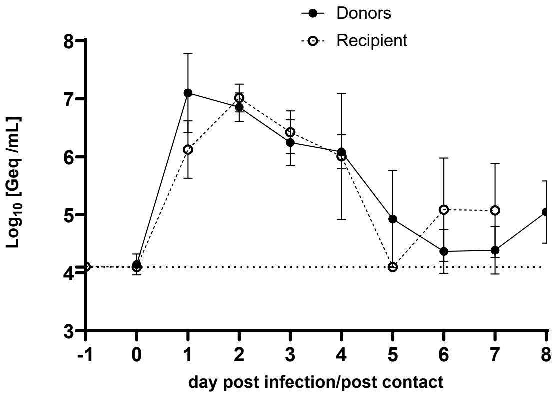


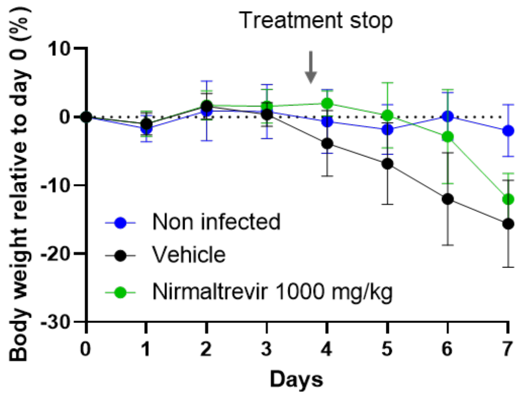
Virus Titre at day 4

Plaque Assay: Lung

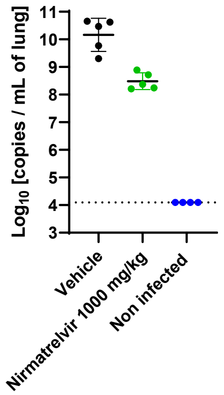


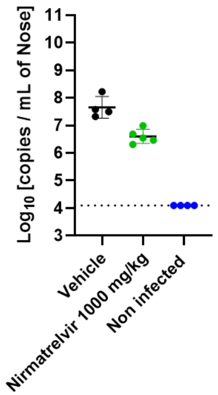
RT-qPCR oral swabs





RT-qPCR: lung and nose





Agenda

1. Evotec Virology Overview
2. Focus on Respiratory viruses
3. Focus on Hepatic viruses (HBV and HDV)
4. NGS & Bioinformatics platforms
5. Integrated virology (Hit ID to PDC)

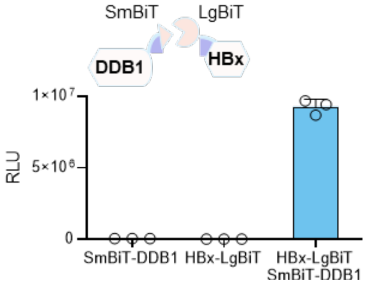
Capability and expertise on hepatitis viruses

From screening to animals

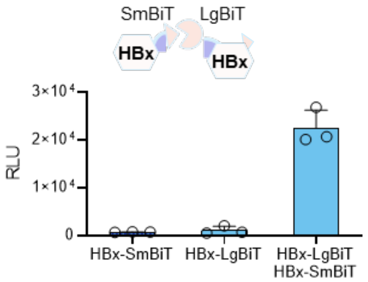
Target based assays

- *In vitro* pharmacology assays
  - SPR-based Assay
  - Thermal shift assay
  - HBx/DDB1 dimerization
  - HBx homodimerization
  - Proteins degradation

HBx DDB1 interaction

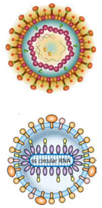


HBx dimerization

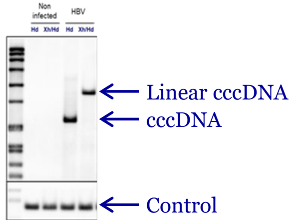


In vitro infection model

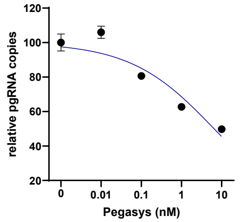
- Cellular models: HepaRG, HepAD38, PHH
- Readouts:
  - Viral DNA and cccDNA
  - Cellular / viral RNAs
  - Circulating DNA and RNA
  - HBe/HBs release
  - Cell viability
  - Cytokines release



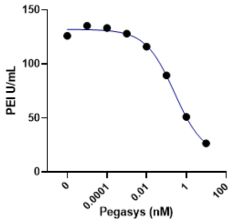
cccDNA



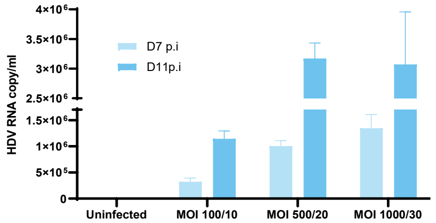
Intracellular pgRNA



HBe /HBs release



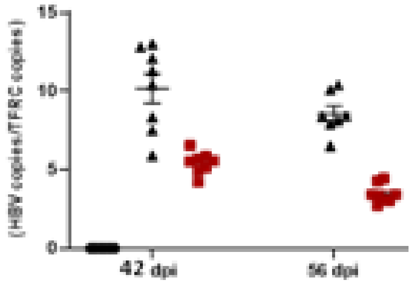
HDV / HBV coinfection (PHH)



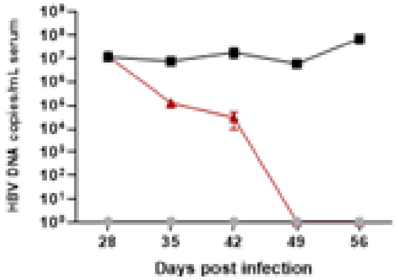
HBV animal model

- Immune competent animals transduced with HBV via AAV carrier
- Viral readouts: HBs, HBe, HBV DNA, pgRNA, cccDNA
- Host readouts: immunoprofiling, cytokines, liver enzymes

Liver HBV DNA



Peripheral blood HBV DNA



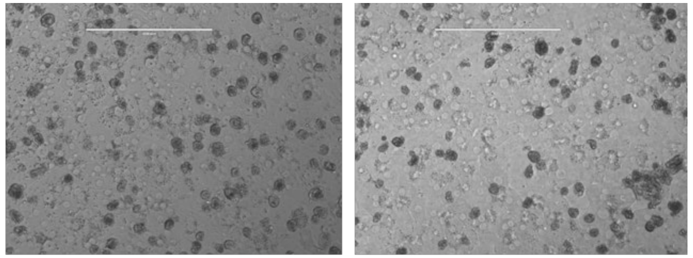
In vitro biology capability and expertise on hepatitis B virus

In vitro infection models

- **Cell lines**
  - **HepaRG:** human hepatic cell line
  - **HepAD38:** inducible liver-derived cell line expressing HBV genome
  - **HepG2-NTCP** stably transfected cell line
  - **PHH:** Primary human hepatocytes

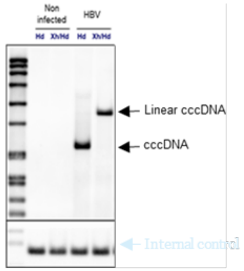
- **Read outs**
  - qPCR, RT-qPCR, ddPCR
  - Southern blot
  - Immunofluorescence
  - Cell viability (CTG, LDH, HAS)
  - Elisa

HBV infection in PHH

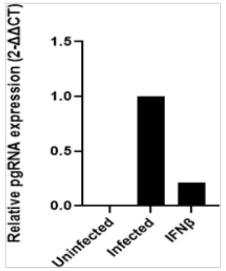


Infection Day | Day 7 post infection

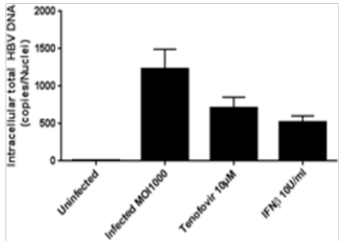
cccDNA



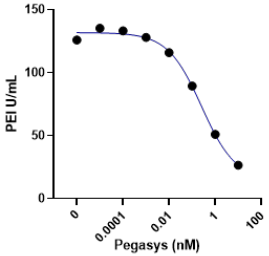
Intracellular pgRNA



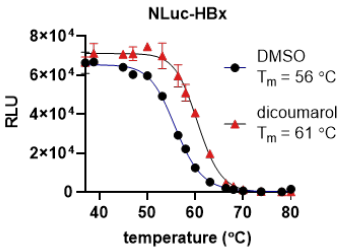
HBV DNA



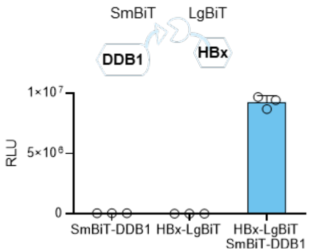
HBe release



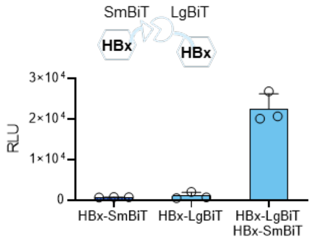
Cellular thermal shift (CTS)



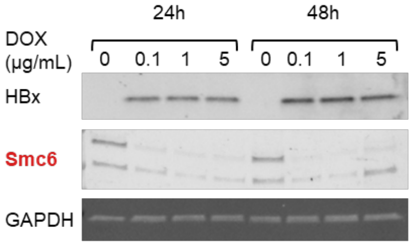
HBx/DDB1 dimerization



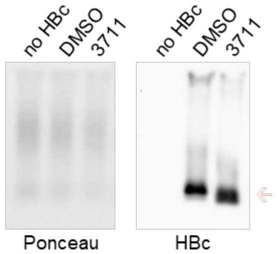
HBx dimerization



Smc6 degradation



Nucleocapsid formation



Various viral strains

- **Hepatitis B** genotype D

In vitro assays

- Antiviral testing – Dose response, incl. cytotoxicity assays
- Viral production and characterization
- Mechanistic studies
- Quantification of Antigens
- Quantification of intracellular viral DNA, RNA and cccDNA
- Quantification of extracellular viral DNA, Hbe and Hbs release and viral burden in tissue
- Multiplexed cytokine assays

Hepatitis B Infection Model

Screening capabilities: HTS in BSL3 laboratory

Assay development | **Primary Screen** | **Analysis** | **Secondary Screen** | **Counter Screen** ▶

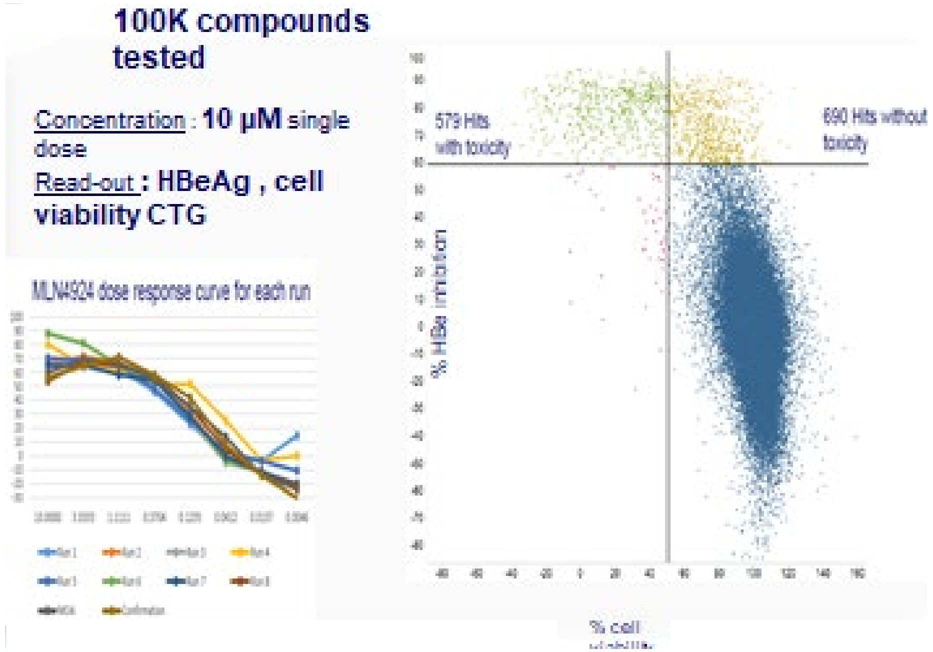
- **Highest quality chemical start points**
- **Best value**

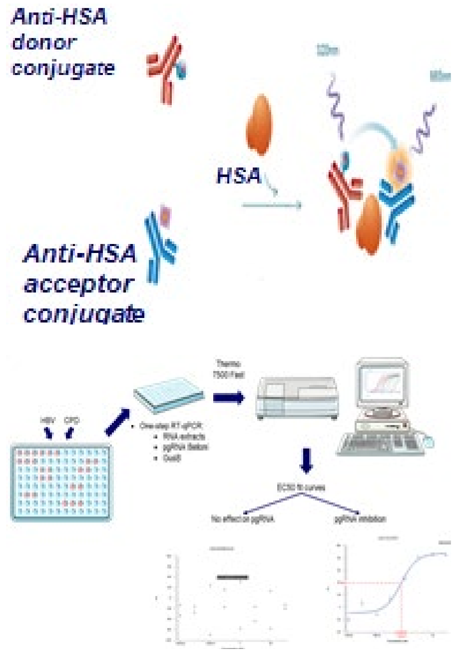
- Choice of cells
  - Primary Human Hepatocytes
  - HepaRG
- HepG2-NTCP
- Plate format
  - 96w
  - 384w
  - 1536w
- Treatment conditions
- Readouts

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

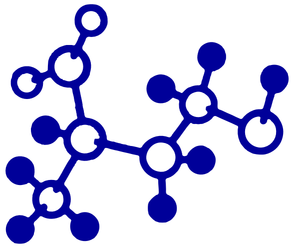
1,269 active hits

Primary screening





hits for follow-up



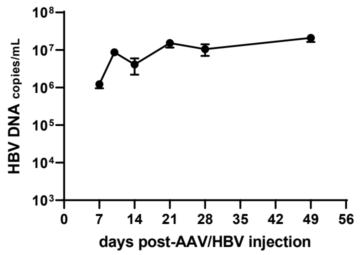
Hepatitis B virus *in vivo* infection model

Viral and host parameters

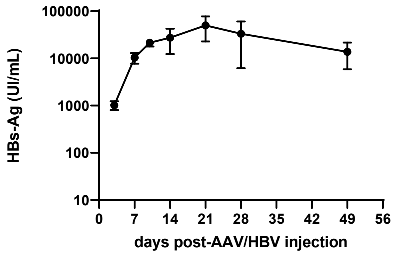
- **Animals**
  - **AAV/HBV-transduced mouse model**
  - **Various routes of treatment** (direct antivirals or host-targeting agents)
- **Infection**
  - Immune competent animals trans-duced with HBV via AAV carrier
- **Viral readouts**
  - Circulating viral DNA, RNA
  - HBeAg, HBsAg, HBcAg
  - Liver viral DNA, RNA and cccDNA
- **Host readouts**
  - Activated immune cells
  - cytokines
  - AST/ALT
  - Anti-HBsAg, anti-HBcAg antibodies
  - **Histology on liver biopsies**
    - Standard coloration (H&E staining, Sirius red staining, HBc-Ag Immunohistochemistry
    - In depth histopathological analysis and/or IHC

Persistence of viremia and viral antigens

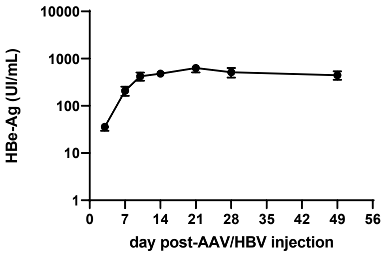
Peripheral Blood HBV DNA level



Peripheral Blood HBs-Ag level

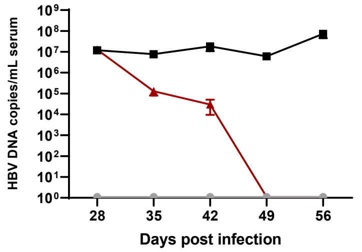


Peripheral Blood HBe-Ag level

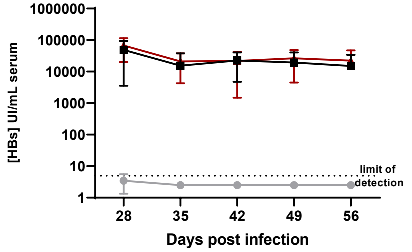


Effect of Nucleoside analogue (Entecavir 0.1 mg/kg)

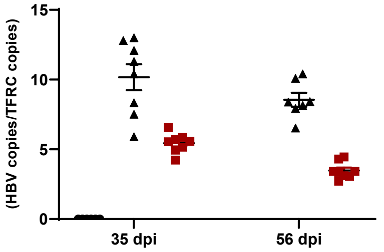
Peripheral Blood HBV DNA level

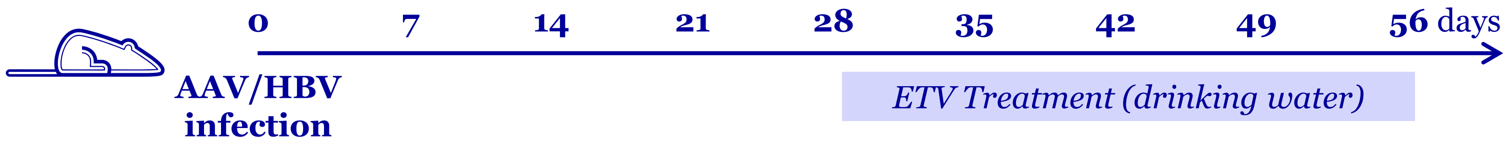


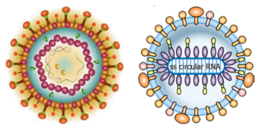
Peripheral Blood HBs-Ag level



Liver HBV DNA level







In vitro biology capability and expertise on hepatitis D virus

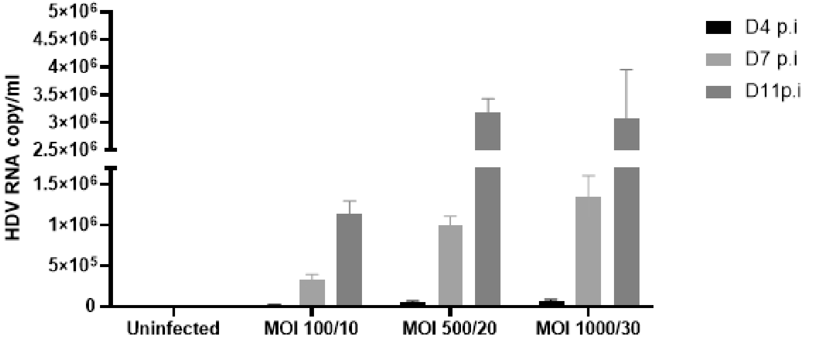
Coinfection, superinfection in Primary human hepatocytes

In vitro infection models

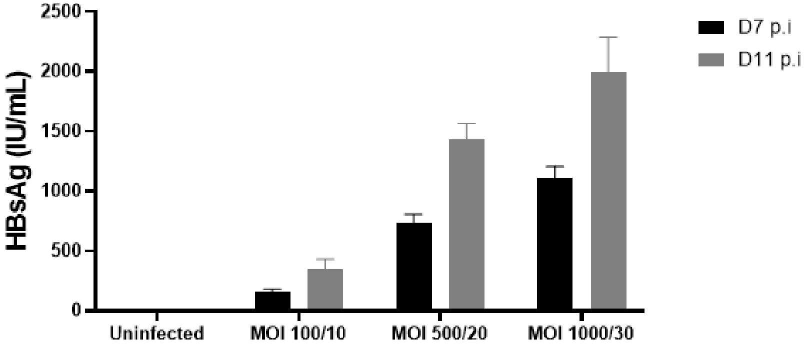
- **Cell lines:** cryoplateable PHH (Multiple PHH donors)
- **Viral strains**
  - **Hepatitis D genotype 1** *(Genotypes 2 to 8 under development)*
- **Different infection protocols**
  - Co-infection HBV/HDV
  - Super-infection HBV/HDV
  - Mono infection HDV
- **Readouts**
  - Intracellular viral DNA/RNA (qPCR, RT-qPCR), cccDNA (SB, ddPCR)
  - Extracellular viral DNA/RNA (q-PCR, RT-qPCR)
  - HBe and HBs release (ELISA)
  - HD-Ag (WB)
  - Host parameters (gene expression, cytokine profiles)
  - Cell viability (CTG, LDH, HSA)

Viral parameters follow-up (co-infection protocol)

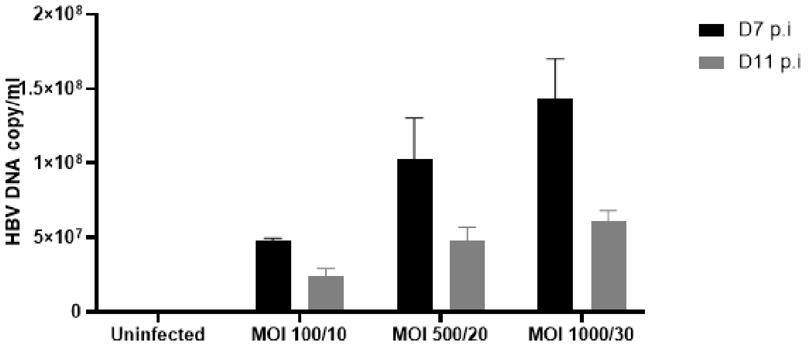
Extracellular HDV RNA



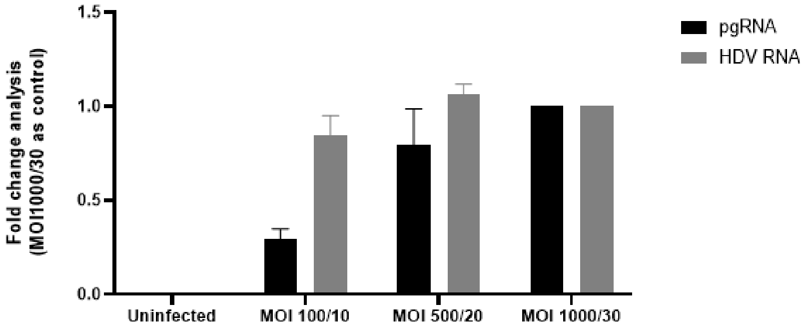
HBsAg secretion



Extracellular HBV DNA

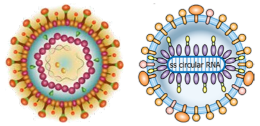


Intracellular HBV and HDV RNA

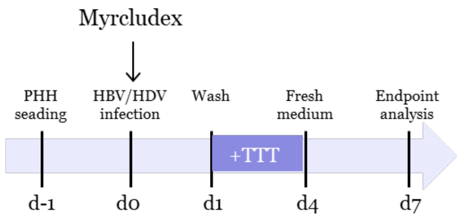


In vitro biology capability and expertise on hepatitis D virus

HBV/ HDV co-infection in Primary Human Hepatocytes (PHH)

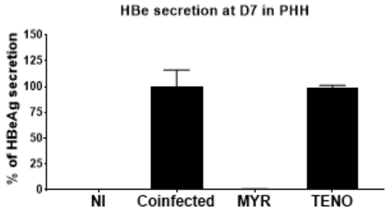


Effect of Myrcludex & Tenofovir

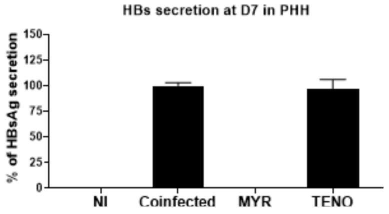


Intracellular and Extracellular viral parameters

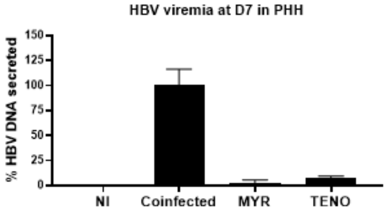
HBe-Ag



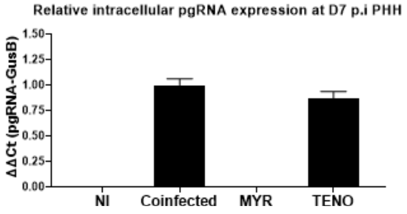
HBs-Ag



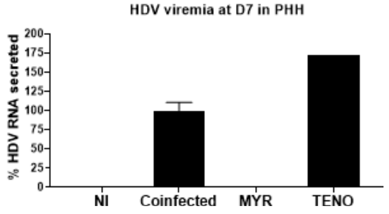
HBV Viremia



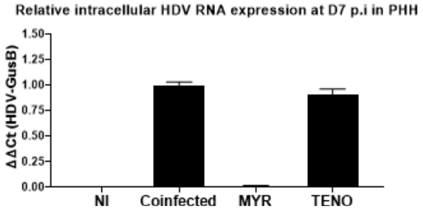
HBV pgRNA



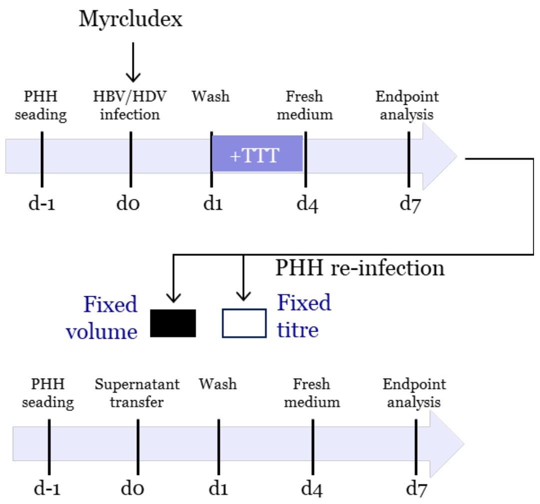
HDV Viremia



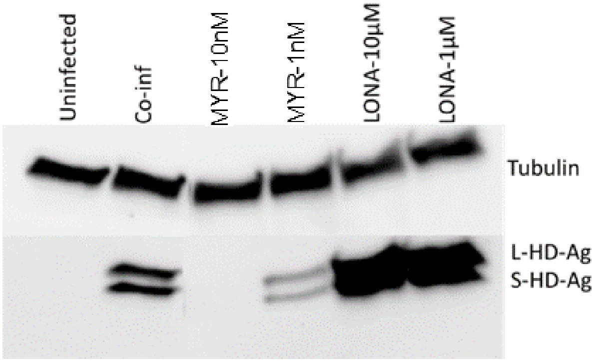
HDV intracellular



Propagation assay protocol

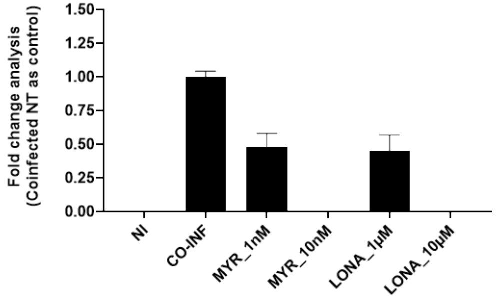


WB (HD-Ag in PHHs)

