

CAR-T capabilities

- For research
 - CAR construct (lentiviral vector (CAR sequence)
 - Viral vector package (concentration and quantification)
 - CAR-T cell culture (isolation, expansion, proliferation, phenotype, activity)
 - Function in vitro (cytokines, cytotoxicity)
 - Animal models (hematological cancers, solid tumors)
 - PD evaluation (tumor growth, cytokine secretion, mouse survival)
 - PK study (CAR-T persistence, phenotype)
- For QC of CAR-T products
 - Purity (activity, immune phenotype, residue, efficiency)
 - Safety (endotoxin, mycoplasma, RCR/RCL, CRS related cytokine levels)
 - Potency (cytokine, cytotoxicity)
 - Identity (cell density, CAR expression, VCN)
- For clinical trials
 - RCL/RCR Assay
 - Vector copy number
 - CAR-T cell immune phenotype
 - Cytokine expression and secretion
 - IFN-γ ELISpot

Vaccine evaluation

- *In vitro* assays
 - Generation and production of vaccines, such as inactivated, attenuated, subunit (proteins or VLPs) and viral vector based vaccines
 - Titration of neutralization Ab in serum
 - Detection of immunoglobulins
 - Assessment of cytokines
 - Virus distribution and PK
 - IHC/HE
- *In vivo* models
 - IFV mouse and ferret infection models
 - RSV mouse and cotton rat infection models
 - HFMD neonatal mouse infection models (EV71 and CA16)
 - HSV-1 mouse infection model
 - Dengue mouse infection mode (subcontract)
 - Zika mouse infection model (subcontract)

Clinical capabilities

- CAP accredited Molecular Testing Laboratory
- Successfully passed CAP inspections in 2018 and 2020 without any findings
- CAP/SCCL/NCCL proficiency testing (HBV DNA and HCV RNA viral load, UGT1A1 genotyping) all one-time passed
- Human genomics, Clinical virology, Oncolytic virus Pharmacokinetics (PK) and Pharmacodynamics (PD), Vaccine immune responses, Cell therapy PK and PD

Platforms

- Genomics: NextSeq 550, 3730xl Genetic Analyzer, ABI 7900HT, QuantStudio™ 6/7 Real-Time PCR System, QX200 Droplet Digital PCR System, COBAS® AmpliPrep/Taqman 48, Qiasymphony, Agilent 2100/Qubit
- Immunology/biomarkers: LumiPulse G1200, Bio-Plex 200 system, SpectraMax M2e, MESO SECTOR S600, AID ELISpot Reader, BD FACS Canton II, BD FACS aria III

Particular Solutions

Clinical virology	Viral load, sequencing, genotyping, TCID ₅₀ , phenotyping, virus isolation and characterization, serology
Vaccines	Detection of antigen/antibody, virus in blood, titration of neutralization Ab, assessment of cytokines, cell responses by FACS and ELISpot
Oncolytic viruses/AAV	Titration of neutralization Ab, PK by PCR/plaque assay/TCID ₅₀ /IHC, transgene expression/functional assays, cytokines and inflammation markers, immune cell activation
CAR-T/TCR-T	Vector copy number/RCL by qPCR, T cell phenotype, cytokine or chemokine expression and secretion, cytotoxicity assay
Genotyping and biomarkers	Biomarkers for NASH, CYP2D6, IL28B, UGT1A9 genotyping, gene expression by RT-PCR, NGS

WuXi AppTec Virology and Viral Vector Platforms

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Virus Platforms

in vitro screening and profiling
in vivo efficacy
clinical virology

Liver-specific viruses:

HBV HCV HDV

Respiratory viruses:

IFV HCoV RSV HPIV HRV HAdV

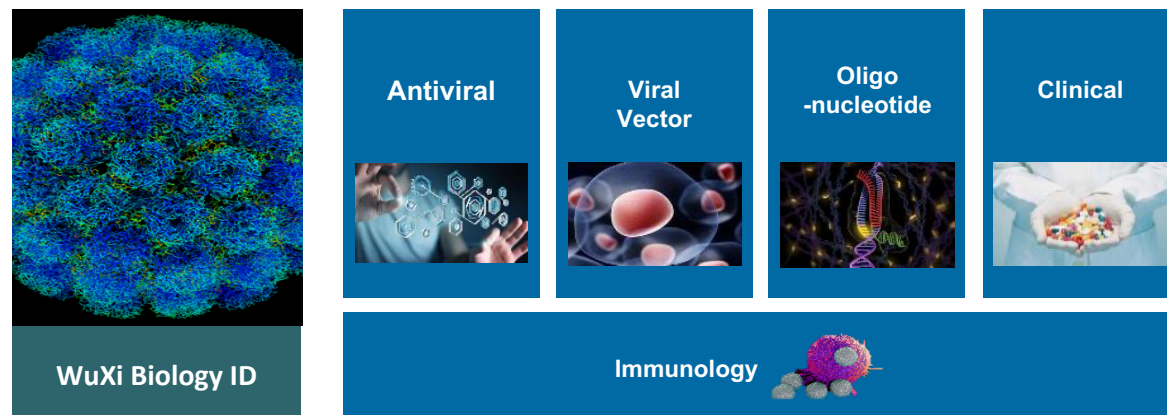
Herpes viruses:

HSV HCMV EBV VZV HHV-6

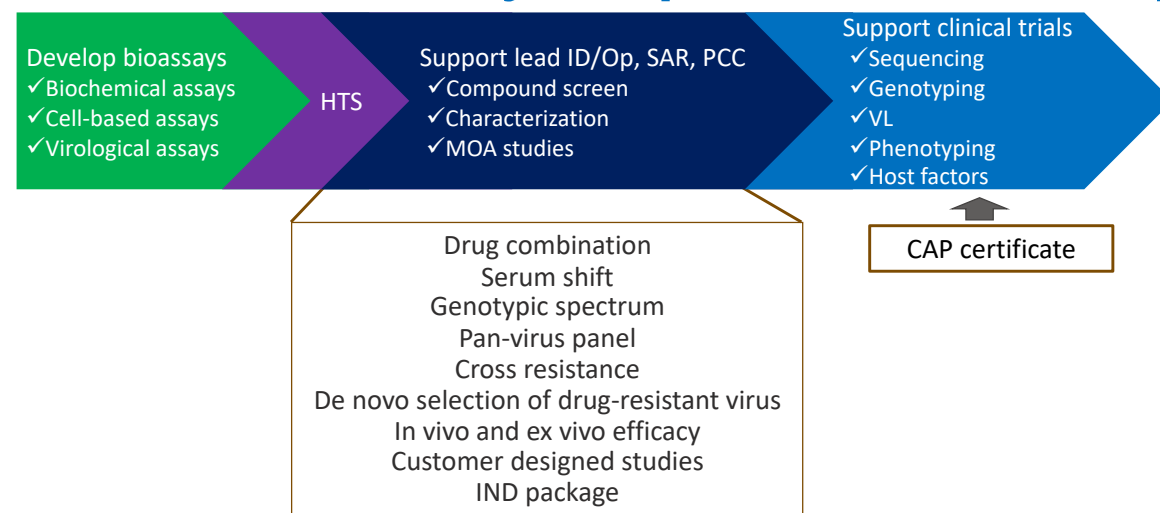
Other viruses:

HIV EV71 CV Zika DENV

WuXi virology platforms



Antiviral: from Assay Setup to Clinical Trial Support



LIVER-SPECIFIC VIRUSES

Hepatitis B virus (HBV): key service capabilities

- Drug screening using HBV producing cell lines
 - HepG2.2.15, DE19, and DES19 cells with qPCR, Southern blot, Northern blot, ELISA, cccDNA, encapsidated pgRNA and DNA
- HBV infectious assays
 - Ex vivo primary human hepatocyte (PHH) culture system
- Reporter assays: hTLR, THP1-Blue™ ISG, TNF-Induced NF-κB-luciferase cell lines
- Core protein expression and capsid assembly quenching system
- Compound profiling
 - Drug combination, serum shift,
 - Clinical isolates and genotypes (up to 5 constructs for each of A to H)
- Drug-resistant mutants
 - Nuc (16 constructs) and CpAM (16 constructs) resistant mutants by transient transfection (qPCR)
 - Construction of drug-resistant mutants, assessment of fitness and drug susceptibility
- Clinical virology
 - Full-length HBV genome sequencing (Sanger and Deep Sequencing), genotyping, VL
 - Phenotyping (full-length, pol, capsid shuttle vectors)
 - New HBV markers: serum HBV RNA, HBcrAg, HBV RNA sequencing
- Animal models
 - Hydrodynamic injection (HDI) mouse model
 - AAV/HBV mouse model
 - Humanized FRG mouse model
 - Duck/DHBV model
 - Transgenic mouse model
 - WHV/woodchuck model
- Immunological assays supporting animal models
 - Isolation of immune cells: lymphocytes from mouse spleen, liver and lymph nodes, PBMC from blood
 - FACS: surface and intracellular markers
 - ELISPOT
 - Cytokines by Luminex, ELISA, MSD and RT-qPCR

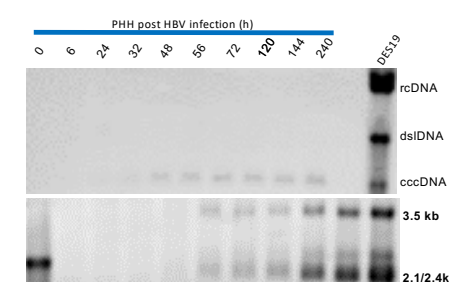
Hepatitis C virus (HCV): key service capabilities

- Screening: stable and transient replicons, and HCVcc with different endpoints
- Profiling
 - Drug resistance: cross resistance, de novo resistance selection
 - Genotypic spectrum
 - Drug combination
 - Serum shift
 - Colony formation
- Clinical support
 - VL (COBAS® AmpliPrep and TaqMan 48 systems)
 - Genotyping
 - Phenotyping (chimera, SDM, fitness, drug susceptibility)
- Biochemistry: viral enzymes

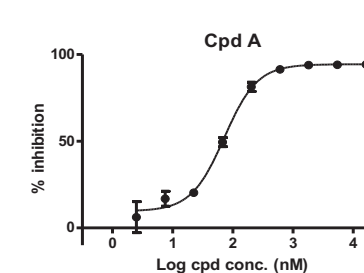
Hepatitis D virus (HDV)

- A cell-based infectious assay

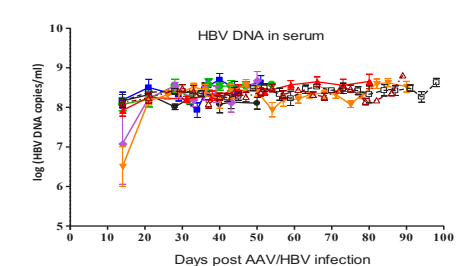
PHH/cccDNA/RNA



HepG2.2.15 (qPCR)



AAV/HBV & humanized FRG mouse

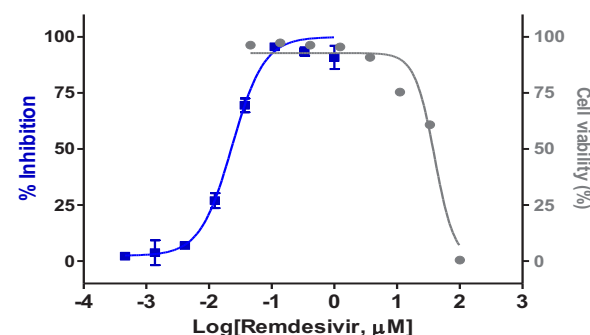


RESPIRATORY VIRUSES

Coronavirus (CoV)