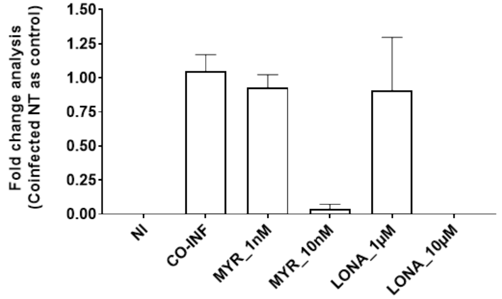


HDV intracellular expression

Fixed volume



Fixed Titre



1 Myrcludex treatment simultaneously with HBV/HDV co-infection

Agenda

1. Evotec Virology Overview
2. Focus on Respiratory viruses
3. Focus on Hepatic viruses (HBV and HDV)
4. NGS & Bioinformatics platforms
5. Integrated virology (Hit ID to PDC)

NGS and Bioinformatics platforms

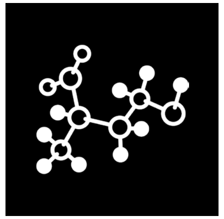
Functional Genomics for Infectious Disease

Available technologies to support drug discovery

Genomics – Microbial whole genome sequencing
(bacteria, viruses)
WGS: Illumina and **Nanopore long-read** technologies
Pool-seq: Variants detection in a population

Transposon sequencing
Tn-seq: saturated transposon library generated with commercial or custom transposon
Sequencing with commercial or custom primers

Transcriptomics
Direct RNA sequencing (Nanopore)
Single **RNA-seq**, dual (mammalian & microbial) RNA-seq
High-throughput **qPCR** (Fluidigm Biomark), **digital PCR**



Metagenomics
Based on the current NGS expertise in extraction and sequencing
Illumina and **Nanopore long-read** technologies
Whole metagenome shotgun and amplicon sequencing (16S)

Under develop-ment

Multiple technologies, fast turnaround time

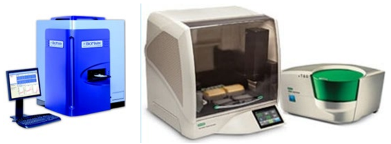
Illumina NextSeq 550
Accuracy
High throughput

Illumina NextSeq 550
Ultra-long reads
Mid throughput

Illumina ISeq
Accuracy
Low throughput

QC controls
Fluorometric
Spectrophotometric
Electrophoretic

Q(RT-)PCR, HT-qPCR & ddPCR
Fluidigm Biomark, Bio-rad QX200

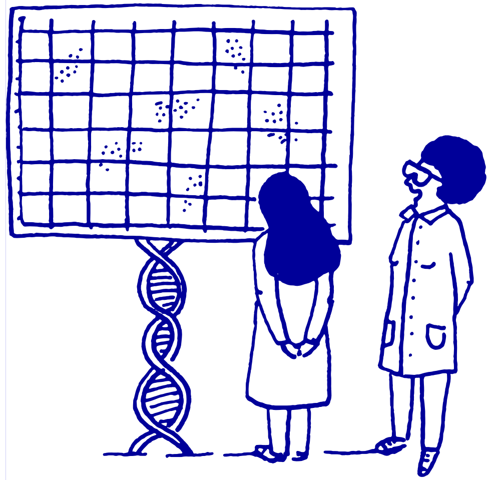


Automation
Automated DNA extraction

NGS and Bioinformatics platforms

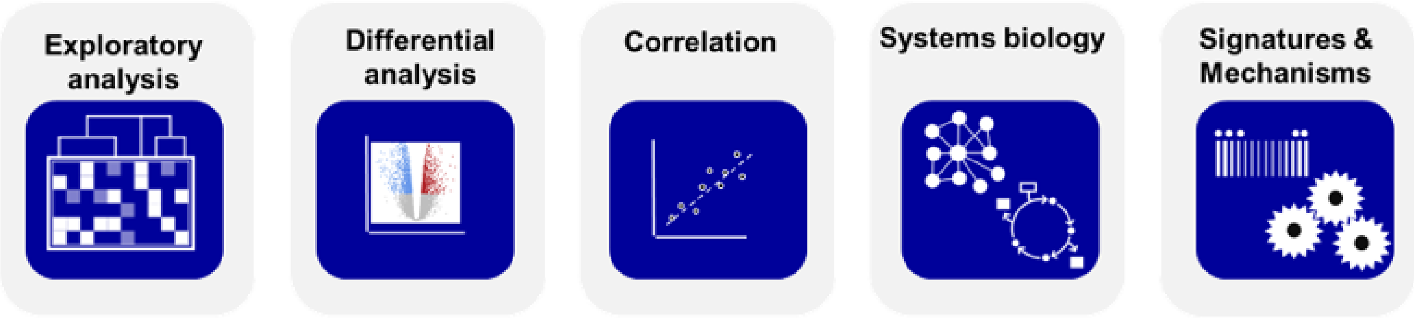
End-to-end workflow

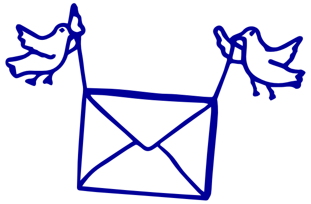
NGS Team
(illumina/ONT)



Bioinformatics Team

Biological question

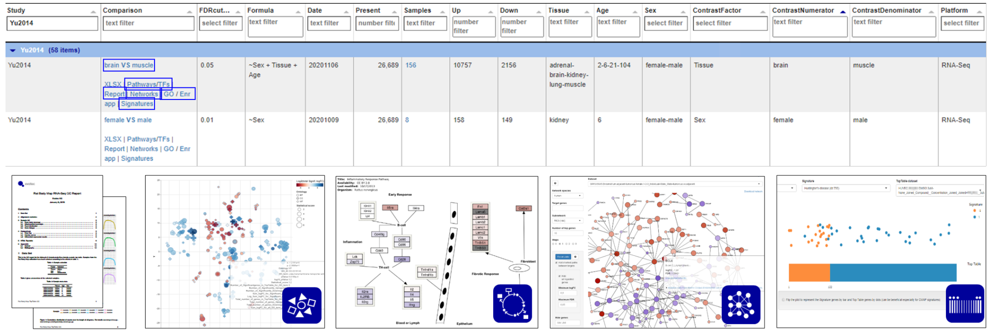




Samples → RNA extraction → Library preparation → Sequencing → **PanHunter** → Data analysis → **Actionable Insights**

Differential gene expression results:

Interactive exploration of downstream analyses results



QC Report | GO term visualization | Interactive pathway maps | Regulated subnetworks | Visualizing signature matches

Agenda

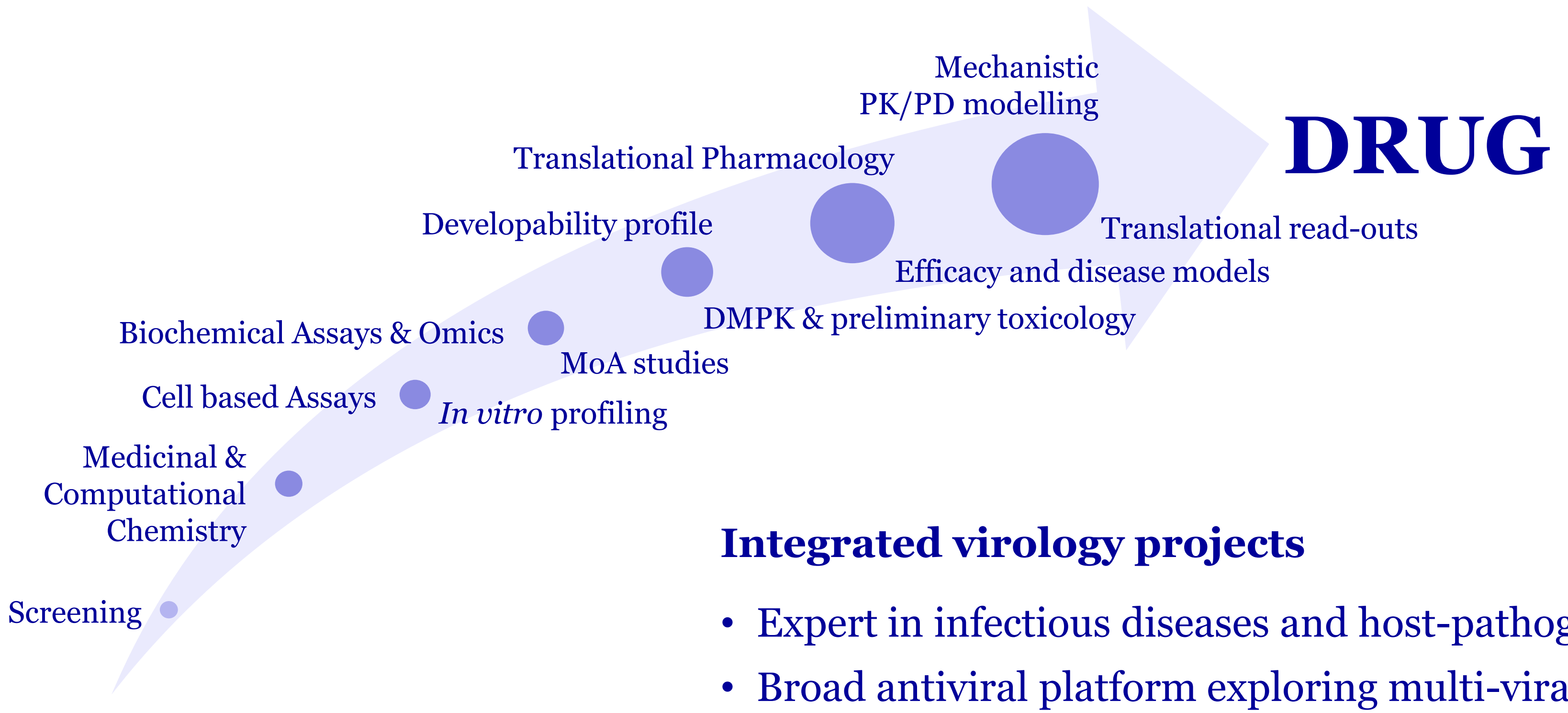
1. Evotec Virology Overview
2. Focus on Respiratory viruses
3. Focus on Hepatic viruses (HBV and HDV)
4. NGS & Bioinformatics platforms
5. **Integrated virology (Hit ID to PDC)**

Agenda

1. Evotec Virology Overview
2. Focus on Respiratory viruses
3. Focus on Hepatic viruses (HBV and HDV)
4. NGS & Bioinformatics platforms
5. Integrated virology (Hit ID to PDC)

Integration of knowledge is key

ONE Project Team



Integrated virology projects

- Expert in infectious diseases and host-pathogen interaction
- Broad antiviral platform exploring multi-viral activity of new molecules
- Disease area specific infrastructure (BSL3, vivarium etc.)
- Exploration across disease areas (virology, respiratory, immunology, fibrosis etc.)
- Single team executing whole project
- Flexibility to react to results and strategic changes

#RESEARCHNEVERSTOPS

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Agenda

BACK UP SLIDES

1. Respiratory viruses
2. Hepatic viruses (HBV and HDV)
3. Immunology capabilities
4. Integrated virology R&D platforms (Hit ID to PDC)

Agenda

BACK UP SLIDES

1. Respiratory viruses
2. Hepatic viruses (HBV and HDV)
3. Immunology capabilities
4. Integrated virology R&D platforms (Hit ID to PDC)

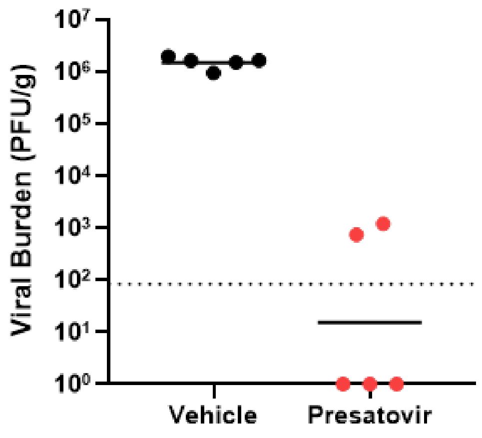
RSV In vivo model

In vivo capability and expertise on RSV

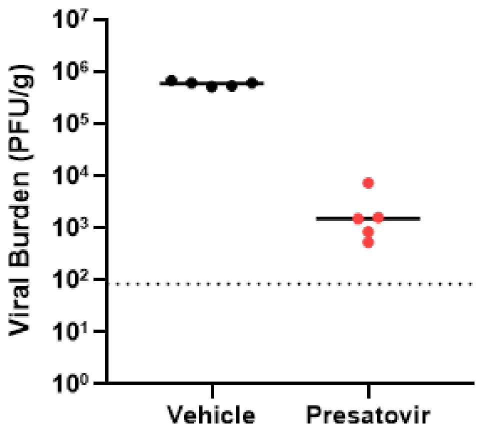
Cotton Rat Infection Model

- 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
- Validated model Read-Outs
  - 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 able to neutralize infection
- Model used in for vaccination and treatment studies

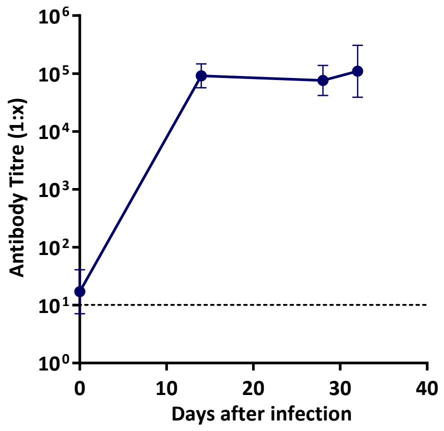
Lung



Nose



RSV specific antibodies



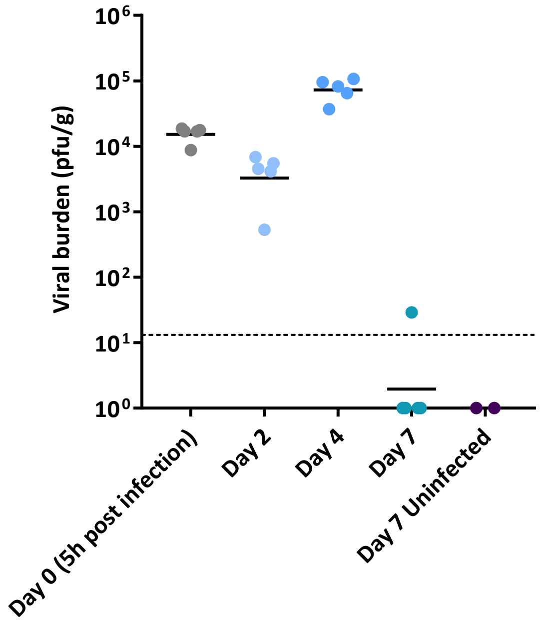
In vivo capability and expertise on RSV

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
- Validated model endpoints
  - 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

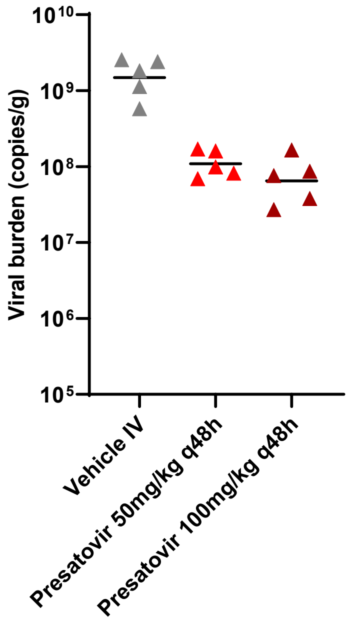
Time course of infection

Plaque assay

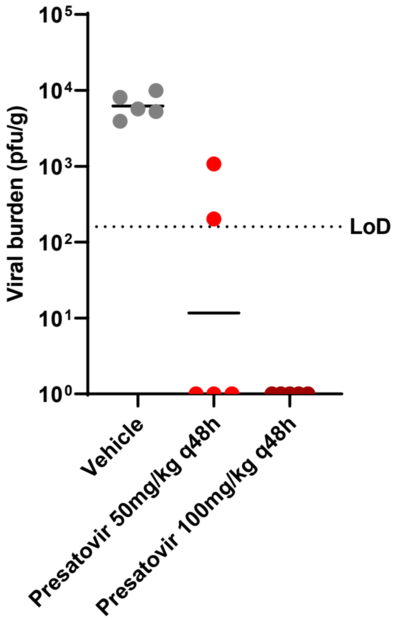


Treatment study

RT-qPCR



Plaque assay



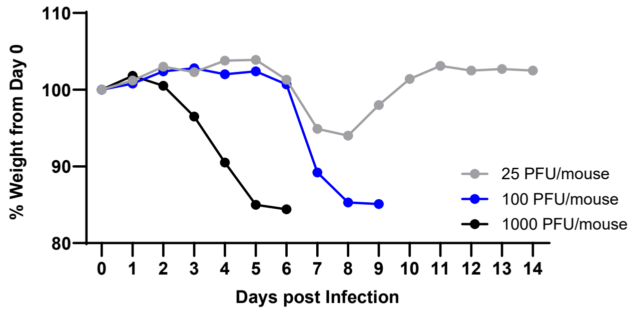
Influenza in vivo model

Influenza *in vivo* model

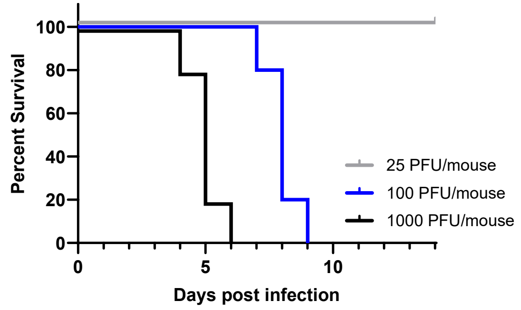
Mouse Infection Model

- **Animals**
  - C57Bl/6 mice, female
  - 8 weeks of age on arrival
  - 5 mice per group
- **Infection**
  - Intranasal infection, 50µL/mouse under isoflurane anaesthesia
  - Infection with mouse adapted Flu A/Puerto Rico/8/34, H1N1
- **Endpoints**
  - Study length: Burden arm 4 days and Survival arm 14 days
  - Weight loss, Survival, Viral lung burden determine by TCID50, NA-TCID50 (based on AVINA), RT-qPCR, WBC analysis, cytokine analysis

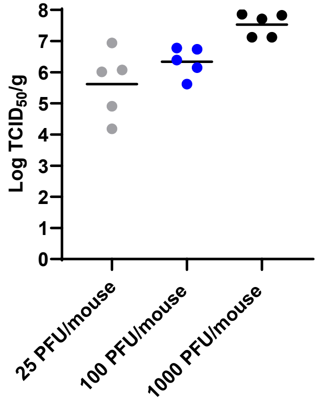
Body weights



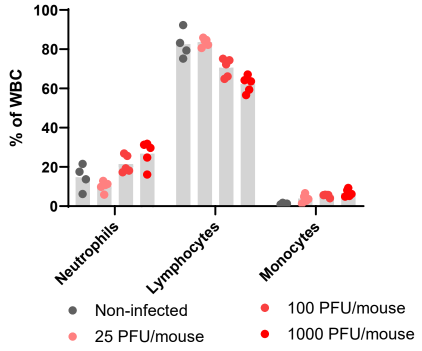
Survival



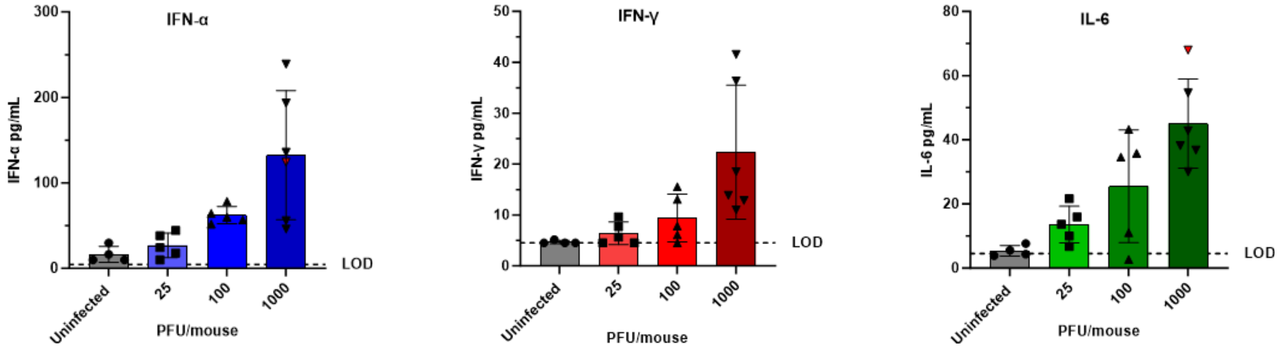
Day 4 Lung burden



White Blood Cells



Example Cytokines



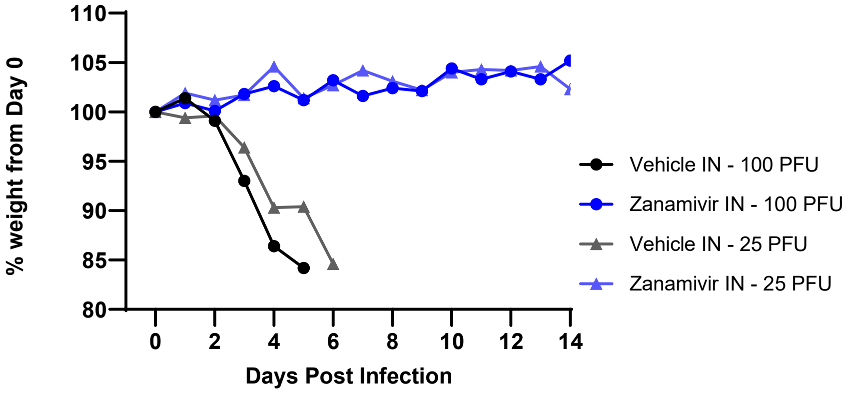
Influenza *in vivo* model

Mouse Infection Model – two different treatment regimen

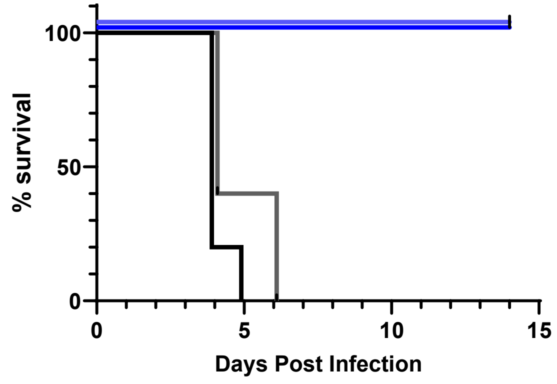
- **Animals**
  - C57Bl/6 mice, female, 8 weeks of age on arrival, 5 mice per group
- **Infection**
  - Intranasal infection, 50µL/mouse under isoflurane anaesthesia
  - Infection with mouse adapted Flu A/Puerto Rico/8/34, H1N1
- **Endpoints**
  - Study length: Burden study 3/4 days, Survival study 14 days
  - Weight loss, Survival, Viral lung burden determine by TCID50, NA-TCID50 (based on AVINA), RT-qPCR, WBC analysis
- **Treatments**
  - Zanamivir intranasally, 12.5mg/kg, q24h
  - 4’-FlU orally, 2mg/kg, q24h

Body weights

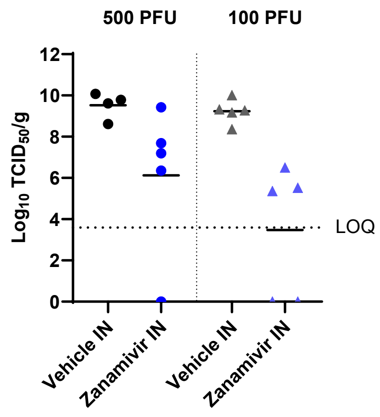
ZANAMIVIR



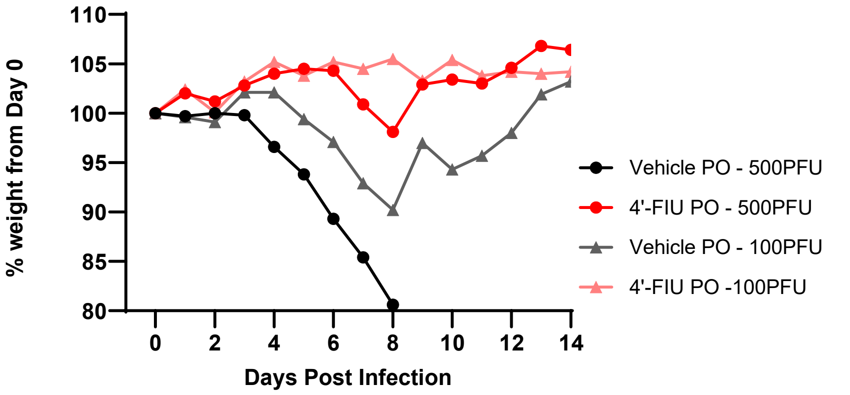
Survival

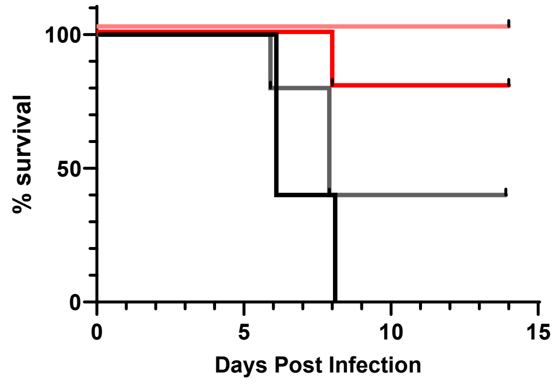


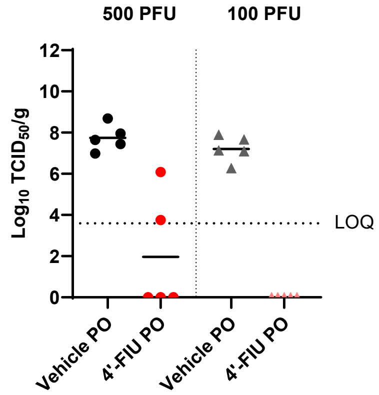
Day 4 Lung burden



4’-FlU





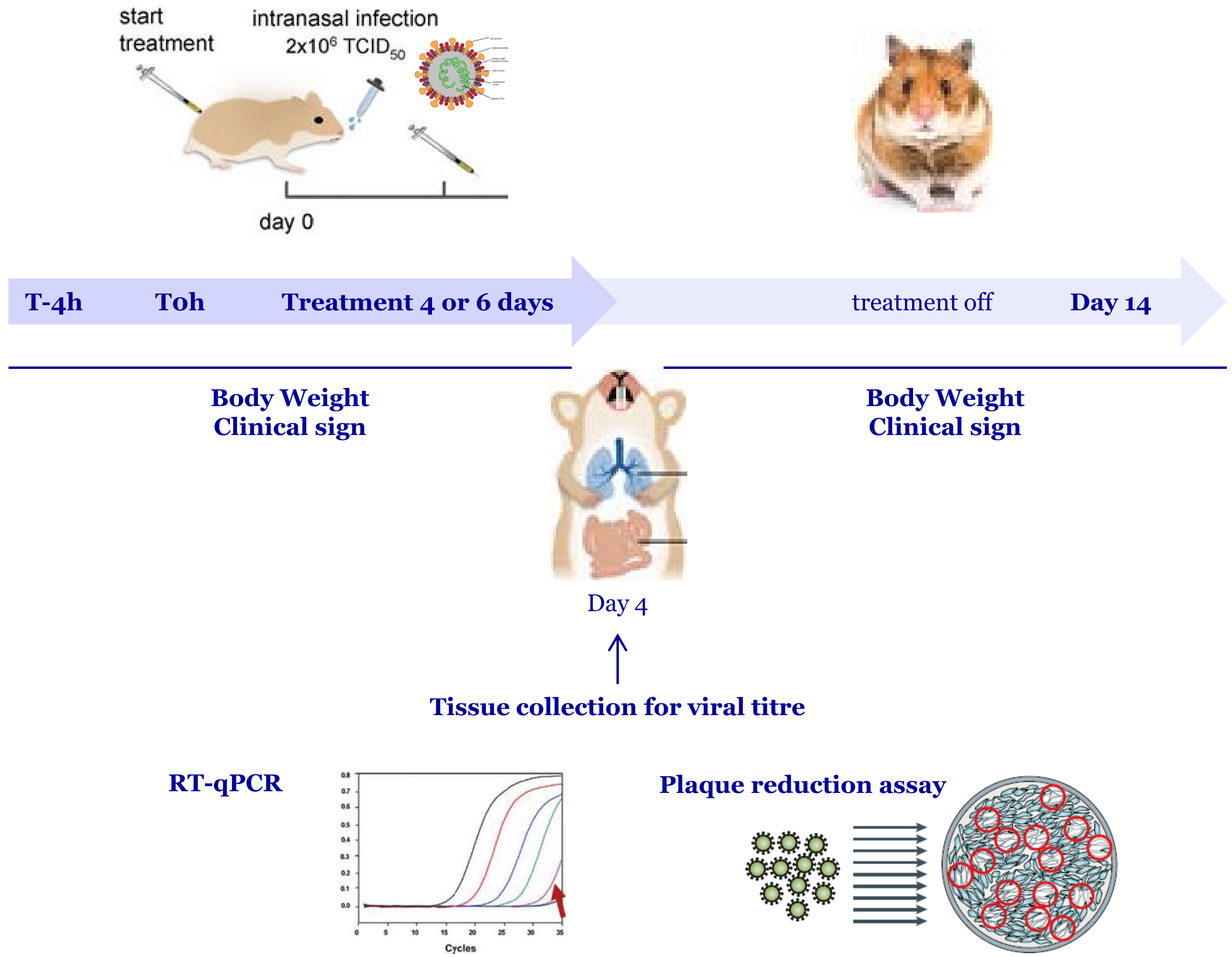


SARS-CoV-2 in vivo model

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

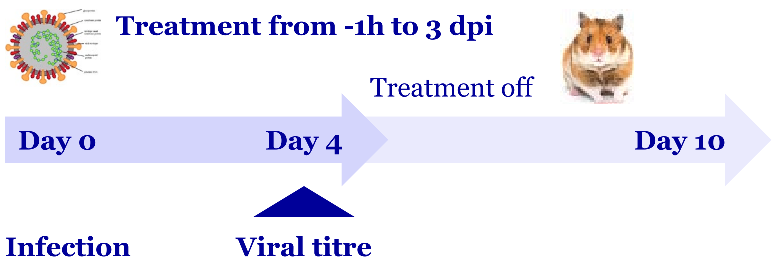
- Histopathology
- Immune response
  - Cytokines
  - Serology
- PK analysis

SARS-CoV-2 Hamster model

Validation with reference compound

Infection

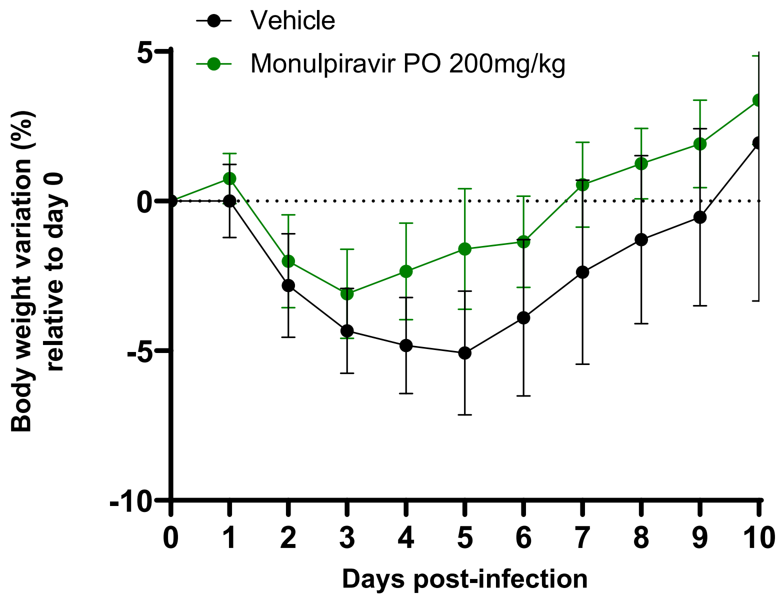
SARS-CoV-2 USA-WA1/2020



Body Weight / Clinical sign

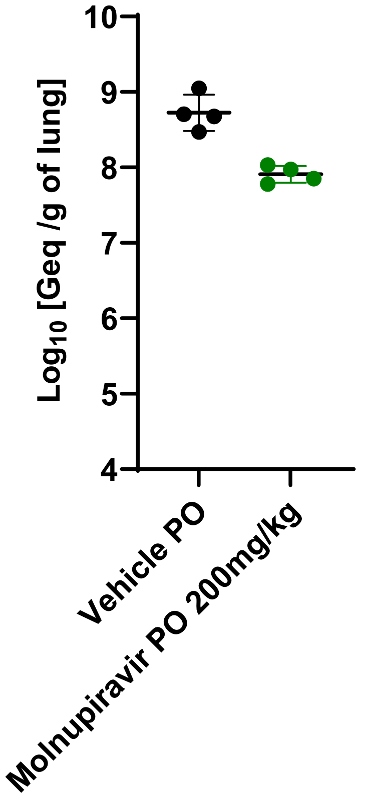
Treatment	Treatment route and regimen	Tissue collection (day post infection)	End of experiment (day post infection)	No. Hamster
Vehicle	PO BID	4	4	4
		–	10	8
Molnupiravir 200mg/kg	PO BID	4	4	4
		–	10	8

Body weight

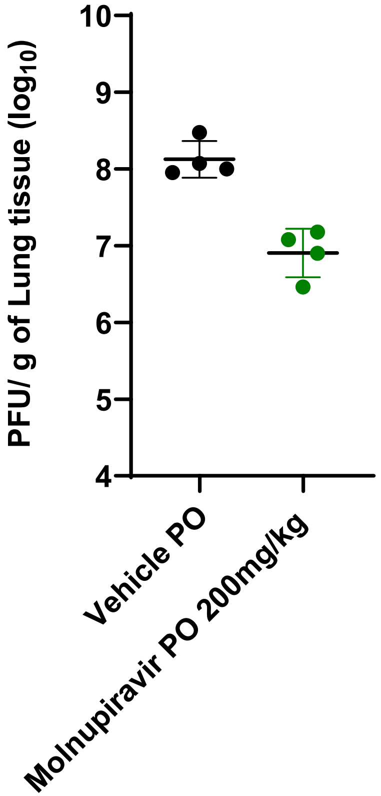


Virus Titre at Day 4

RT-qPCR



Plaque Assay



SARS-CoV-2 Hamster model

Validation of transmission model

Infection

SARS-CoV-2 USA-WA1/2020

Day 0 Day 1 Day 2 Day 3 Day 4 Day 5 Day 6 Day 7 Day 8

Infection

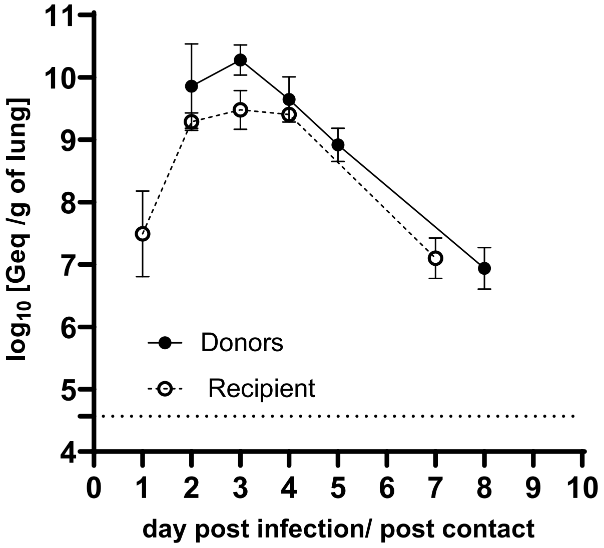
Co-housing

Lung/Nose viral titre

Body Weight / Clinical signs / Oral swabs

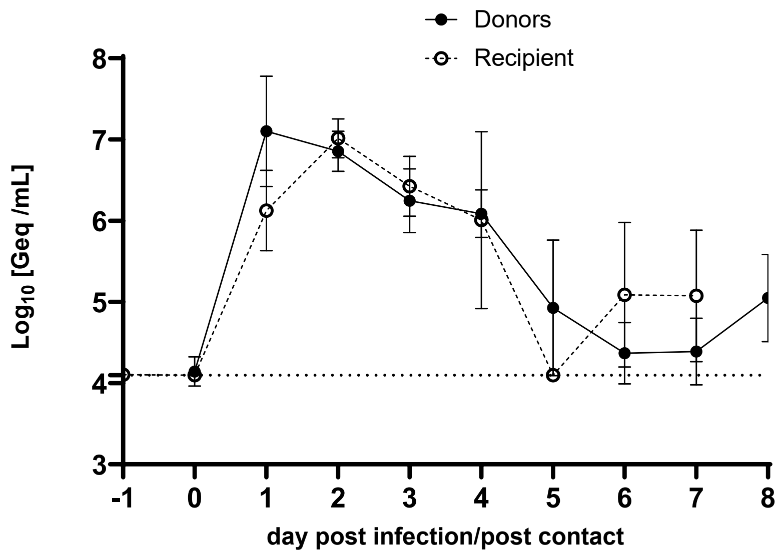
Kinetics of virus titre in lung

RT-qPCR



Kinetics of virus titre in oral swabs

RT-qPCR

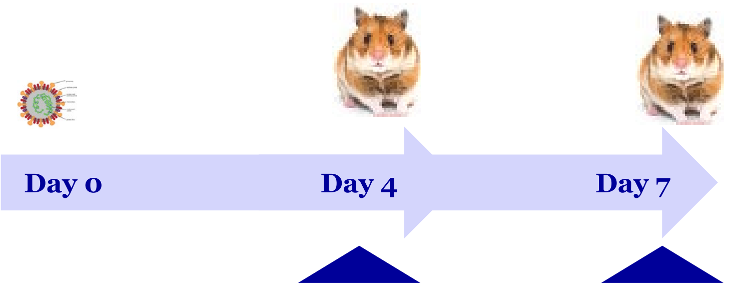


SARS-CoV-2 Hamster model

Validation of long disease model

Infection

SARS-CoV-2 USA-WA1/2020

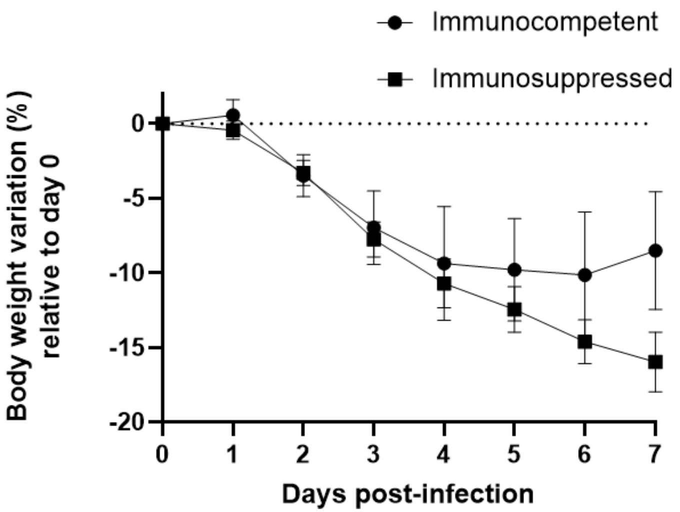


Infection	Viral titre	Viral titre

Body Weight / Clinical signs

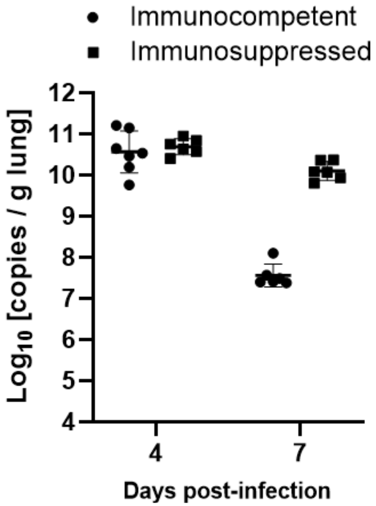
	Tissue collection (day post infection)	End of experiment (day post infection)
Immunocompetent	4	7
Immunosuppressed	4	7

Body weight

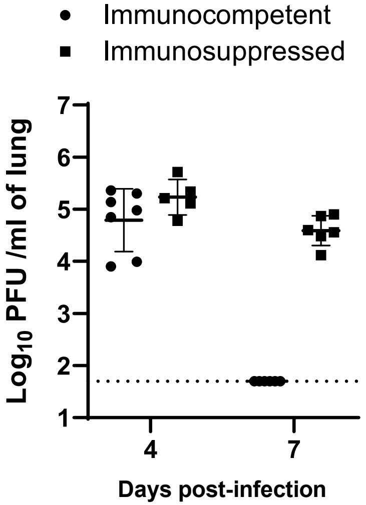


Virus Titre at Day 4 and Day 7

RT-qPCR



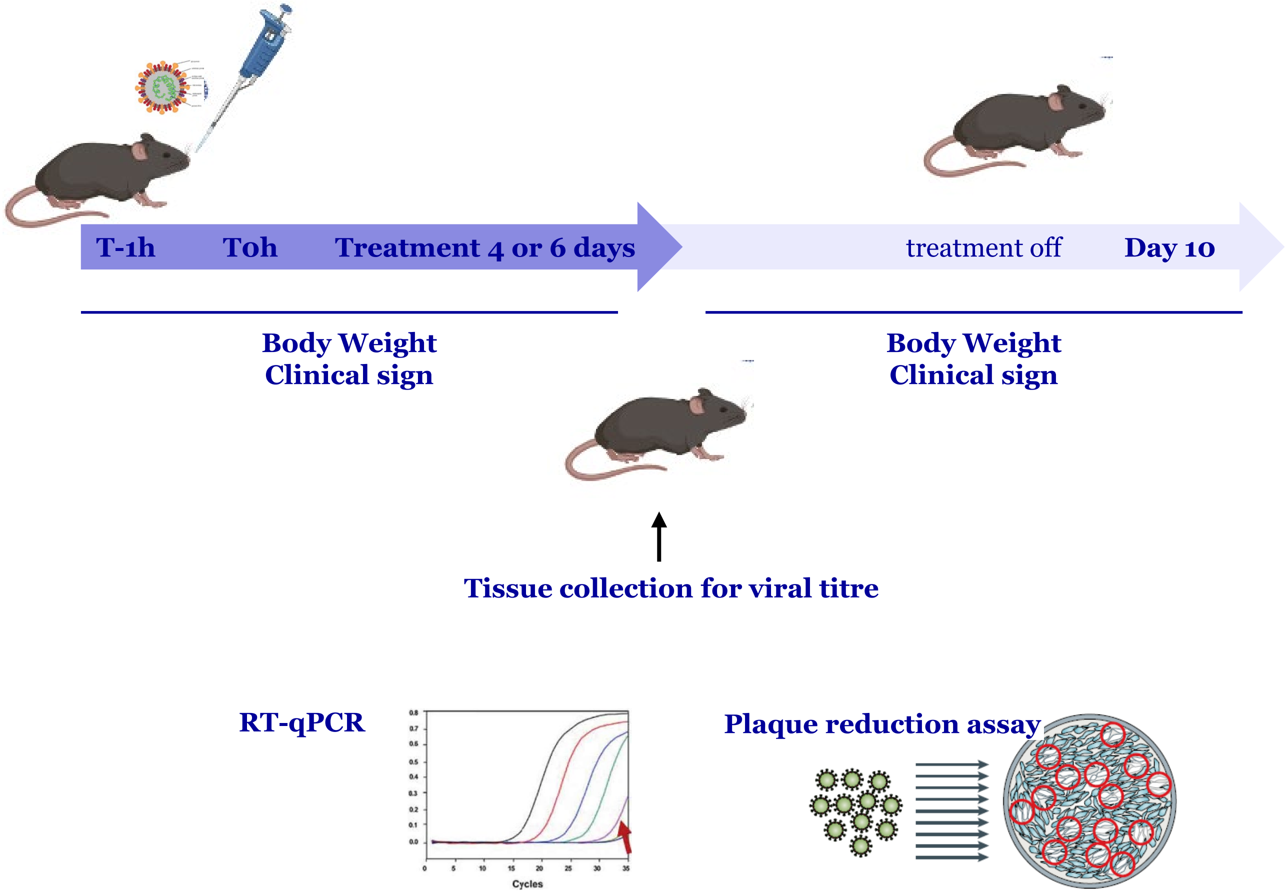
PFU



SARS-CoV-2 Mouse Model

Study design

- Humanised K18-ACE2 mouse
- Infection intranasally
- Several strains in development
- Dose range
- Monotherapy or combination
- Treatment with small molecule, Biology, Vaccine
- Bespoke dosing regimen
- Bespoke treatment du-ration & Study duration



Standard endpoints

- Virus titre in tissues (lung, trachea, nose)
  - RT-qPCR
  - Plaque assay
- Clinical observations
  - Body weight
  - Scoring

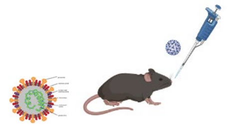
Additional endpoints

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

SARS-CoV-2 Humanised ACE2 mouse model

Infection

SARS-CoV-2 USA-WA1/2020



Day 0 → Day 4 → Day 7

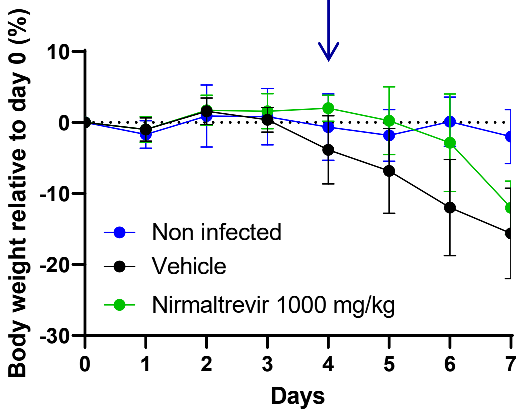
Infection | Viral titre

Body Weight / Clinical signs

	Tissue collection (day post infection)	End of experiment (day post infection)
Vehicle	4	7
Nirmaltrevir	4	7

Body weight

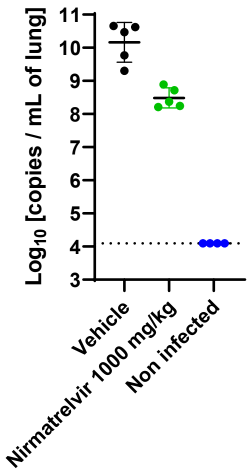
Treatment stop



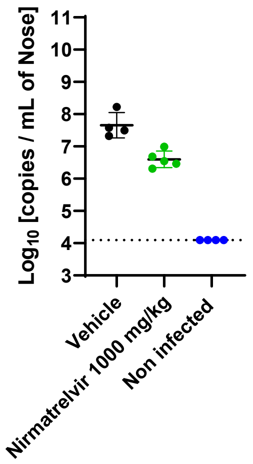
Virus Titre at Day 4

RT-qPCR

Lung



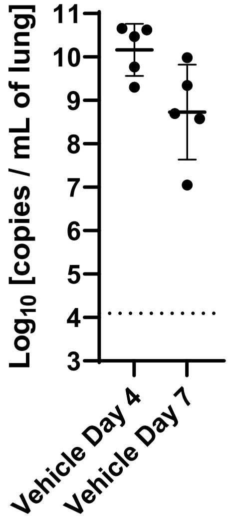
Nose



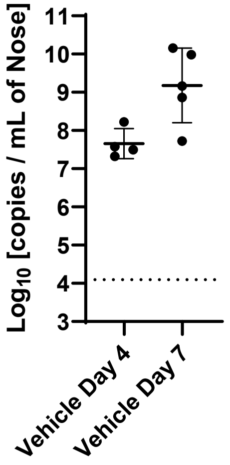
Virus Titre Day 4 vs Day 7

RT-qPCR

Lung



Nose



Agenda

BACK UP SLIDES

1. Respiratory viruses
2. Hepatic viruses (HBV and HDV)
3. Immunology capabilities
4. Integrated virology R&D platforms (Hit ID to PDC)