- SARS-CoV-2 assays
 - Mpro enzymatic assay
 - SARS, MERS, 229E, OC43, HKU1 and NL63 CoV Mpro assays
 - PLpro enzymatic assay
 - SARS and MERS PLpro assays
 - RdRp enzymatic assay
 - Spike or RBD and ACE2 binding assay
 - Spike protein-mediated pseudovirus (PsV) receptor binding/entry
 - SARS PsV assay
 - SARS-CoV-2 cell-based live virus assays: available in collaborator labs

229E

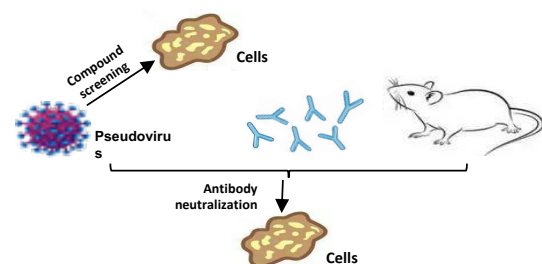


Influenza virus (IFV)

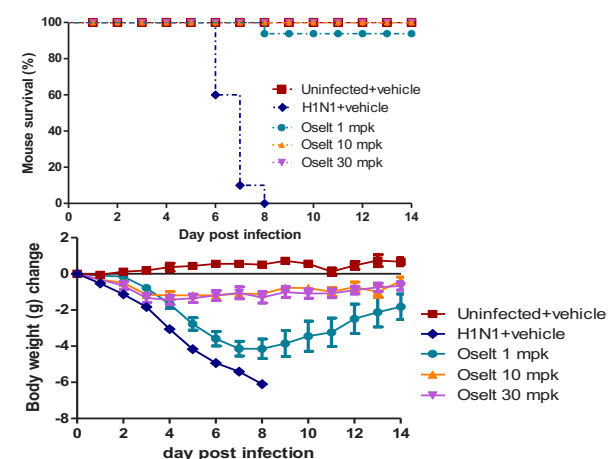
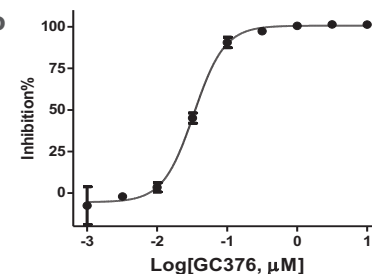
- Screening/profiling
 - A mini panel of H1N1, H3N2 and B strains
 - H7N9, H5N1 (subcontract lab)
 - Cell-based CPE, plaque and qPCR
 - De novo selection of drug resistance
 - Serum shift and drug combination
 - Oseltamivir, VX-787 and Baloxavir resistant viruses
- In vivo model
 - Mouse nasal infection with mortality, body weight and VL
 - Ferret nasal infection with body weight and viral load
- Biochemistry
 - Neuraminidase, hemagglutinin, polymerase
- Purification of viral protein and replication complex from infected hen eggs

- Cell-based human general coronavirus (hCoV) assays
 - 229E (α) CPE and RT-qPCR assays
 - OC43 (β) CPE and RT-qPCR assays
- Animal model
 - OC43 (β) mouse infection model with mortality and viral load
- Human proteases: a panel of 23 human protease assays

Pseudovirus



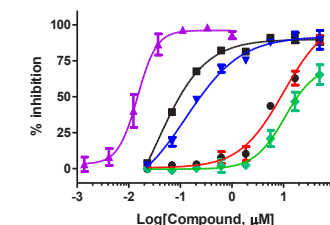
Mpro



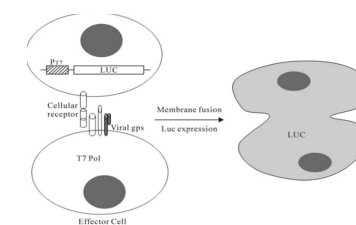
- H1N1: i.n. inoculation
- Oseltamivir: po, bid, 1st dose at 4 h prior to infection, 7 days