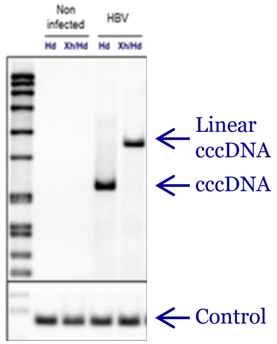
Biology capabilities and expertise on hepatic viruses

Platform for hepatitis B and Hepatitis D viruses

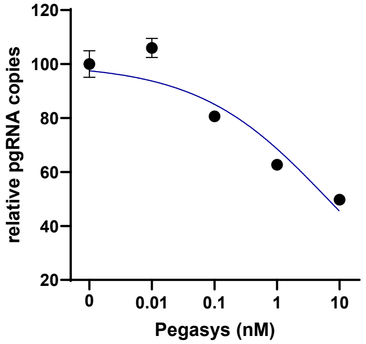
Antiviral *in vitro* assays

- **Cells**
  - **Primary Human Hepatocytes**
  - HepaRG
  - HepaD38
- **Readouts**
  - Viral DNA
  - cccDNA
  - Cellular / viral RNAs
  - Circulating DNA
  - Circulating RNA
  - HBe/HBs release
  - Cell viability
  - Cytokines release
  - Target specific assays

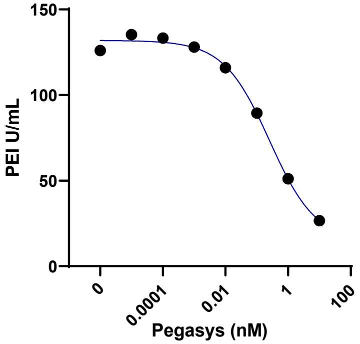
cccDNA



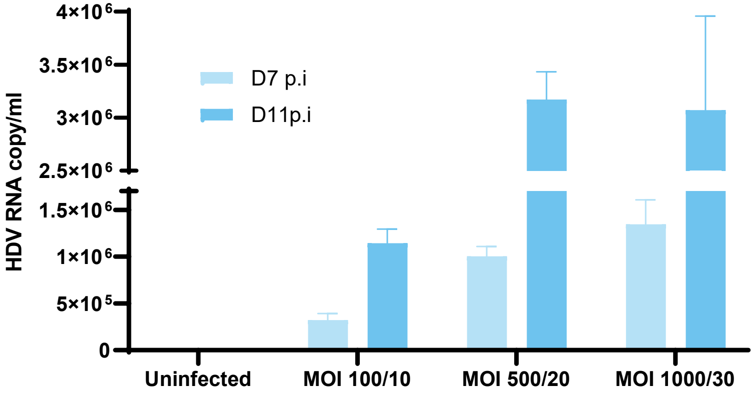
Intracellular pgRNA



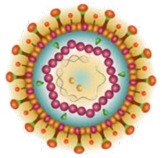
HBe /HBs release



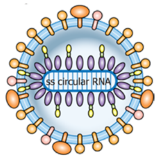
HDV / HBV coinfection of primary hepatocytes



HBV



HDV

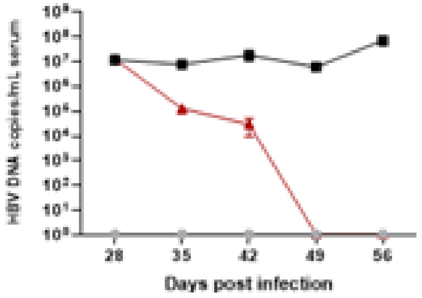


HBV mouse model

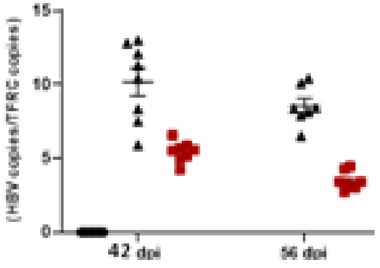
Immune competent animals transduced with HBV via AAV carrier

- **Viral readouts:** HBs, HBe, HBV DNA, pgRNA, cccDNA
- **Host readouts:** immunoprofiling, cytokines, liver enzymes

Peripheral blood HBV DNA



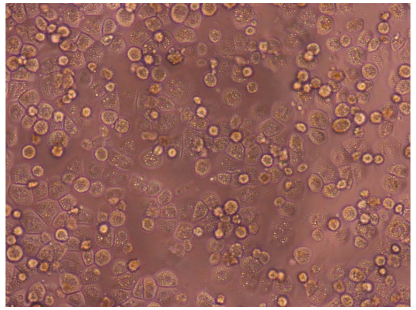
Liver HBV DNA

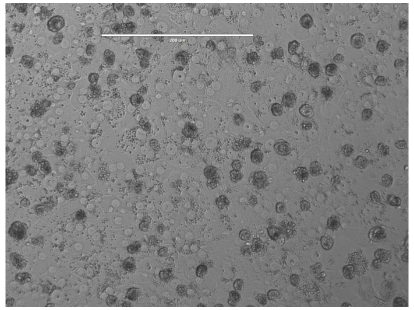


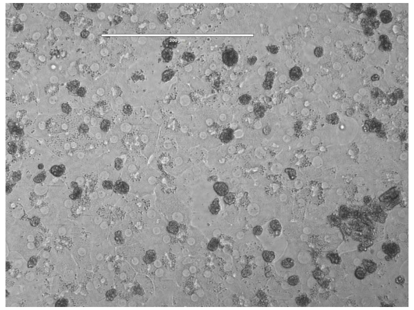
Hepatitis B virus *in vitro* infection model and read-outs

Primary Human Hepatocytes (PHH) infection model

HBV infection in Primary Human Hepatocytes (PHH)


Post-thawing


Infection Day

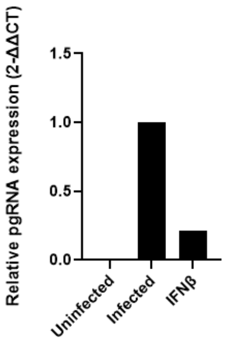

Day 7 post infection

- **Standard protocol:** (can be adapted)

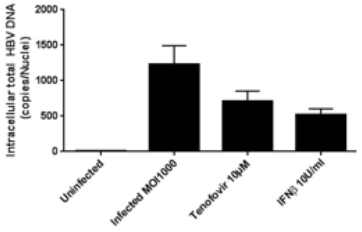
- **Readouts:**
  - Intracellular viral DNA and RNA (qPCR, RT-qPCR and ddPCR), cccDNA (Southern blot, ddPCR and qPCR)
  - Extracellular viral DNA (q-PCR); Hbe and Hbs release (ELISA, WB)
  - Cell viability (CTG, LDH, HSA)

Intracellular and Extracellular viral parameters

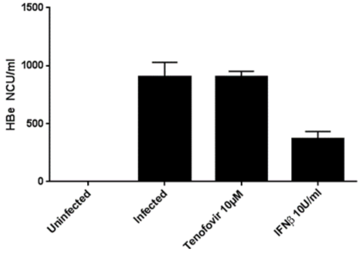
HBV pgRNA



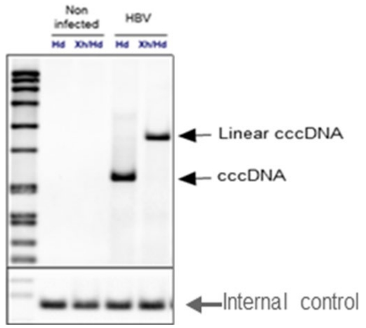
HBV DNA

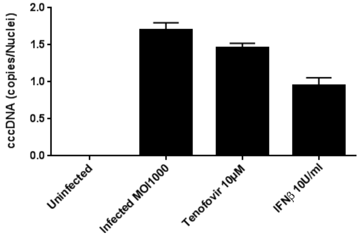


HBe-Ag release

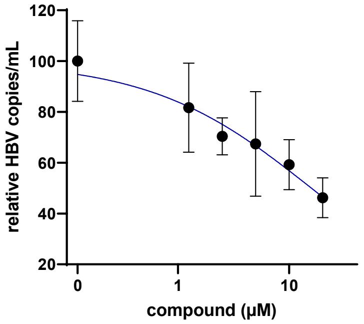


HBV cccDNA southern Blot and PCR





Circulating DNA



Hepatitis B virus *in vitro* infection model and read-outs

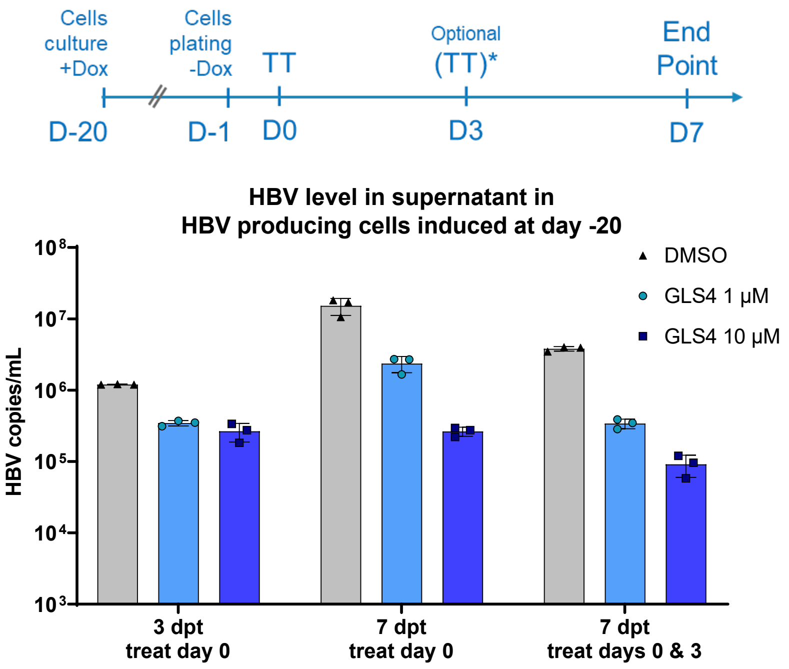
Anti-HBV activity of test articles in HBV *in vitro* models

HepAD38 cells: HBV expression models

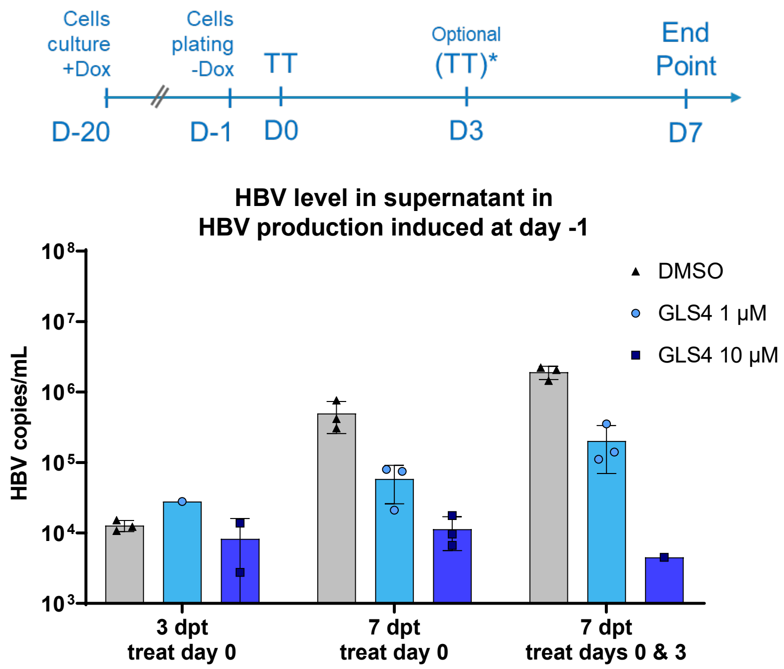
- Under licensing conditions
- **Treatment**
  - On well-induced HBV producing cells or on freshly-induced HBV expressing cells
  - Applied once or multiple times during HBV expression period
  - Dose range effect of single drug (8 to 10 dose levels) or multiple drugs
- **Read-out:**
  - Cell viability
  - Extracellular and Intracellular HBV DNA
  - Intracellular HBV pgRNA

Different Treatment designs can be adapted

Treatment on well-induced HBV producing cells



Treatment on freshly-induced HBV expressing cells



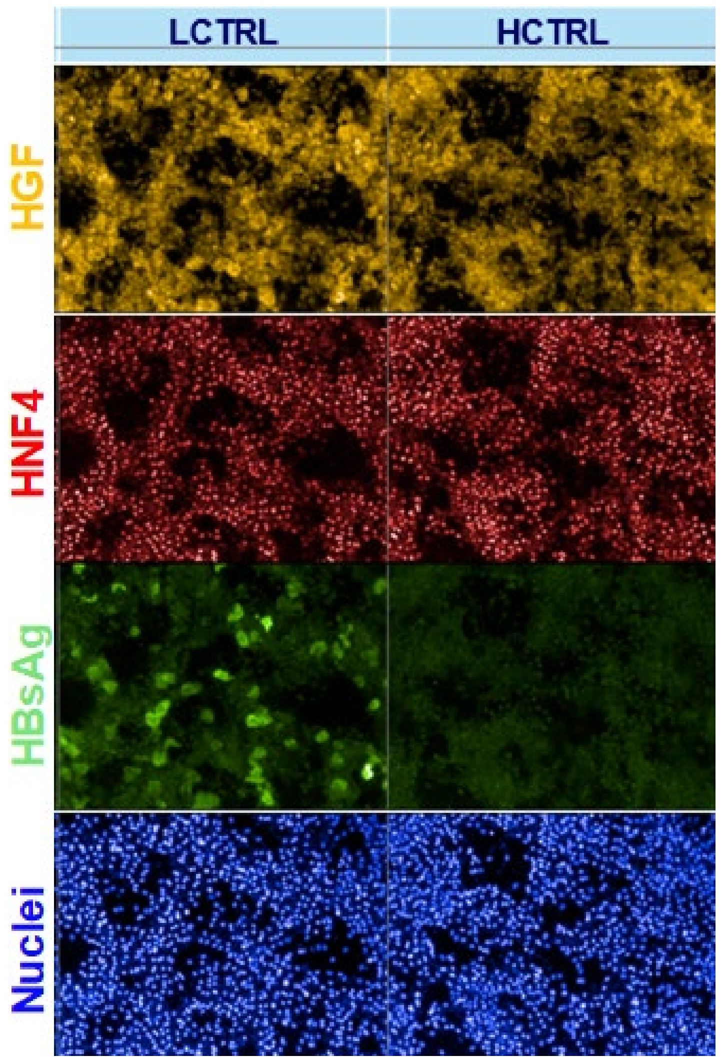
Internal data with the HepAD38 model including the evaluation of a capsid inhibitor during the model development.

Hepatitis B Infection Model

Screening capabilities: assay principle using HBs-Ag marker

- 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.

First Week	Second Week	Third Week	Fourth Week
Cell plating	Infection	Compound addition	Read-out



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

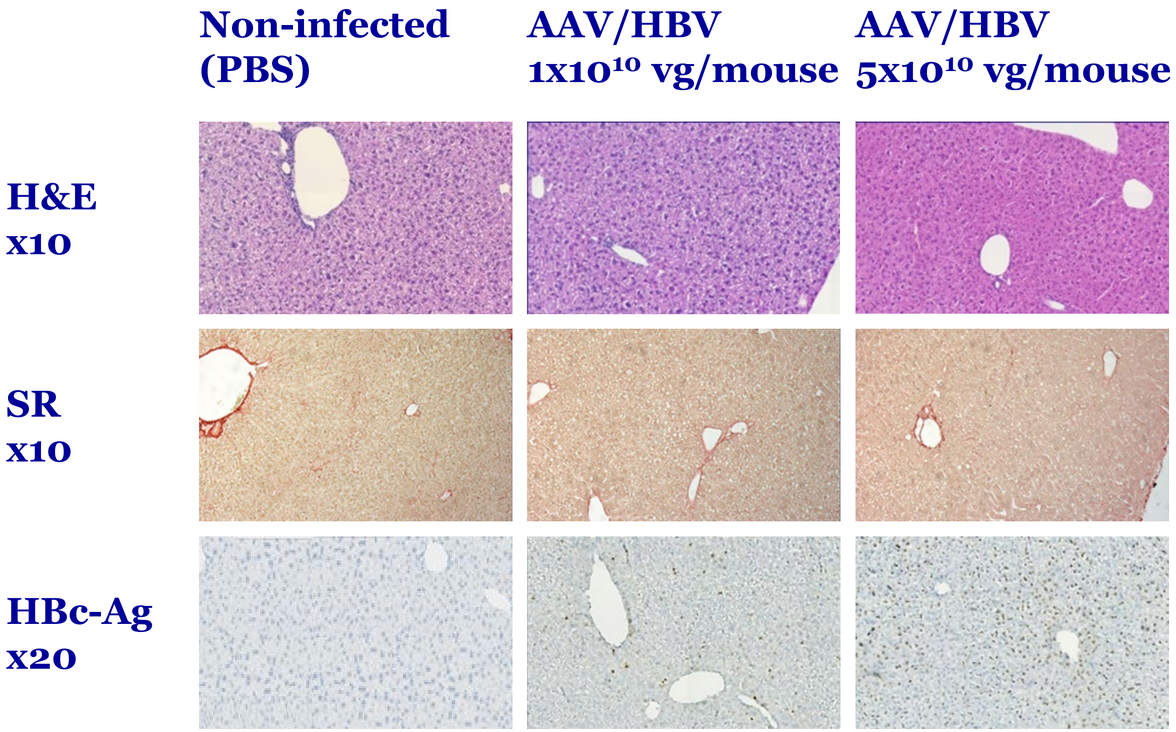
Hepatitis B virus *in vivo* infection model

Histopathological analysis

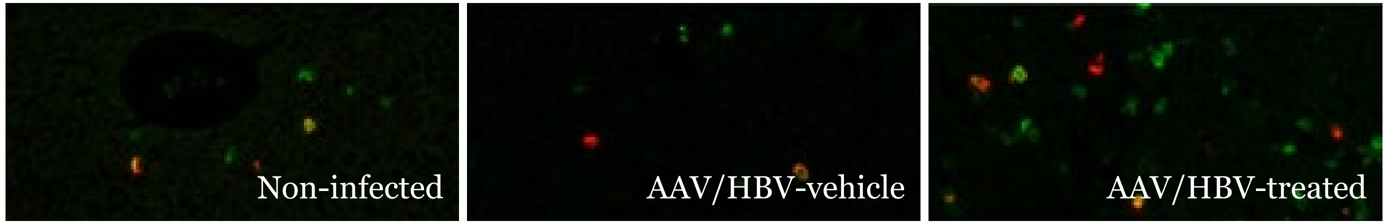
- **Histology on liver biopsies from AAV/HBV mice**
  - Organ weight and macroscopic observation performed at the necropsy
- **Standard histology of the liver is based on:**
  - H&E staining
  - Sirius red staining
  - HBc-Ag Immunohistochemistry

Our histological data are in accordance with published data and confirmed AAV/HBV model features

- **In depth histopathological analysis and/or IHC** can be performed on our histology platform in Toulouse. Already validated:
  - HBc-Ag quantification (%HBc+ cell)
  - CD3/CD8 co-staining for total lymphocyte infiltrate quantification and CD4 and CD8 population quantification

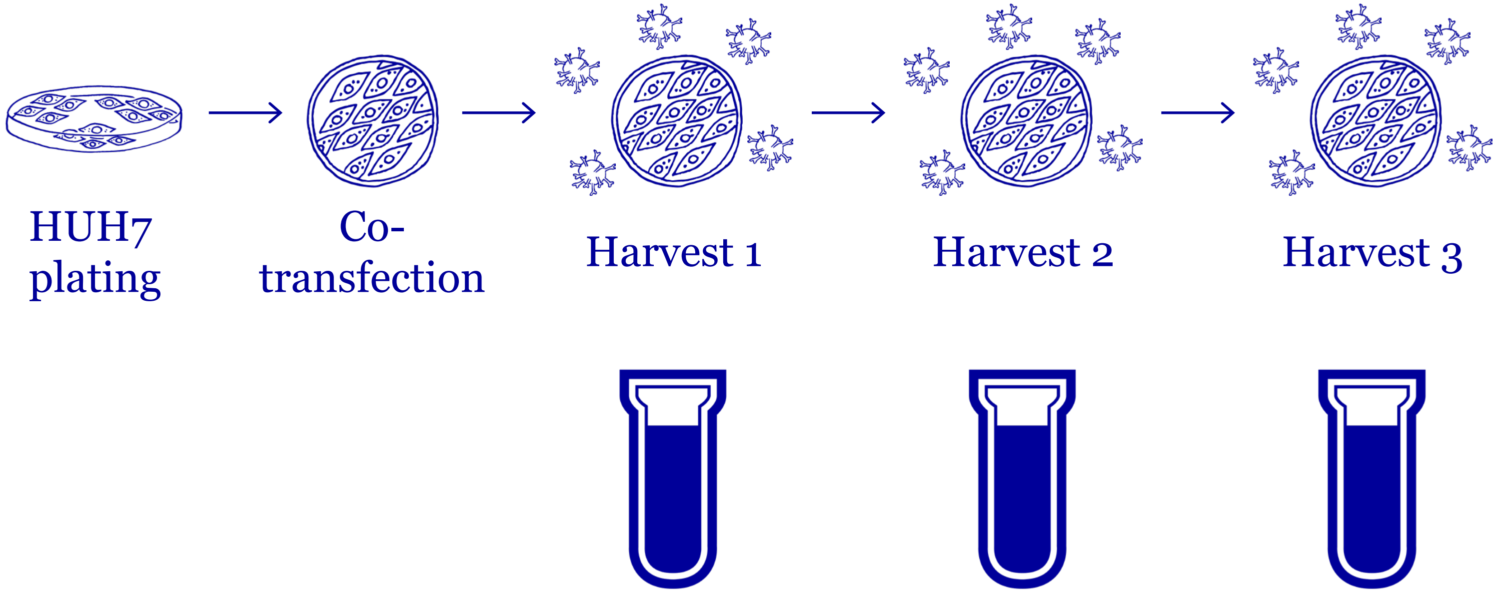


CD3/CD8 co-staining



Ongoing: production and characterization of different HDV genotypes

Viral production ongoing



Plasmid name	Geno-type	Origin	Genebank accession No.	Genome size
pcDNA3.1-HDV-1	1	Ethiopia	U81989	1,679 bp
pcDNA3.1-HDV-2	2	Japan	X60193	1,683 bp
pcDNA3.1-HDV-3	3	Peru	L22063	1,677 bp
pcDNA3.1-HDV-4	4	Japan	AB118847	1,678 bp
pcDNA3.1-HDV-5	5	Guinea-Bissau	AB183331	1,687 bp
pcDNA3.1-HDV-6	6	Nigeria	AM183329	1,680 bp
pcDNA3.1-HDV-7	7	Cameroon	AM183333	1,674 bp
pcDNA3.1-HDV-8	8	Ivory Cost	AM183327	1,681 bp

Agenda

BACK UP SLIDES

1. Respiratory viruses
2. Hepatic viruses (HBV and HDV)
3. Immunology capabilities
4. Integrated virology R&D platforms (Hit ID to PDC)

Broad and deep immuno-virology capabilities and expertise

Several assays available to monitor innate and adaptive immunity

Technologies and readouts

- **Multiparametric flow cytometry:**
  - Extracellular
  - Intracellular
  - Nuclear
  - Phosphoflow
- **Immune cell isolation**
  - Magnetic isolation
- **Multiplexed cytokine assays**
  - CBA, MSD platform
- **Live cell imaging** (Incucyte)
- **RT-qPCR**
- **ELISPOT**

Immunological assays & Immunomodulatory drugs

Innate immune responses

DC activation assays
(activation markers, cytokine secretion)

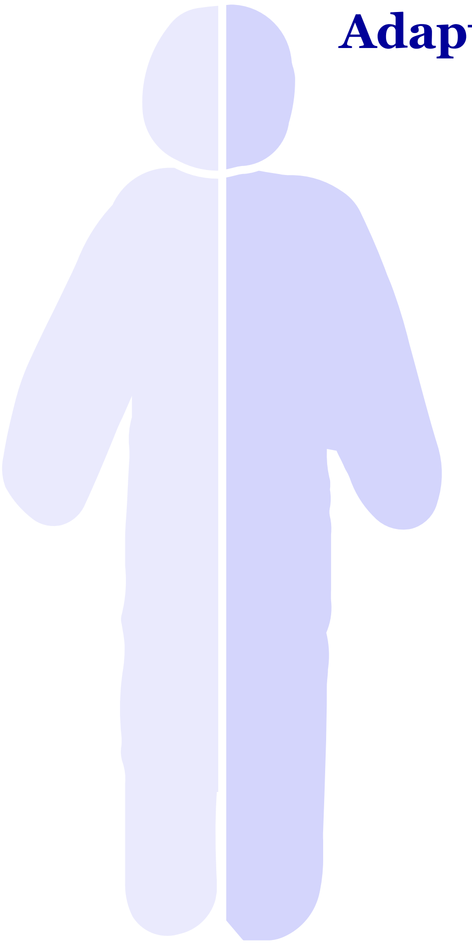
Macrophage activation assays (M1, M2) (activation markers, cytokine secretion)

NK activation assays
(activation markers, proliferation, cytotoxicity, cytokine secretion)

Cytotoxicity assays with mAbs
(ADCC, CDC, ADCP)

PRRs activation
Several reporter systems available

Whole blood (CRS evaluation)



Adaptative immune responses

T CD4/CD8 activation assays

- Activation markers
- MLR “mixed lymphocyte reaction”
- Proliferation
- Cytokine secretion

B cell activation assays

- Activation markers
- Proliferation
- Antibodies secretion

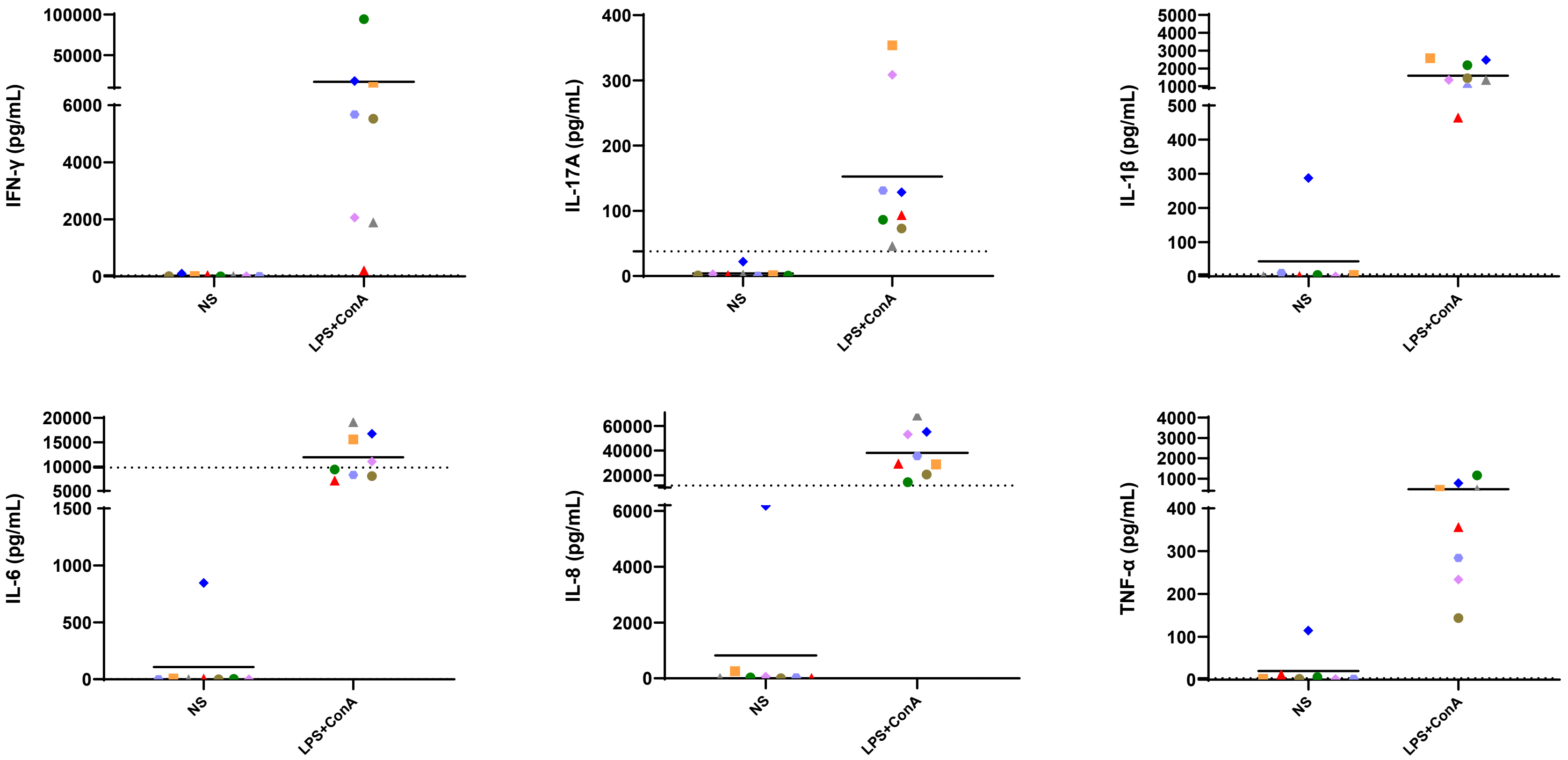
Innate immunity

Whole blood activation assay

- **Whole Blood from healthy donors**
- **Cytokine release profiling**
  - For safety assessment of immunomodulators and biomarker characterization
- **Various matrices**
  - Human whole blood or PBMC
  - Other species (mouse, NHP…)
  - In liquid or plate-bound formats
- **Multiplexed cytokine analysis using MSD platform (mesoscale technology)**

Case study: Cytokine release syndrome assessment

- Whole blood activation with LPS + Concanavalin A during 24h – 8 donors
- Monitoring of pro-inflammatory cytokine release by MSD:



Innate immunity

Dendritic cell activation assay

Protocol:

Human whole Blood

↓ Ficoll gradient

hPBMC

↓ Magnetic isolation

monocyte

↓ Differentiation GM-CSF + IL-4 (6 days)

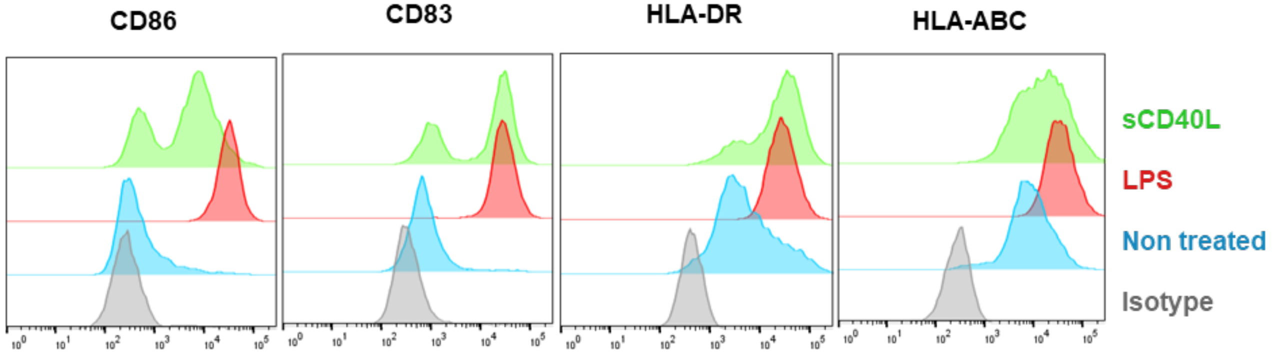
moDC

Endpoints:

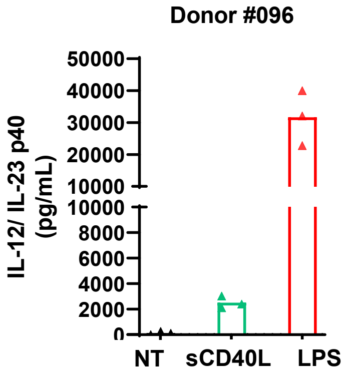
- **Activation markers** (CD86, CD83, HLA-DR, HLA-ABC ...)
- **Cytokine release** (IL-12p70, IL-12/IL-23p40, IL-1β, IL-6, IL-8...)

Case study: moDC activation

- MoDC treatment with LPS or sCD40L during 24h
- Monitoring of activation markers by flow cytometry:



- Monitoring of IL-12/IL-23p40 secretion by ELISA:

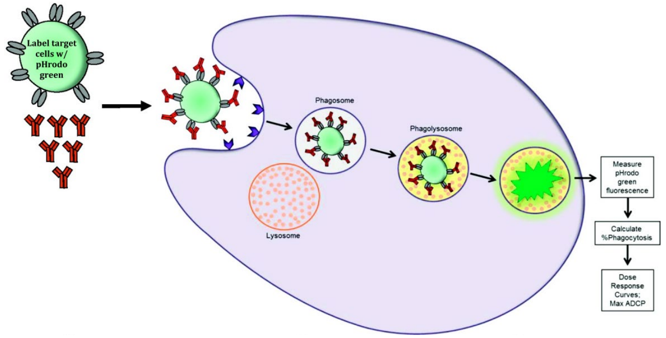


Innate immunity

Macrophage phagocytosis

Protocol:

- Target cell labelled with pH rodo dye
- Effector cells: Macrophages M2c differentiated from monocytes



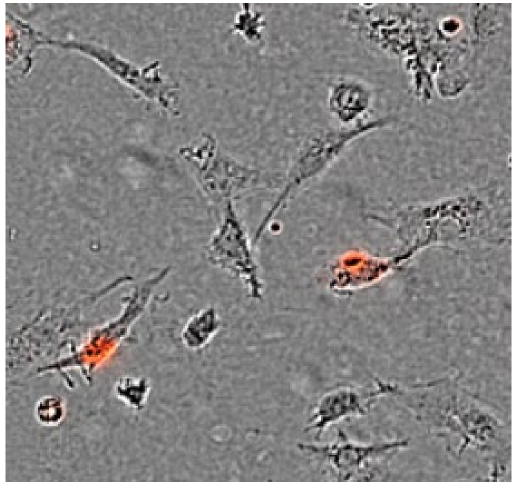
Fluorescence increase upon target cell phagocytosis

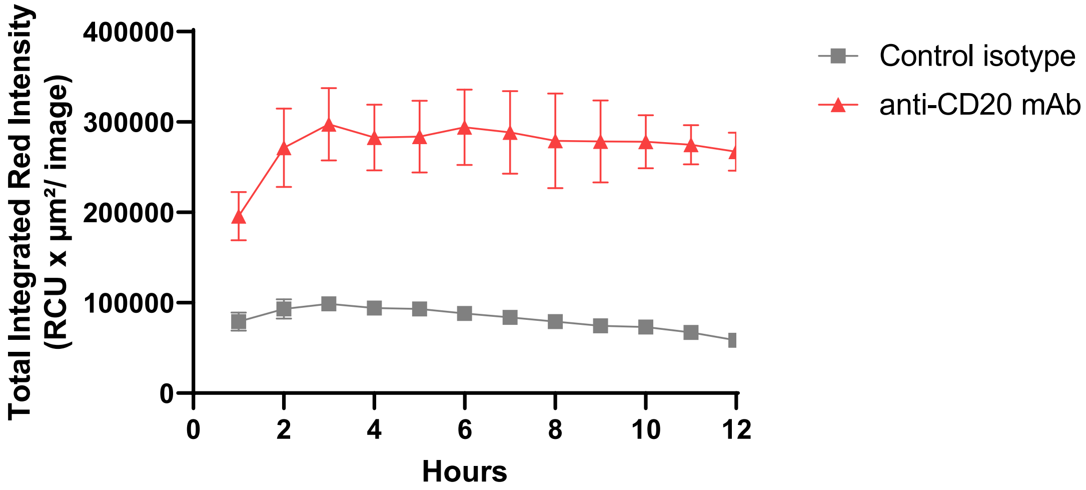
Endpoints:

- **Phagocytosis** = Target cell pH rodo + (Incucyte)
- **Genotyping FcγRIIA 131 H / R** by PCR
- **Cytokine release**

Case study: ADCP (Antibody Dependent Cell Phagocytosis)

- Target cell : Raji labelled with pH rodo Red dye
- Target cell : Raji + Effector cells: M2c (ratio 1:1) + anti-CD20 mAb: Rituximab
- Phagocytosis monitored by Live imaging (Incucyte):





Adaptative immunity

B cell activation assay

Protocol:

Human whole Blood 

↓ Ficoll gradient

hPBMC 

↓ Magnetic isolation

B cell 

↓ TT + IL-21 (14 days)

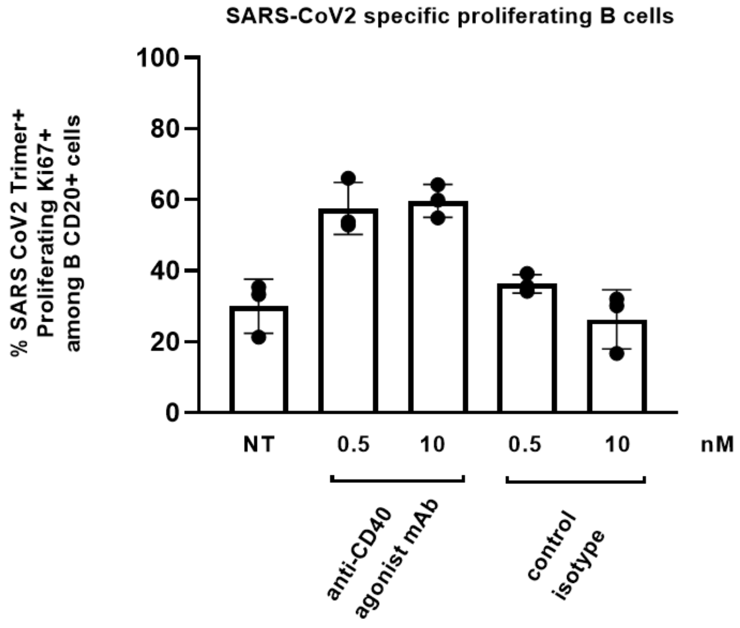
Monitoring of B cell specific & non specific responses

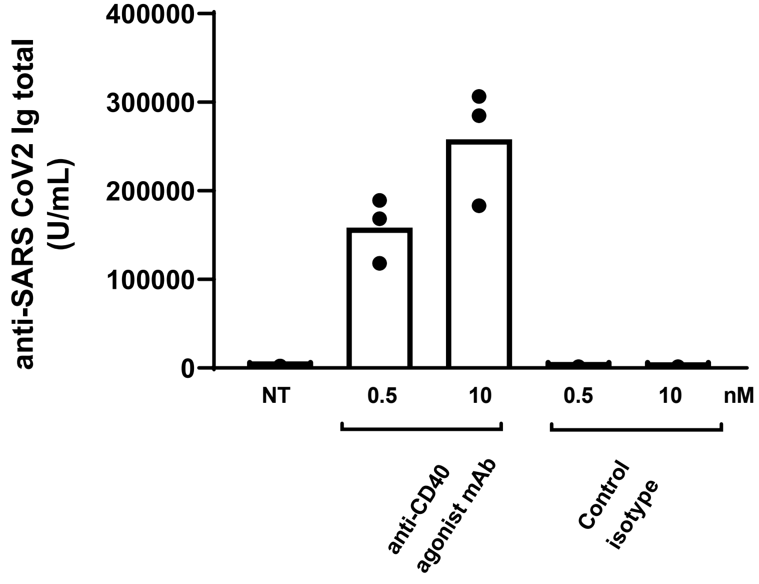
Endpoints:

- **Proliferation** (Ki67, CFSE by flow cytometry)
- **Mabs secretion** (IgM, IgA, IgG…by ELISA)
- **Cytokine release**
- **Activation markers** (CD69, CD86 -flow cytometry)

Case study: Specific SARS-CoV2 B cell recall

- B cell (from COVID-19 vaccinated donor) treatment with anti-CD40 mAb agonist during 14 days
- Monitoring of:
  - SARS-CoV2 specific B cells proliferating by flow cytometry
  - Anti-SARS CoV2 Ig total secretion by ELISA



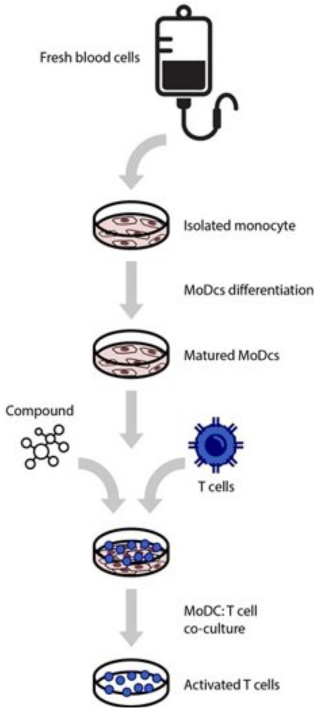


Adaptive immunity

T cell activation assay

Protocol:

- Monitoring of T cell specific and non-specific responses
- Monitoring of T cell sub-populations (TCD4 Th1/ Th2/ Th17/ Treg - T CD8)

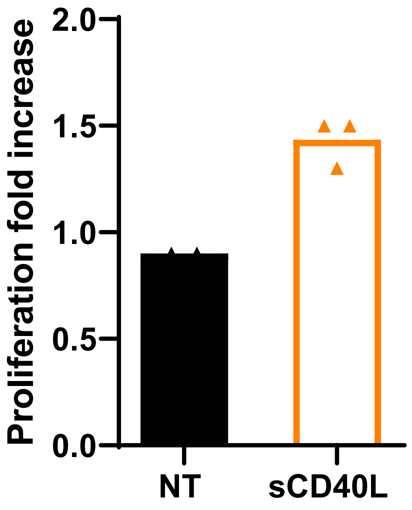


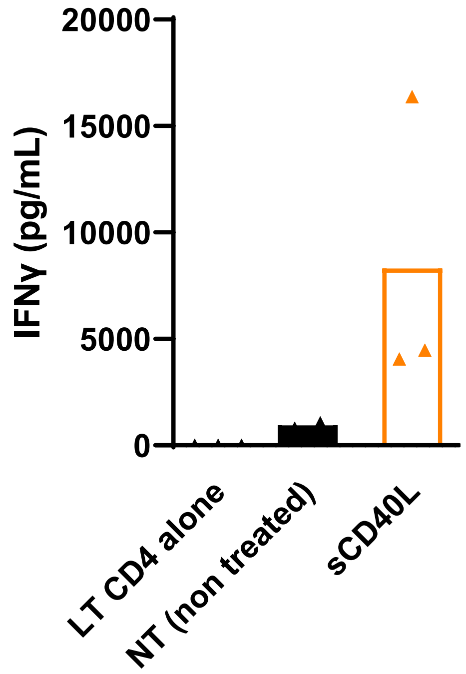
Endpoints:

- **Proliferation** (Ki67, CFSE by flow cytometry)
- **Cytokine release** (IL-2, IFN-γ, TNF-α…)
- **Activation markers** (CD69, CD25- flow cytometry)

Case study: Mixed lymphocyte reaction

- moDC co-culture with naive TCD4 (ratio 1:10) from different donors + TT during 7 days
- Monitoring of:
  - T CD4 cell proliferation by ATP quantification and
  - IFN-γ secretion by ELISA:





Internalization assay

Protocol:

Primary cells
or Cell line

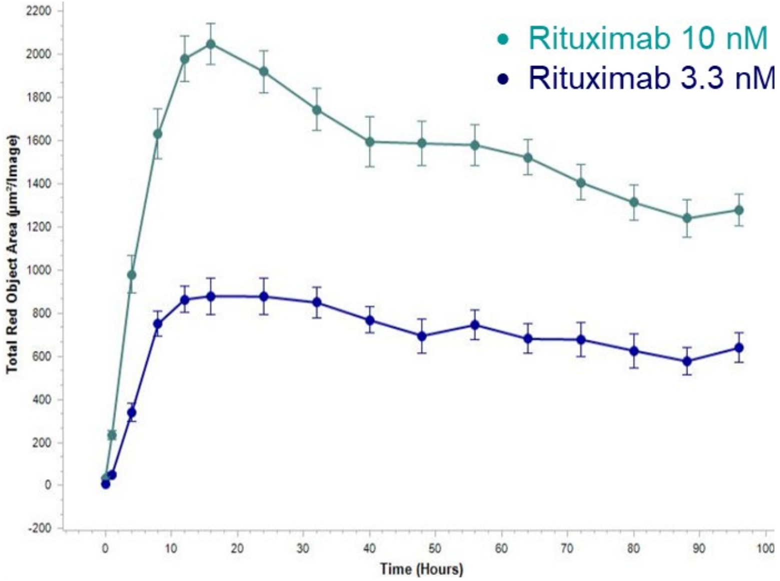
Monitoring of Protein or mAb internalization in kinetic

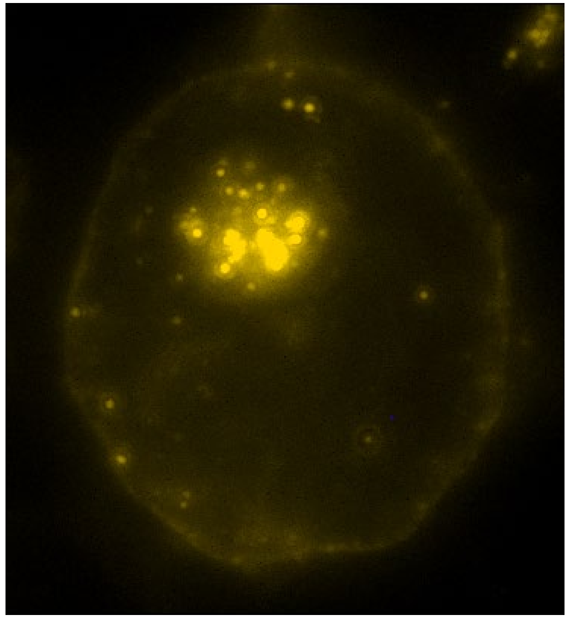
Endpoints:

- Internalization using Live imaging (Incucyte)
- Internalization using fluorescence microscopy (Nikon Zeiss)

Case study: Internalization assay

- B cell or Raji cell line incubated with Rituximab-Fabfluor pH Red or Compound X-AF546
- Monitoring of Internalized Red object increase using Incucyte technology:





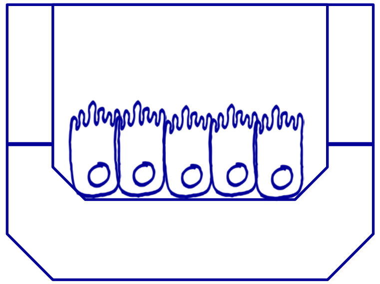
Monitoring of cell localization of compound X-AF456 by fluorescence microscopy

Host – Pathogen *in vitro* model

Airway model

Protocol:

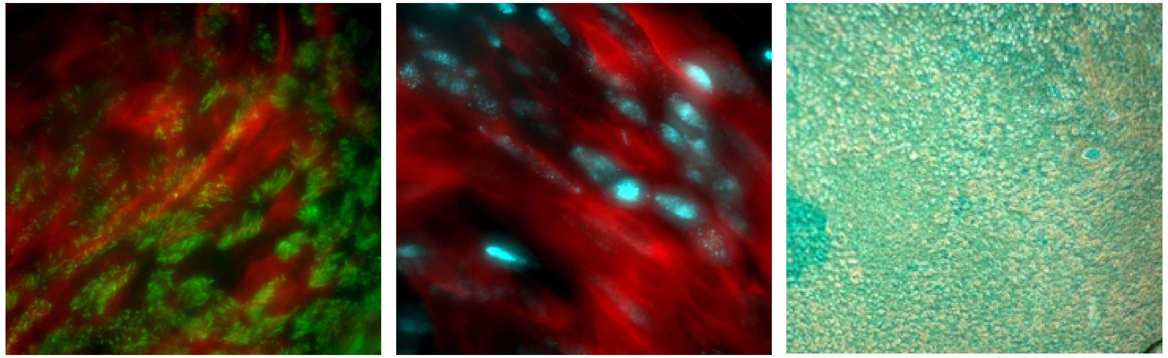
Airway Epithelial primary cells differentiation in transwell in Air-Liquid interface during 28 days



- Donor primary cells from different anatomical sites (Nasal, Tracheal, Bronchial or Small airway cells)
- Highly relevant model to address pharma-cology, toxicology and biology demands

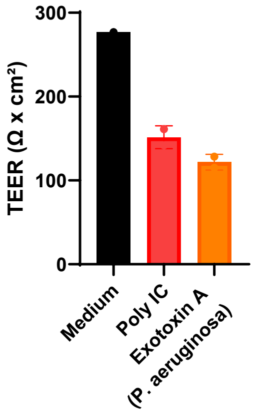
Case study: Airway model caracterization

Human *in vitro* model representing the airway epithelia containing beating cilia, goblet and basal cells and secreting mucus:



Ciliated cells = AC tubulin;
Goblets cells = MUC5AC;
Basal cells = KRT5;
Mucus = Alcian blue staining

Airway *in vitro* model responsive to viral and bacterial stimuli:



Host – Pathogen *in vitro* model

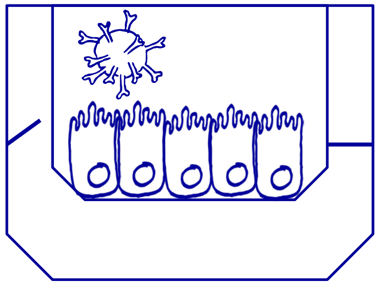
Airway model

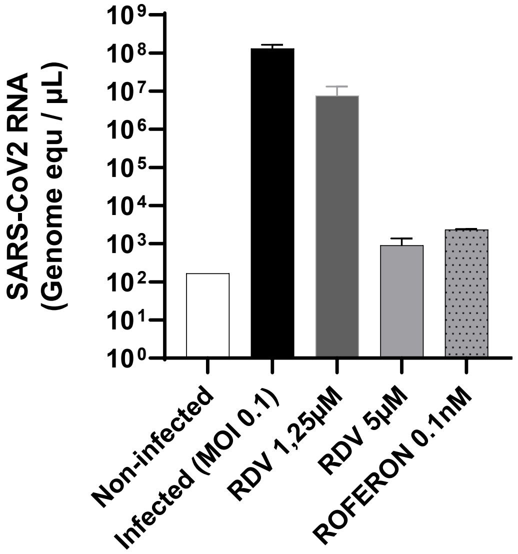
- Human *in vitro* model representing the airway epithelia containing beating cilia, goblet and basal cells
- Donor primary cells from different anatomical sites (Nasal, Tracheal, Bronchial or Small airway cells)
- Highly relevant model to address pharmacology, toxicology and biology demands
- Model available for SARS-CoV2, RSV, HPIV, Influenza A
- Can be adapted for other pathogens including bacteria

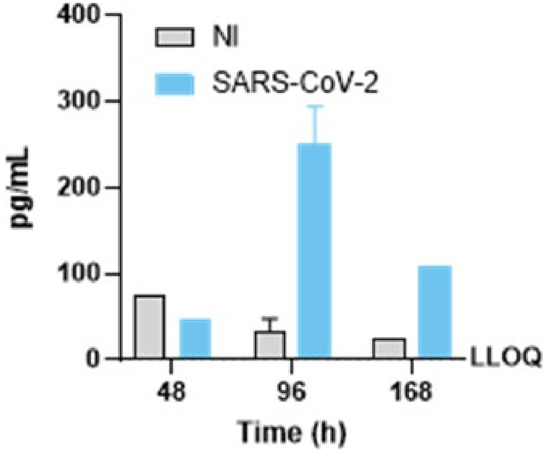
Endpoints:

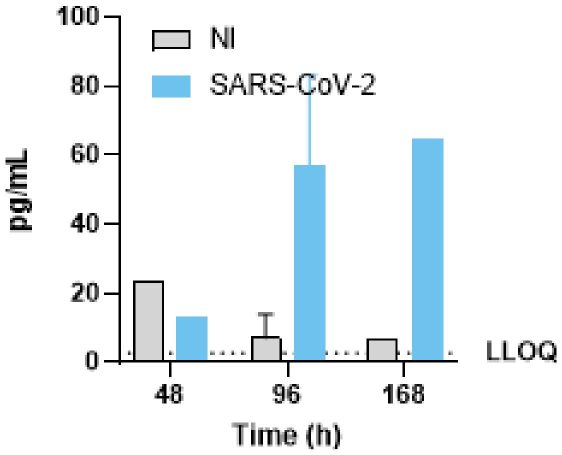
- **Pathogen read out:**
  - Viral load
  - Viral titer by RT-qPCR
- **Host read out:**
  - Barrier integrity (TEER)
  - Paracellular permeability (FITC-dextran)
  - Cytokine release
  - Cytotoxicity (LDH)

Case study: SARS-CoV2 infection in airway model







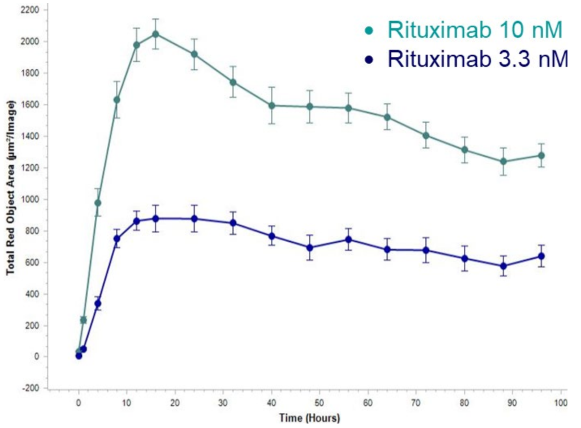


Immuno-virology case study

Assay for Target exploration, Cytokine profiling and immune cells activation evaluation

Target expression, activation and Internalization assays

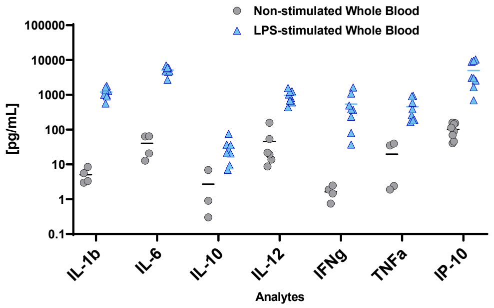
- Flow cytometry on various cell populations for target expression and activation assessment
- Reporter Cells system for target binding and activation
- Internalization assay using Live imaging (Incucyte)



Rituximab internalization monitored with the Red fluorescence increase

Cytokine release profiling

- For safety assessment of immunomodulators and biomarkers characterization
- Various matrices:
  - Human whole blood or PBMC
  - Other species (mouse, NHP…) or other cell types possible
- In liquid or plate-bound formats
- Multiplexed cytokine analysis using MSD platform (mesoscale technology)



Whole blood from 8 human donors stimulated with LPS (10 ng/mL): multiplex cytokine analysis (values <LLOQ in Non-stimulated condition are not represented)

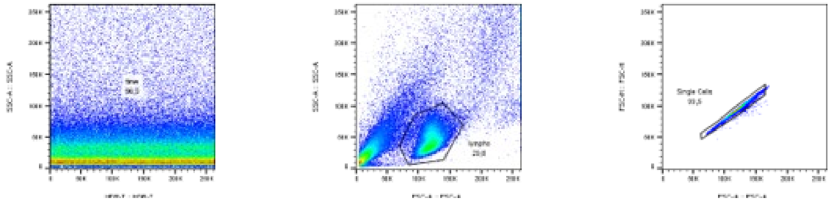
MOA on immune cell population

- PBMCs or isolated immune cells
- Proliferation, differentiation & activation assays

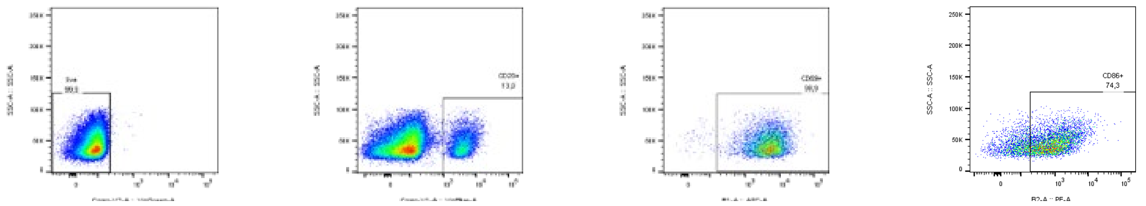
Multiparametric flow cytometry:

- Gating on LyB (CD20+)
- Read out of activation markers (CD69, CD86)

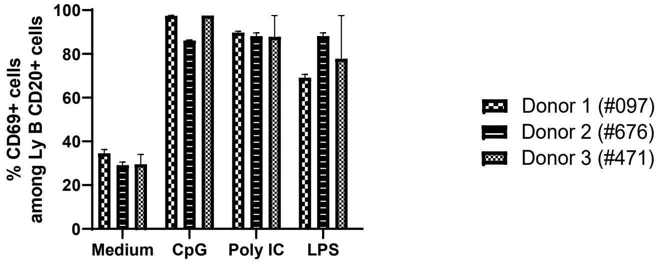
Time **Lymphocytes** **Single cells**



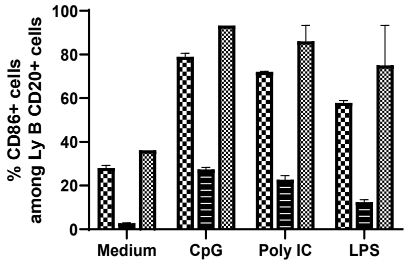
Live **LyB CD20+** **CD69** **CD86**



CD69 expression on B cells



CD86 expression on B cells



Agenda

BACK UP SLIDES

1. Respiratory viruses
2. Hepatic viruses (HBV and HDV)
3. Immunology capabilities
4. Integrated virology R&D platforms (Hit ID to PDC)

Complementary approaches and modalities to tackle viruses

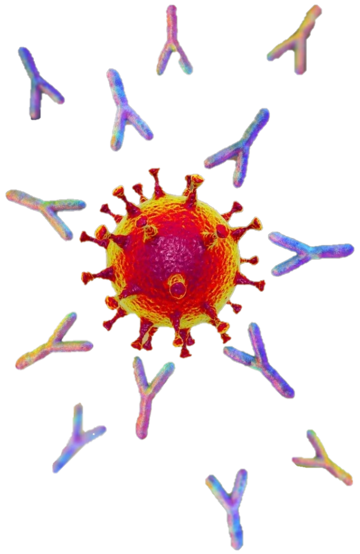
Developing tailored therapeutics that delay or prevent escape and resistance

Boosting innate and adaptive immunity

Host targeting platform

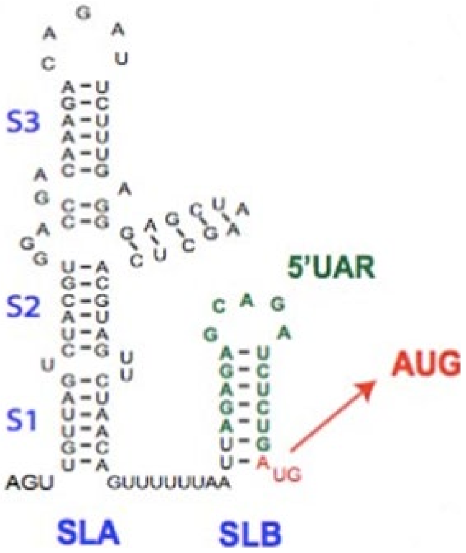
Preventing Viral entry

Neutralizing antibodies



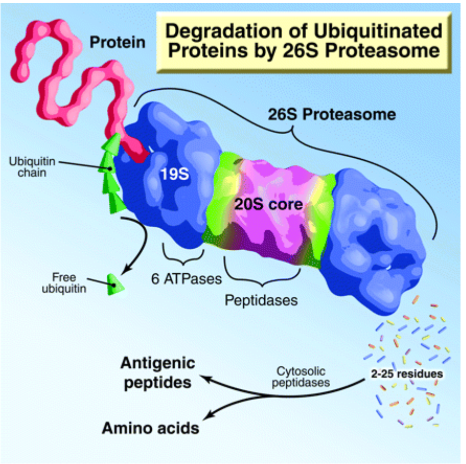
Targeting key viral RNAs structures

RNA targeting with small molecules



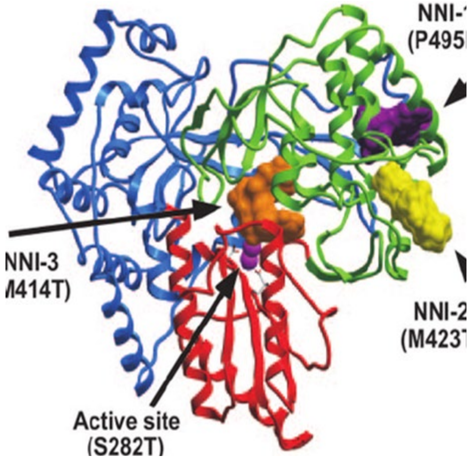
Degrading proteins not accessible to traditional therapeutics

Targeted protein degradation



Preventing replication & assembly

Inhibiting key enzymes



Drug discovery platforms to tackle endemic and emerging viruses

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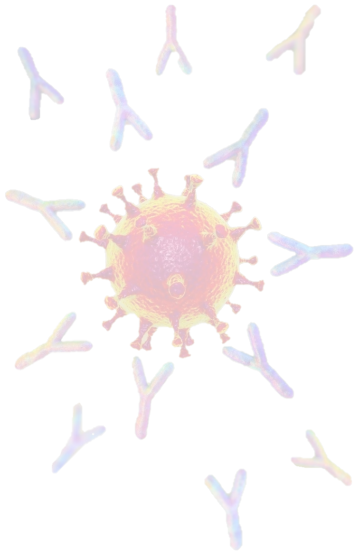

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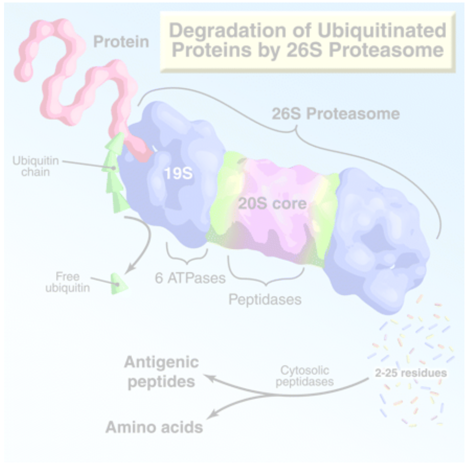

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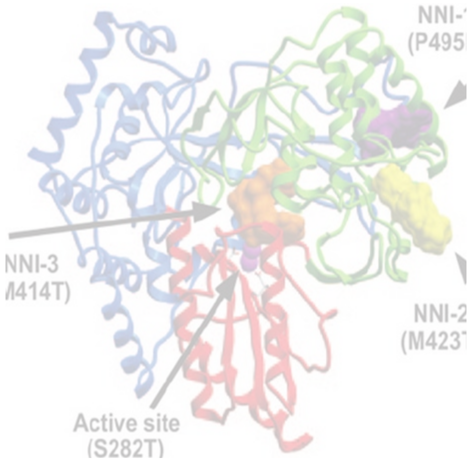
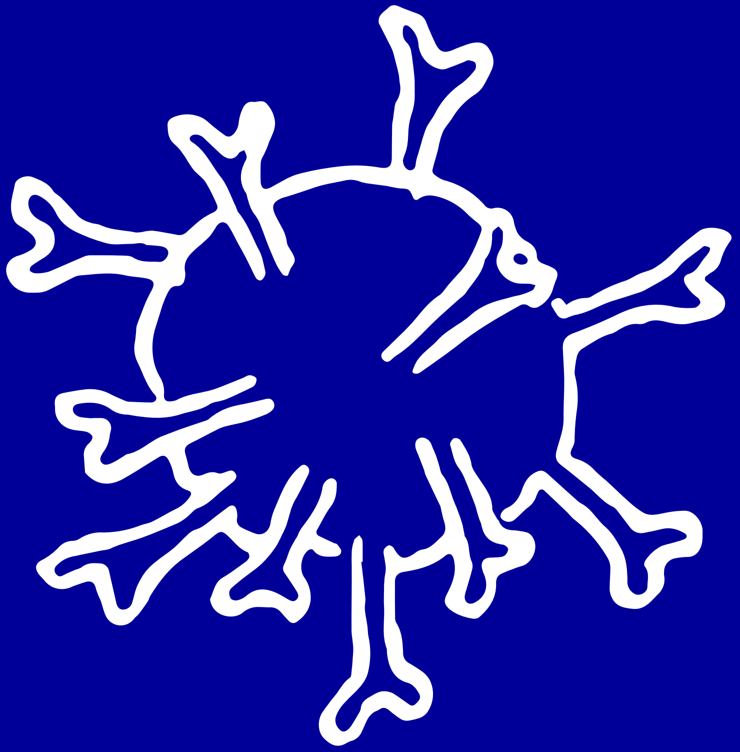


evotec

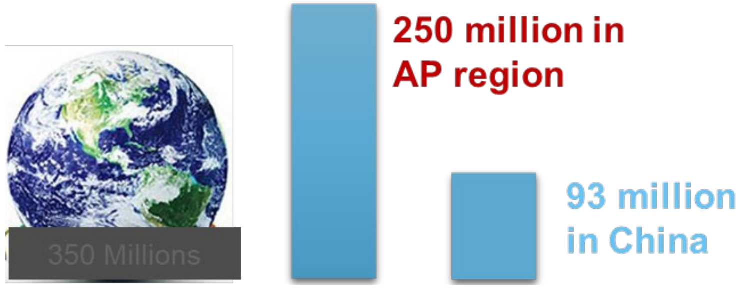
#RESEARCHNEVERSTOPS

A bifunctional immune modulator exhibits potent antiviral activity in HBV infection models



Show case

HBV infection causes chronic hepatitis B (CHB) with significant global health burden and no cure

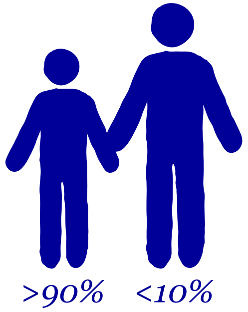


250-350

million chronically infected patients

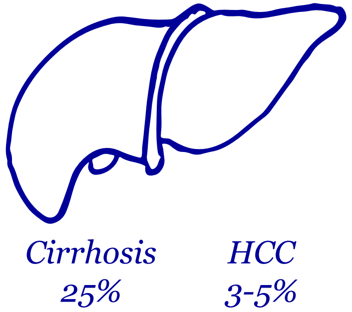
>90%

of neonates develop CHB



<10%

of adults develop CHB



>800,000

deaths annually

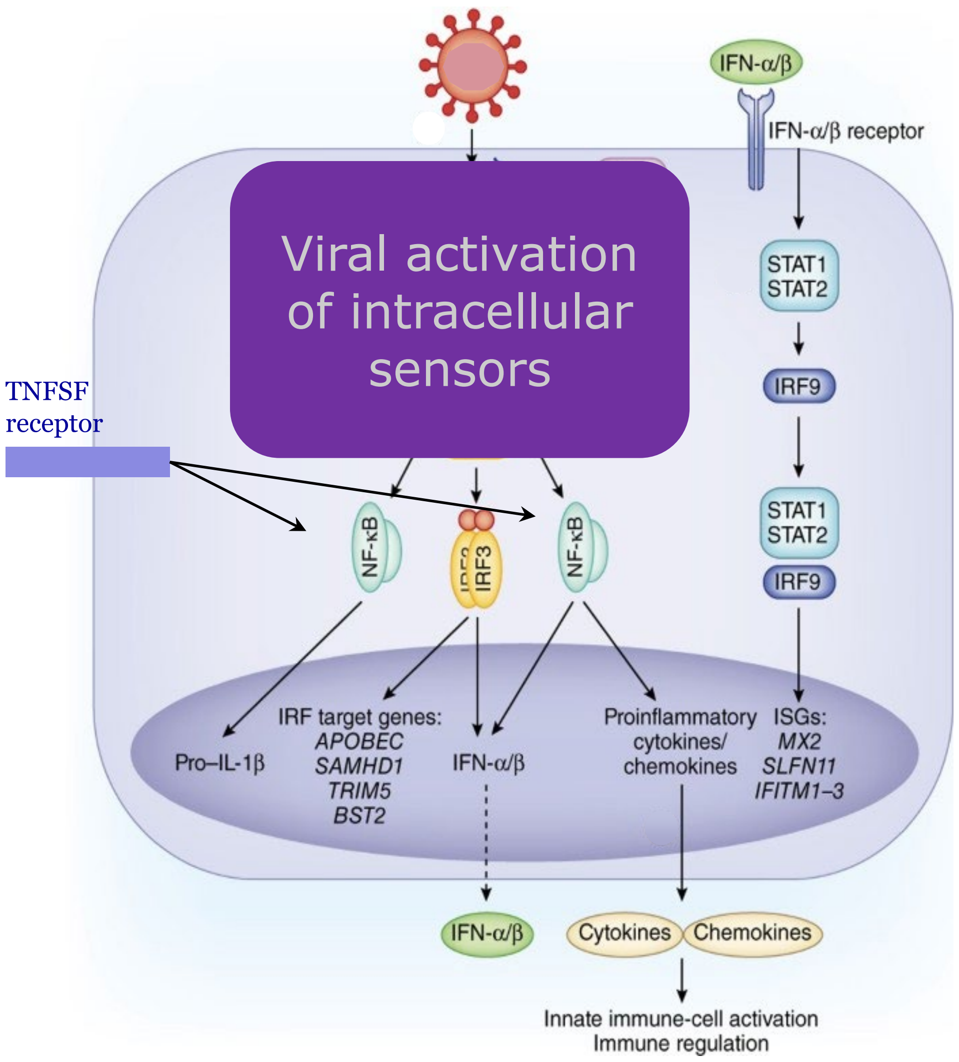
2

approved treatment modalities

T and B-cell dysfunction and exhaustion are typical features of persistent HBV infection

Boosting the IFN pathway and restoring an adaptive immune response are required to achieve cure

Efficient antiviral response requires full activation of intracellular signals to induce a full spectrum of antiviral proteins



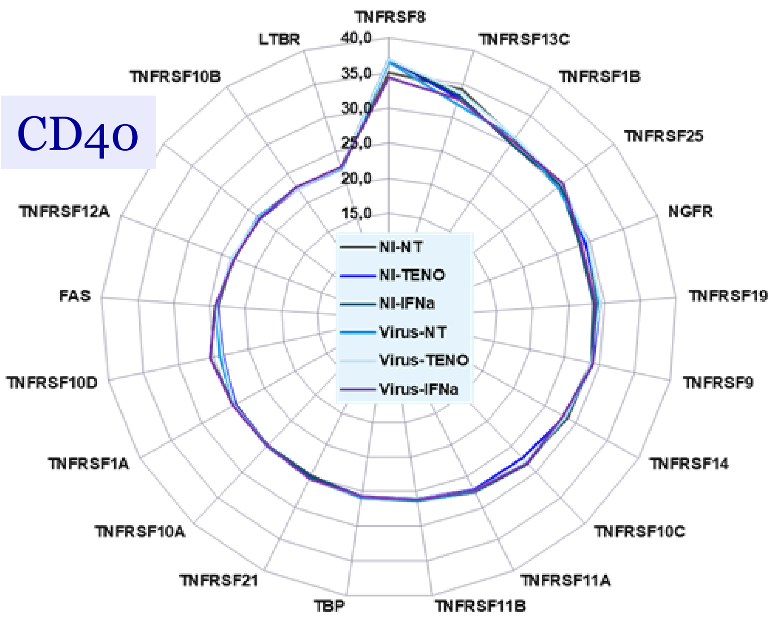
Type I interferons are active, but have limited efficacy:

- Viruses block of the innate immune response
- High levels of IFN are needed to produce suboptimal therapeutic benefit
- IFN induces tolerability issues

Figure adapted from Altfeld and Gale, 2015 Nature Immunology

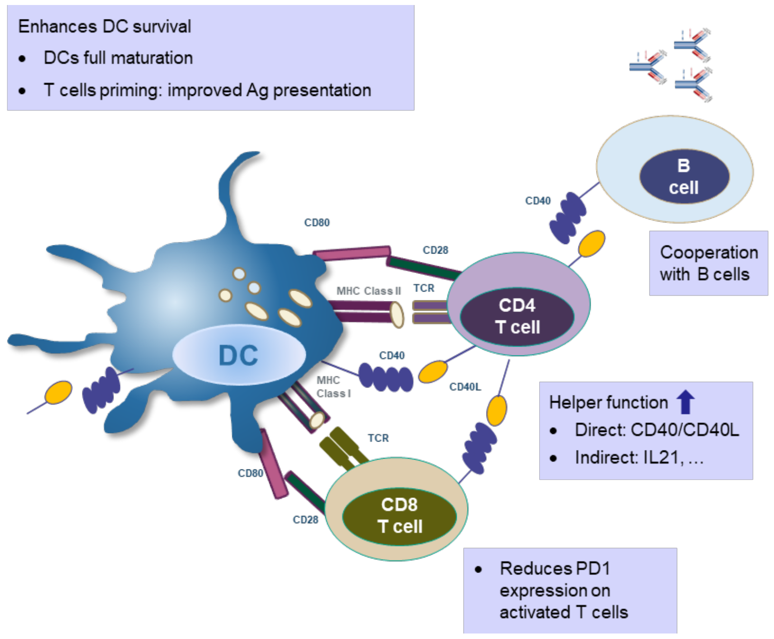
Combination of CD40 agonism and IFN synergistically increases ISGs without increasing inflammatory markers

Several TNFRs are expressed in hepatocytes



RT-PCR (Ct) analysis of TNFRSFs in hepatocytes

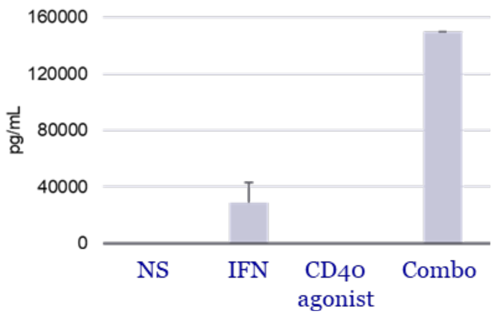
CD40 is a key player of adaptive immunity



The **direct** role of CD40 activation on HBV infection in hepatocytes is not known

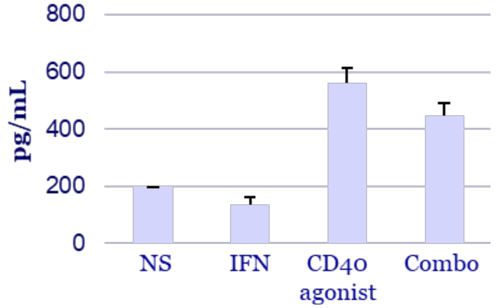
Enhancement of innate immunity *in hepatocytes*

CXCL10 release



No enhancement of inflammatory markers

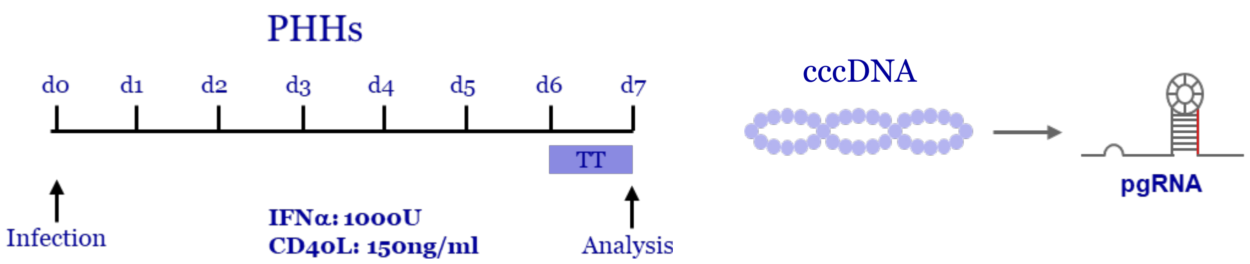
IL8 release



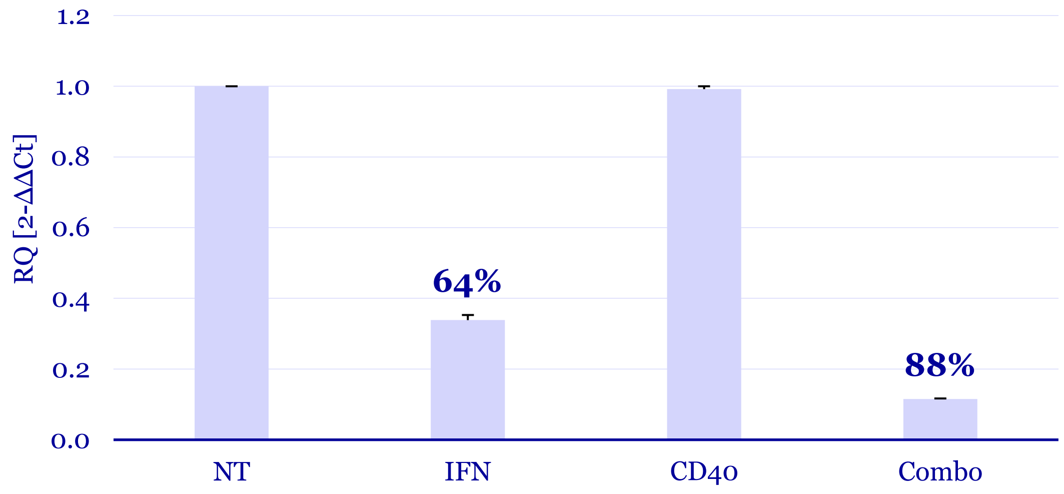
CD40 agonism &Type I IFN translate to potent antiviral activity

The combination produces a more robust and complete antiviral response

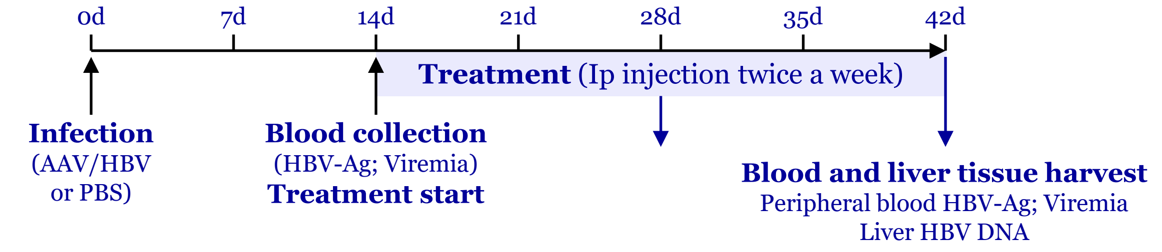
Effect on HBV infected primary human hepatocytes (PHH)



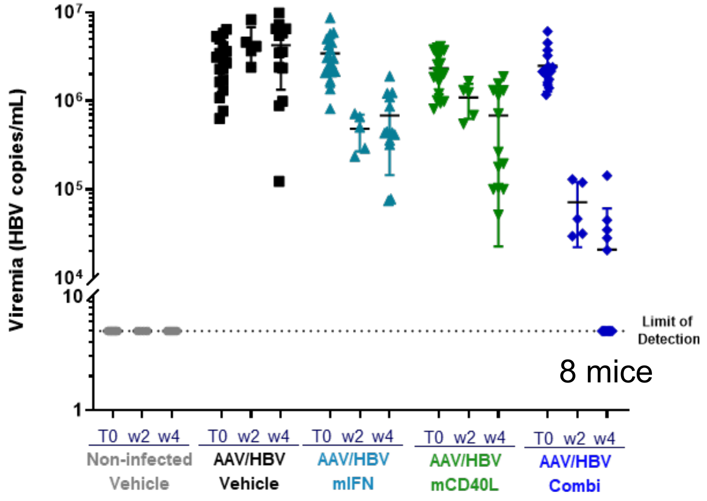
pgRNA



Target validation in the AAV/HBV model



Circulating HBV DNA (d28+d42)

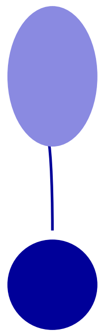


How can this combination be translated to the clinic for the benefit of patients suffering from Chronic Hepatitis B?

Bifunctional molecules combining CD40 agonism and IFN activity recapitulate potent antiviral activity

Generation of single bifunctional molecules

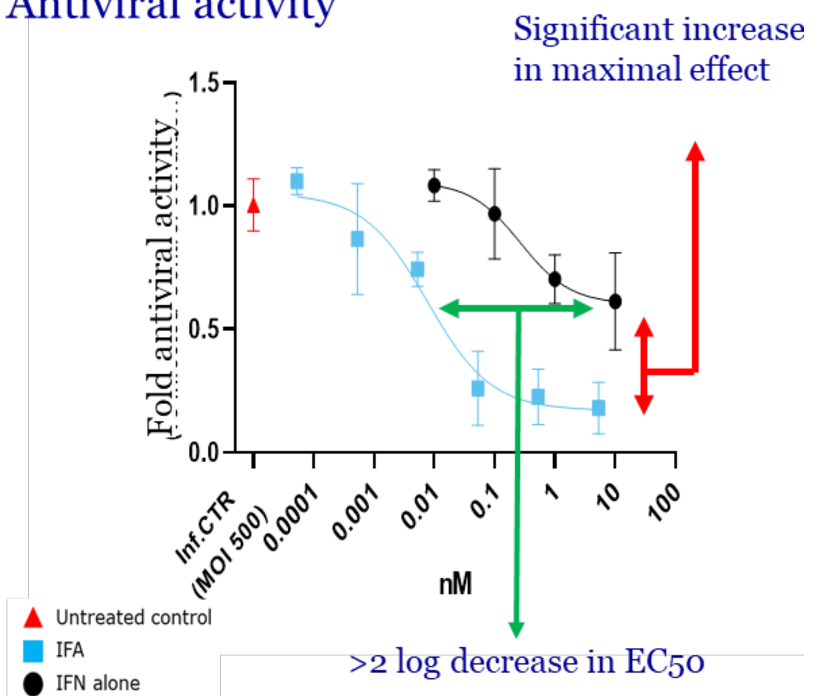
- fuses IFN to a CD40 agonist
- Simplify dosing and clinical development



- Maintain both CD40 and IFN activities

Example of molecule highly potent against select viruses including HBV

Antiviral activity



Ready to initiate phase 1 studies

- **Robust manufacturing** process established
- **The lead molecule is well tolerated in NHP**
- **IND enabling studies** completed
- **CTA approved** to initiate phase 1 i.v in HV

- **Proprietary Know-How and Patent Families**
- **Technical expertise** to tailor-make immunomodulators addressing additional combinations

Drug discovery platforms to tackle endemic and emerging viruses

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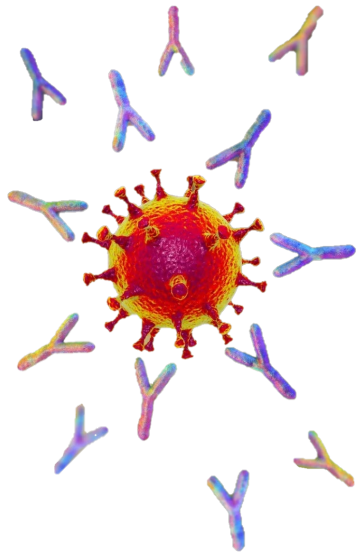
Neutralizing antibodies



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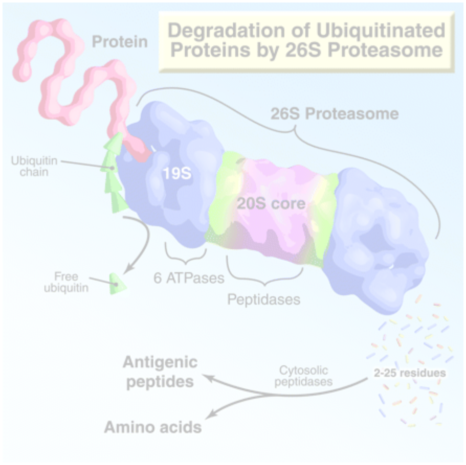
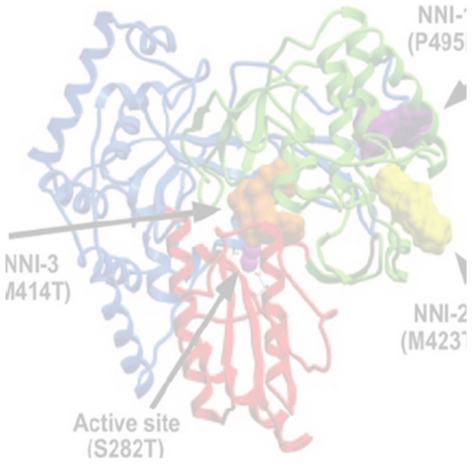


Preventing replication & assembly

Inhibiting key enzymes



J-HAL library screening to identify potent and broadly neutralizing Abs

PoC studies with SARS-CoV-2 anti-Spike antibodies

Evotec Antibodies Discovery Platforms

Hybridoma platform

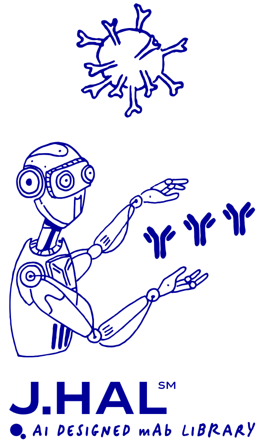
Natural immune repertoire

J.HAL

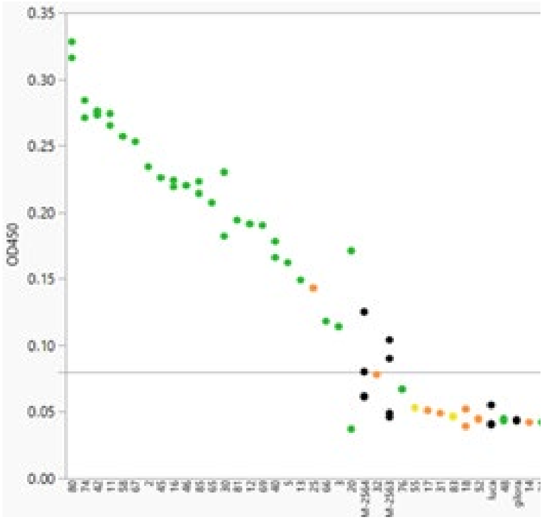
In future:

B cell technology

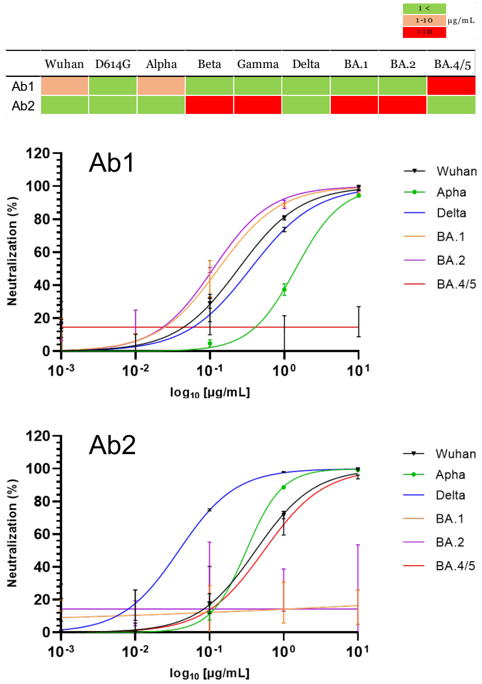
De novo mAb design



Robust & target-specific dose-dependent binding



Potent and broadly neutralizing antibodies



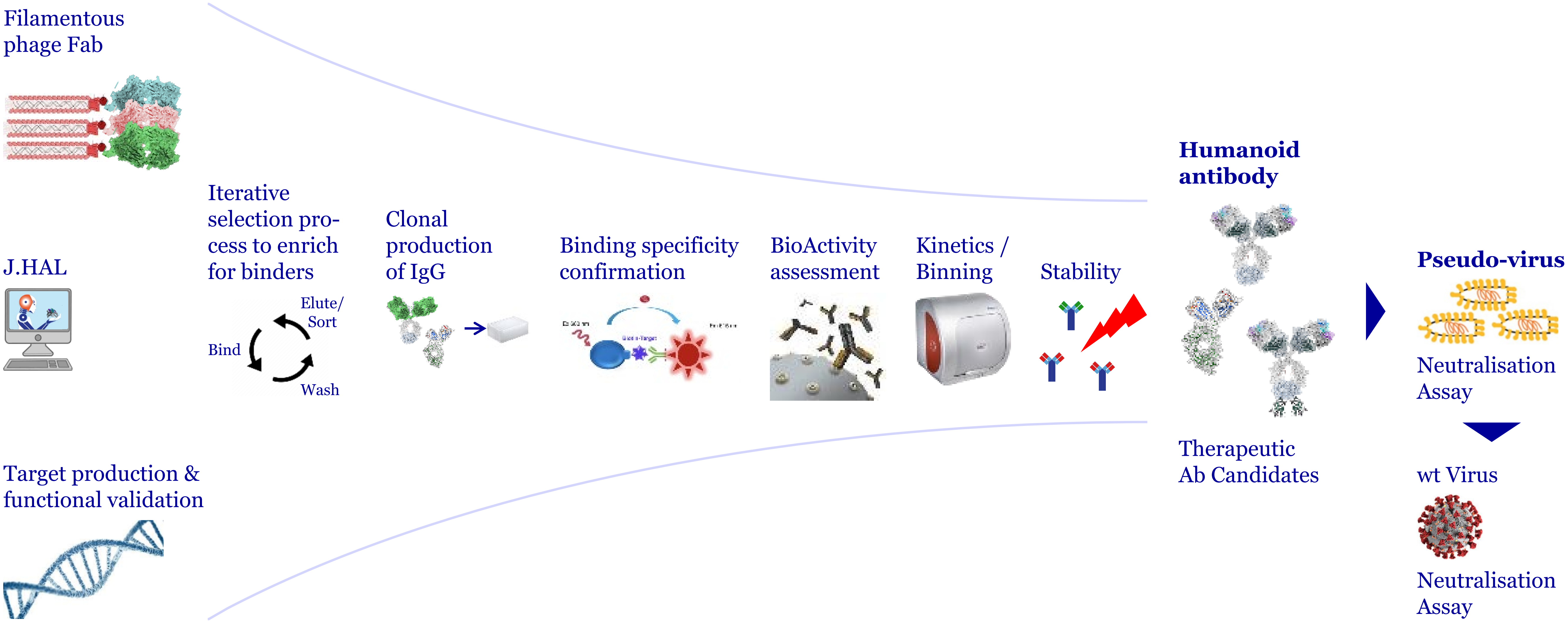
High potential for manufacturability

- Reduced Immunogenicity
- Extended half life
- Thermal & chemical stability
- Inherent high solubility
- Favorable colloidal stability
- Productivity/Cost

J.HAL® library-derived neutralizing anti-SARS-CoV-2 antibodies showed ***good binding profile, broad spectrum anti-viral activity without need of affinity maturation and high potential for manufacturability***

J.HAL: Biased libraries to find the best therapeutic

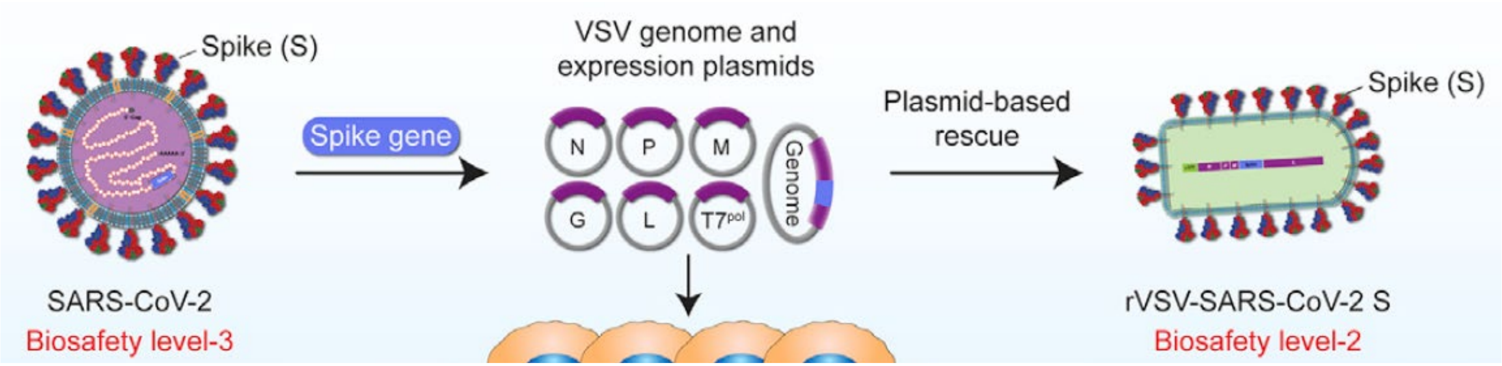
A novel, cost-effective and accelerated approach to therapeutic antibody discovery



VSV-Pseudoviruses platform

A safe toolbox to identify antiviral strategies interfering with viral entry

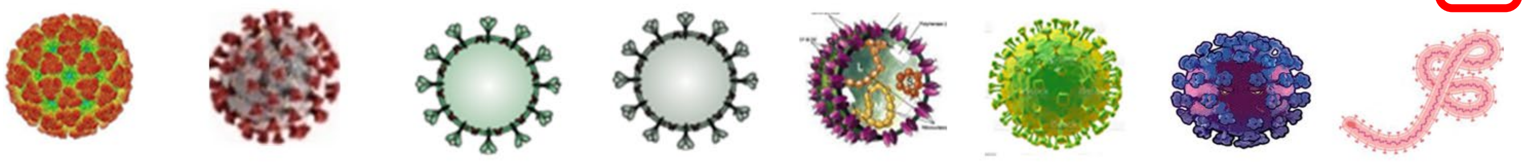
VSV pseudoviruses



- Safer and cost-effective alternative to live viruses
- Support working on BSL3 and BSL4 viruses within a BSL2 environment
- Potential to evaluate therapeutic / vaccine efficacy against different variants

VSV pseudoviruses pipeline

CHIKV SARS-CoV-2 SARS-1 MERS CCHF Nipah Lassa Ebola[1]



BSL3 BSL4

- Various pseudoviruses already generated, characterized and validated
- Potential to apply to many other virus
- Facility to test several variants

1 In development

Drug discovery platforms to tackle endemic and emerging viruses

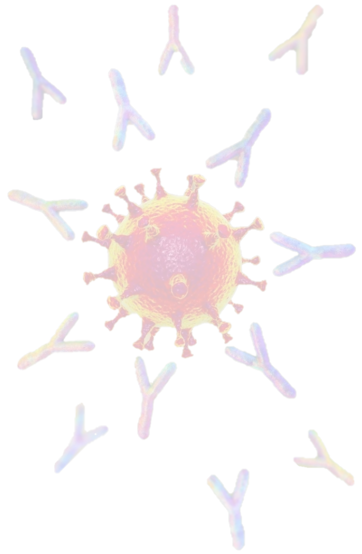
Developing tailored therapeutics that delay or prevent escape and resistance

Boosting innate and adaptive immunity

Host targeting platform

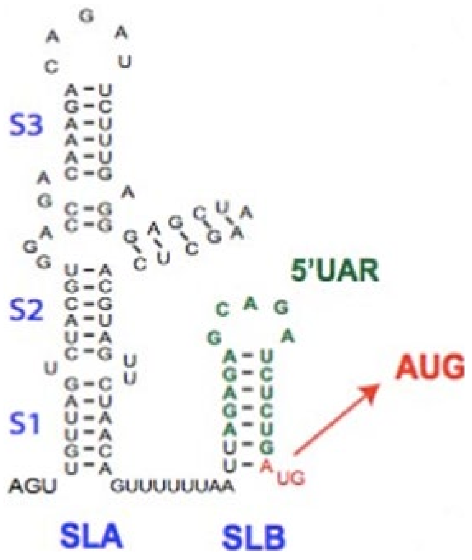
Preventing Viral entry

Neutralizing antibodies



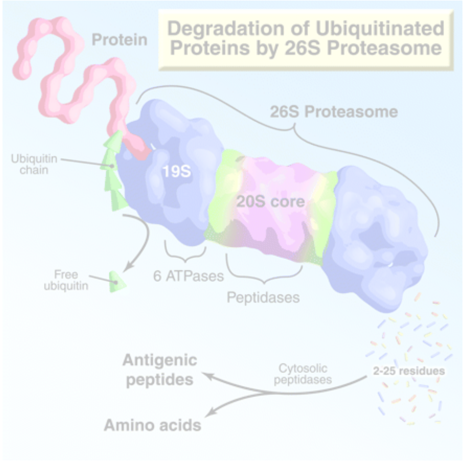
Targeting key viral RNAs structures

RNA targeting with small molecules



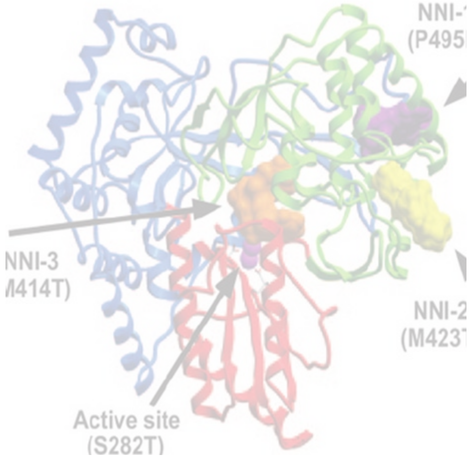
Degrading proteins not accessible to traditional therapeutics

Targeted protein degradation



Preventing replication & assembly

Inhibiting key enzymes



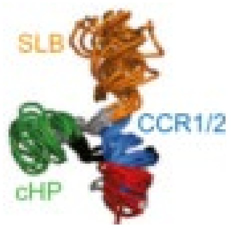
RNA targeting platform

Small molecules to targeting key viral RNA elements

Targeting regulatory RNA with small molecules is an emerging area of drug discovery

- Targeting RNAs with small molecules offers opportunities to modulate numerous cellular processes
- Messenger and non-coding RNAs adopt 3D structures that confer varied functional roles

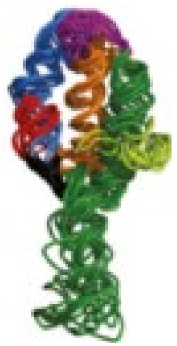
5'UTR 1&2



Envelope 4

NS2A 8

NS2B 10



3'UTR 24

Viral RNA potential as a drug target

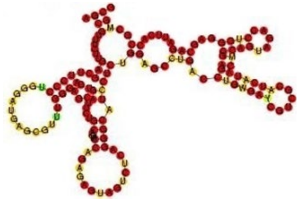
- Multiple highly structured functional elements
- Key roles for RNA elements throughout virus infectious cycle
- Highly selective pressure, which reduces risk for resistance

Transcription regulation

- HIV TAR element
- **HBV epsilon, others**

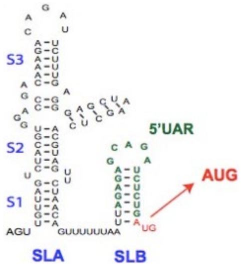
IRES Translation

- **EBV**, HCV, others



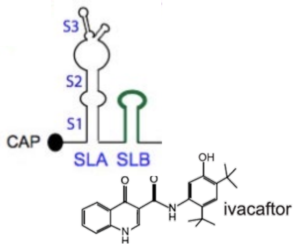
Genome cyclization

- **Dengue virus** YFV, Japanese encephalitis



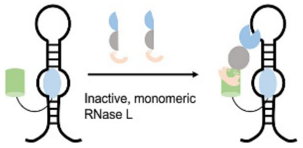
Ribosomal frameshift

- ChikV, HIV, RSV SARS-CoV-2, WNV...



Ribozyme: Enzymatic activity

- **Hepatitis D virus**



https://cen.acs.org/articles/93/i39/Molecule-Screen-Finds-Ligand-Flip.html
Dethoff et al. PNAS 2018

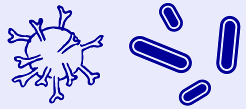
RNA targeting platform

From RNA structure analysis to Lead identification and optimization

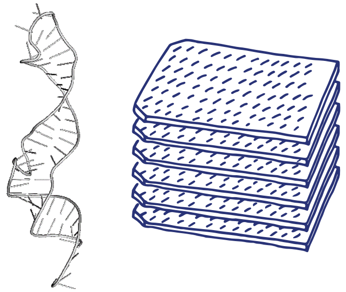
Structural data

Druggability assessment

Target enablement
3D structure
Functional validation

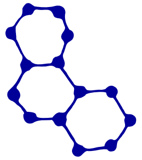


Pilot screen

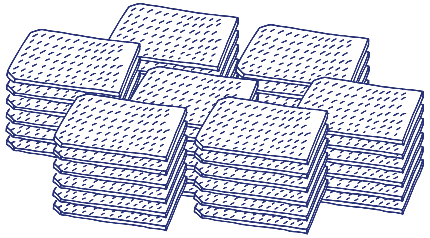


+ RNA-focused library

HITS Small molecules



Hit validation
Affinity
Pathogen inhibition



Full-deck screens
on validated RNA targets

Hit characterization
Lead optimization

45+
# of completed RNA target specific binder screens

15+
# of active targets

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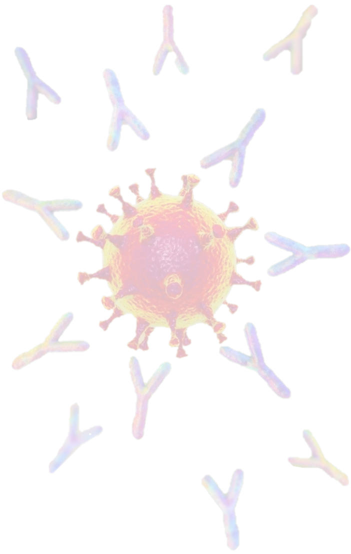

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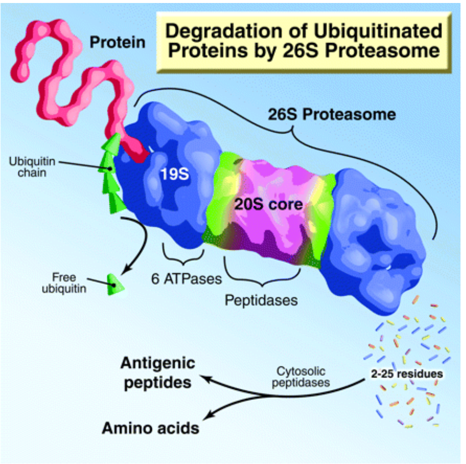
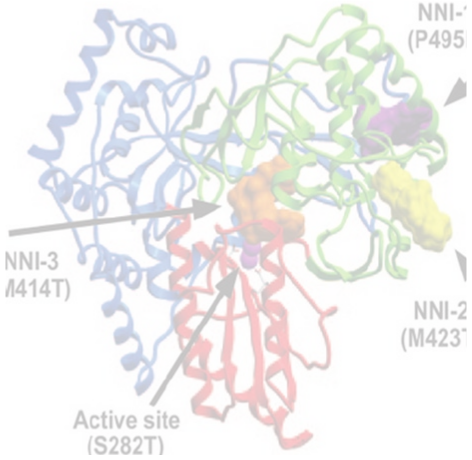


Preventing replication & assembly

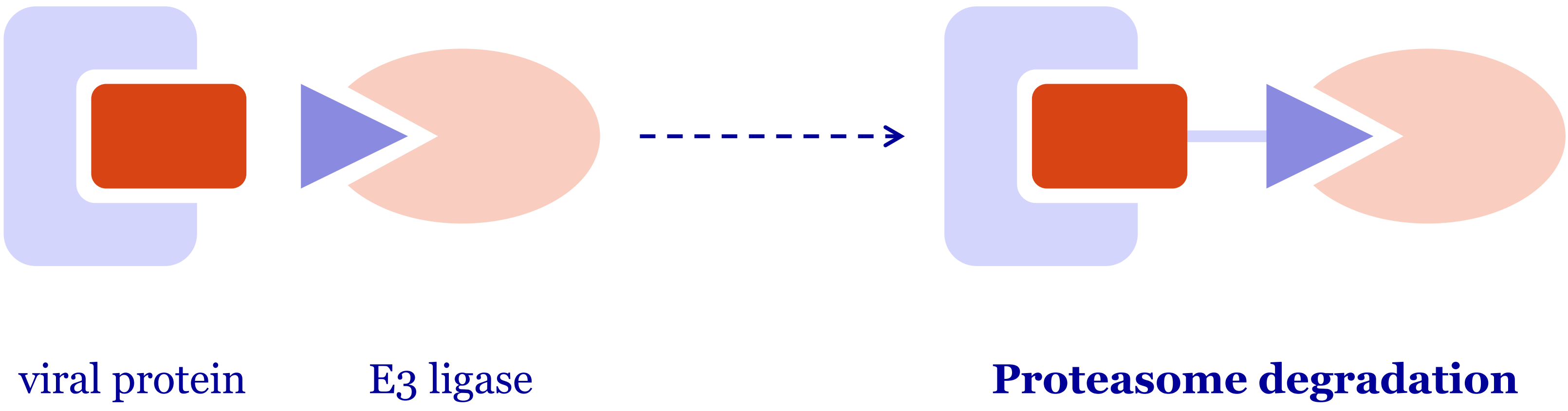
Inhibiting key enzymes



Targeted protein degradation to target undruggable viral proteins

Overcoming major challenges of antiviral drug discovery

- Targets “undruggable” proteins
- Applicable to any viral protein
- Resulting in complete silencing of viral activities
- Is less likely to lead to resistance
- Oral administration



- **Reporter cell lines expressing the target of interest**
  - Choice of tag (luminescent, fluorescent) & position
  - Full characterization of protein kinetics, turnover, activity
  - CRISPR knockout of E3 ligase of interest

- **High-throughput degradation assays**
  - 384-well plate format, DC_{50} and Dmax determination
- **Live degradation** kinetics (fluorescence/luminescence)

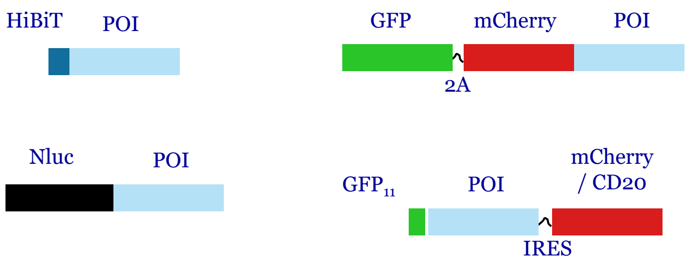
- A panel of **additional assays** to study targeted protein degradation
  - Cellular E3 ligase engagement
  - Binding affinity to viral protein (e.g. SPR, MST etc.)
  - Ternary complex formation (NanoBRET)
  - Ubiquitination (NanoBRET) / Ubiquitinomics
  - Proteomics in infectious model
  - Chemical competition / E3 ligase modulation

A panel of assays to evaluate small molecule degraders activities

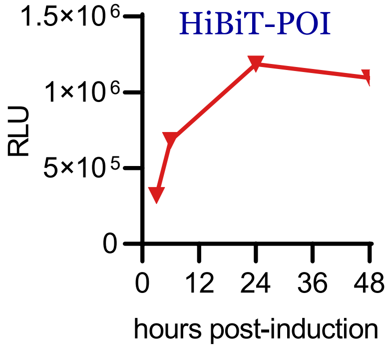
On-demand generation of reporter cell lines expressing the target of interest

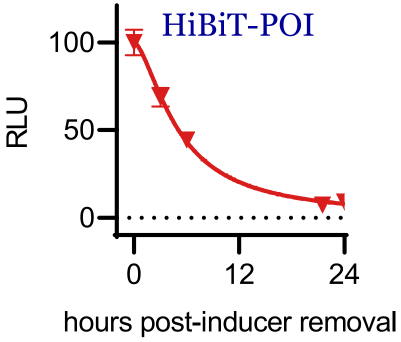
Multiple choices of tags

luminescent fluorescent

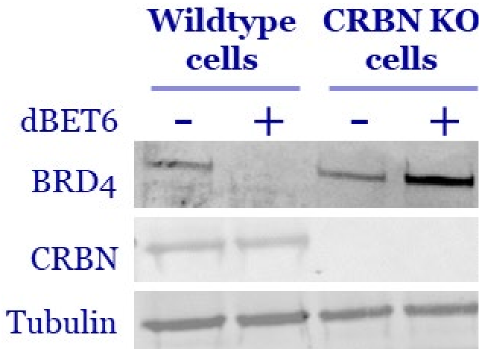


Full characterization of protein kinetics, turnover, activity

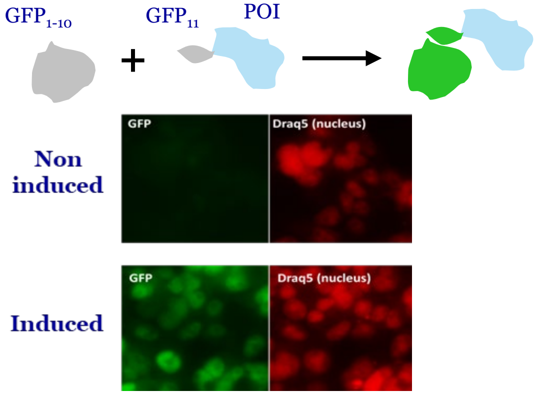




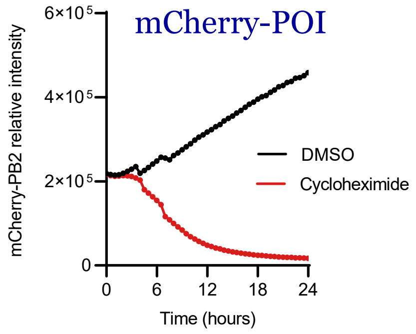
CRISPR knockout of E3 ligase of interest

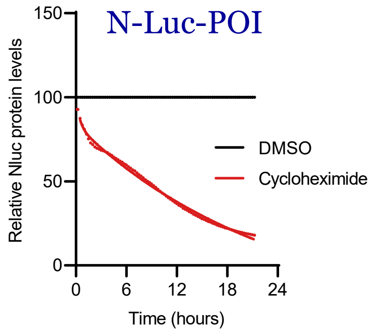


Stable cells expressing split GFP-POI



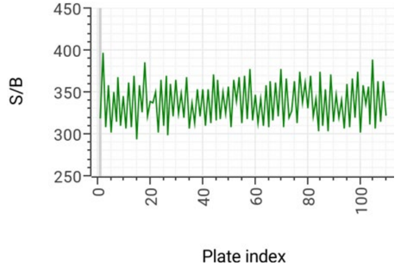
Live degradation kinetics (fluorescence/luminescence)



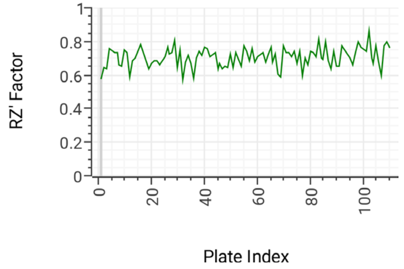


Successful High-throughput screenings

S/B = 336



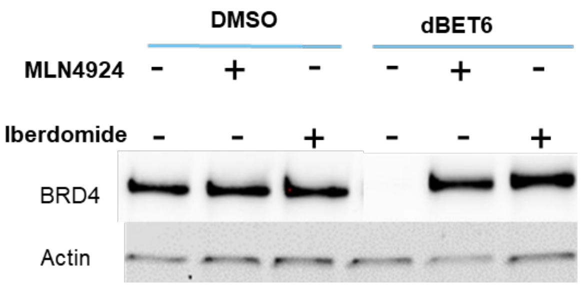
RZ' = 0.7



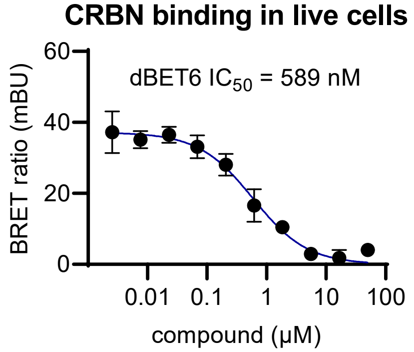
A panel of assays to study targeted protein degradation

Flexibility in reporter type, full characterization

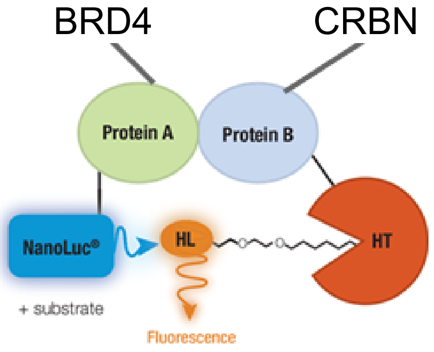
Chemical competition / E3 ligase modulation

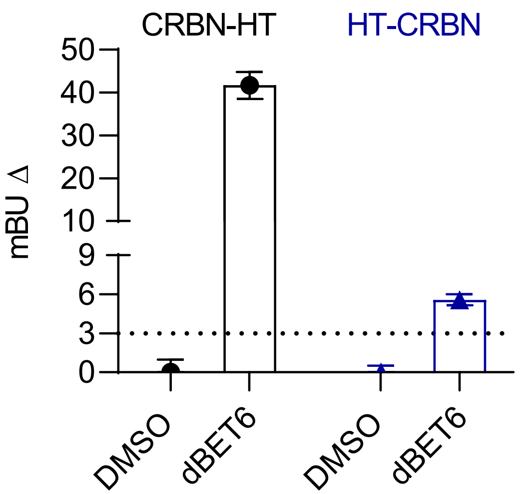


E3 ligase cellular engagement

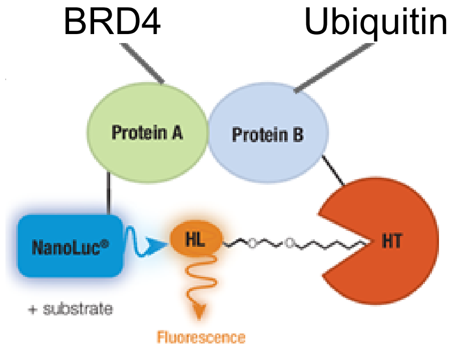


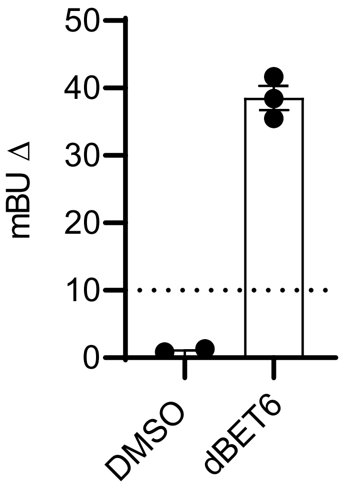
Ternary complex formation (NanoBRET)



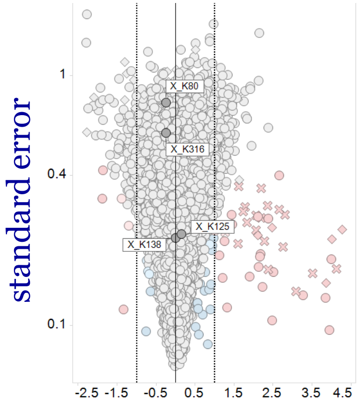


Ubiquitination (NanoBRET)





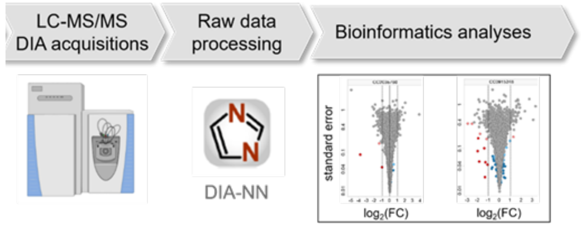
Ubiquitinomics



Pulldown of ubiquinated peptides

Nluc-POI peptides well detected in stable cells

Proteomics in infectious model



dBET6 = CRBN-dependent BRD4 degrader, binds CRBN ; GLS4 = HBV capsid assembly modulator, binds HBc
NanoBRET system image source: Promega, with adaptation; HL = HaloTag NanoBRET 618 Ligand; HT = HaloTag protein
MLN4924 = neddylation inhibitor; Iberdomide = CRBN ligand; 14a = VHL-dependent CRBN degrader

Drug discovery platforms to tackle endemic and emerging viruses

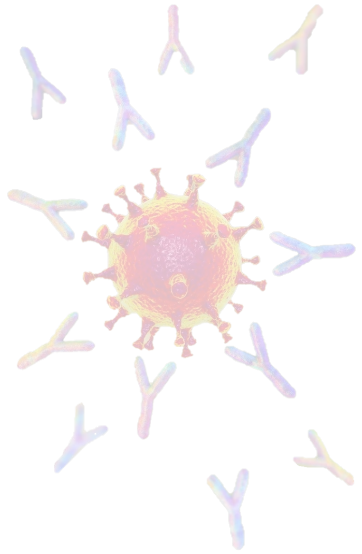
Developing tailored therapeutics that delay or prevent escape and resistance

Boosting innate and adaptive immunity

Host targeting platform

Preventing Viral entry

Neutralizing antibodies

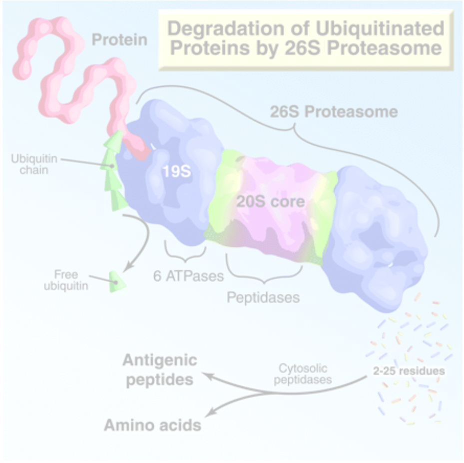


Targeting key viral RNAs structures

RNA targeting with small molecules

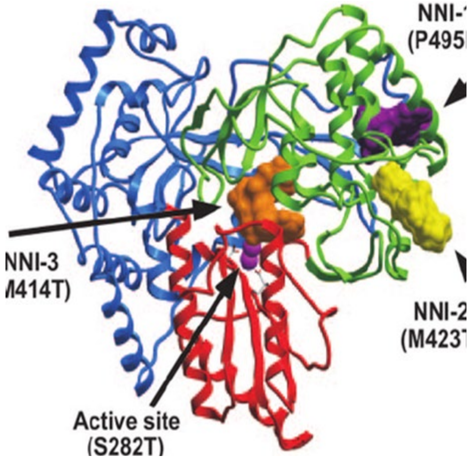
Degrading proteins not accessible to traditional therapeutics

Targeted protein degradation



Preventing replication & assembly

Inhibiting key enzymes



Novel platform to rapidly identify broadly acting small molecule antivirals

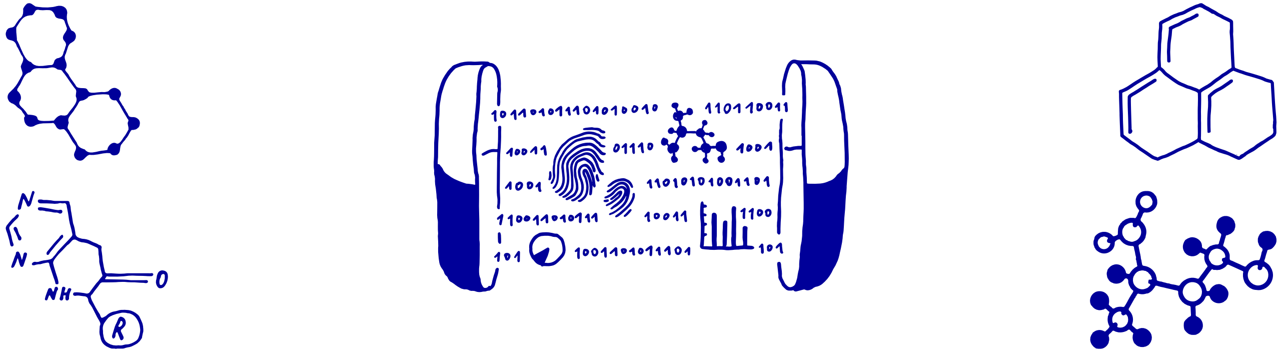
Enabling technologies applicable across virus families

Establishing an antiviral focused library to accelerate antiviral research and development

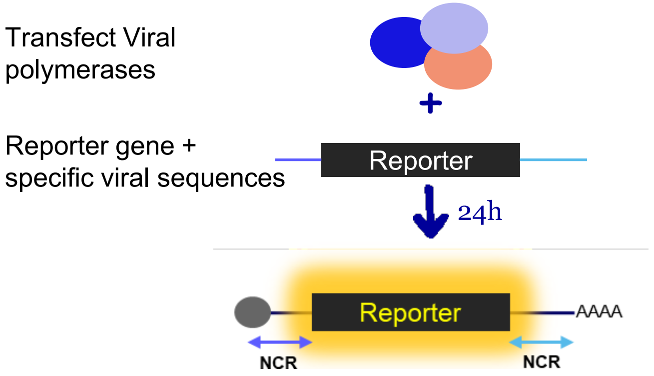
Initial focus and Poc: RNA-dependent RNA polymerases

- Validated targets (HIV, HCV, IAV…)
  - Conserved structures[1]
  - **Permanent need:** New variants / single mutations can lead to drug resistance[2]

Library building approach: Literature, ligand-based pharmacophore modelling and machine learning

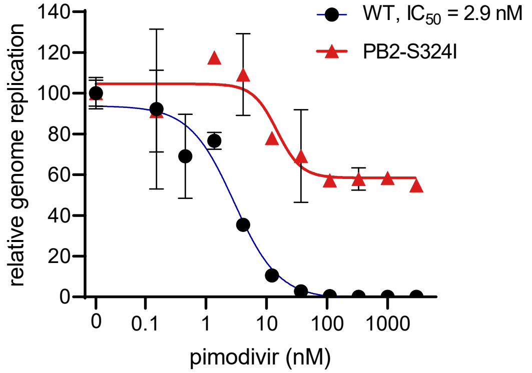


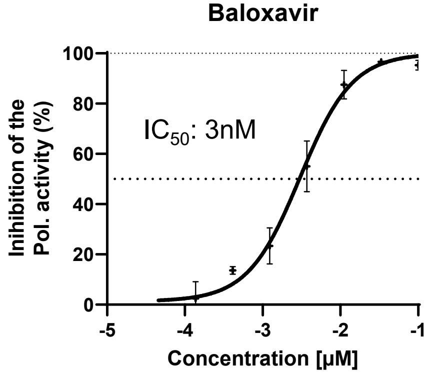
In cellulo minigenome polymerase assay



- Applicable to many viruses
- Work on BSL4 viruses in BSL2 labs
- Easy to test variants with natural or induced resistance mutations
- Adaptable to HTS: ongoing on influenza

Validation of Influenza minigenome with 2 ref cpds





1 Krammer & al. 2018. DOI: 10.1038/s41572-018-0002-y
2 Te Velthuis & al. (2018). DOI: 10.1007/978-1-4939-8678-1_17
NCR: non-coding regions used by the viral polymerases for genome replication

Novel platform to rapidly identify broadly acting small molecule antivirals

Building a pan-viral screening platform

Many viruses of concern

Virus of concern	Biosafety level	Design	Assay development
Influenza	2		
Nipah[1]	4		
CCHF[1]	4		
Hendra[1]	4		
Dengue	3		
Lassa[1]	4		
Ebola[1]	4		
MERS-CoV[1]	3		
SARS-CoV-1[1]	3		
SARS-CoV-2[1]	3		
RSV	2		
Chikungunya virus	3		

Approach: One-stop shopping

Building library of 5-10,000 cpds

Building mini-genomes for selected viruses

Screening of different minigenomes

Identify broad inhibitors

Chemical optimization

1 WHO priority pathogens