Respiratory syncytial virus (RSV)

- Viral infection assays
 - RSV A: Long and A2
 - RSV B: B9320 and B18537
 - Clinical isolates: RSV A and B
 - CPE, plaque, ELISA
- Profiling: drug resistance, genotypic spectrum, drug combination
- MOA studies
 - De novo selection of virus resistance
 - F protein-based cell fusion assay
- Mouse nasal infection (A2) model with VL in lung by the plaque assay
- Cotton rat nasal infection model with VL in lung by the plaque assay



- RSV: Long strain
- Cells: HEp-2
- Compound treatment: 5 days
- Endpoint: CPE



RSV F protein mediated cell fusion assay

Human rhinovirus (HRV)

- HRV 1B (Subtype A) and HRV 14 (Subtype B) strains, cell-based CPE assay
- Mouse nasal infection model with viral load in lungs, cytokines and H&E stain

Human parainfluenza virus (HPIV)

- HPIV 3, cell-based CPE assay
- HPIV GFP reporter assay under development

Human adenovirus

- AdV 14 cell-based CPE assay
- RCA assay

Porcine reproductive and respiratory syndrome virus (PRRSV)

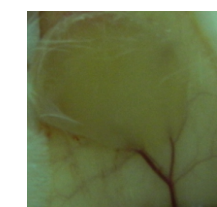
- Cell-based CPE assay

HERPES VIRUSES

Herpes viruses

- Herpes simplex virus (HSV)
 - HSV-1 and HSV-2, cell-based CPE assays
 - *In vivo* model: (1) mouse intranasal and vaginal infection lethal model, mortality, body weight and viral load (2) Mouse ocular latent infection and activation model
 - *Ex vivo* model: isolation and cultivation of latently infected ganglia neurons
- Human cytomegalovirus (HCMV)
 - Cell-based CPE and reporter replication assay
 - *In vivo* model: NOD SCID mouse, sponge virus implant with VL by plaque assay

- Epstein-Barr virus (EBV)
 - Cell-based qPCR assay
- Human herpes virus 6 (HHV-6)
 - Cell-based qPCR assay
- Varicella-zoster virus (VZV)
 - Cell-based CPE and qPCR assays

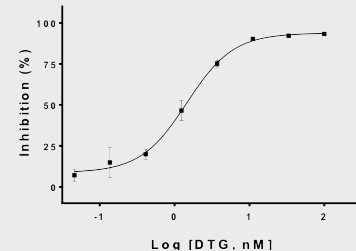
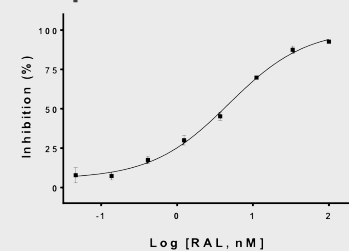


Gelfoam implants from an NOD SCID mouse (9 days post implantation)

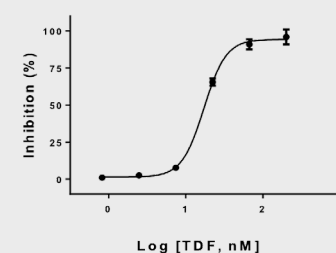
HIV-1

- HIV-1 IIB or BaL, MT-4 cell-based CPE assay
- HIV-1 pseudovirus reporter assay
- Reference: AZT (Retrovir), TDF (Viread), RAL (Raltegravir) and DTG (Dolutegravir)

HIV pseudovirus



HIV IIB



Hand-foot-mouth disease viruses

- Enterovirus 71 (EV71)
 - Cell-based CPE assay
 - EV71 Shenzhen/120F1/09
 - EV71 MAV-W
 - EV71 mouse lethal and VL qPCR model
 - Transgene mouse model for EV71 clinical strain (under validation)
- Coxsackie virus (CV)
 - Cell-based CPE assay
 - A16 SZ05
 - CV A16 G08-M
 - B3 Nancy
 - A16 mouse lethal model and VL qPCR model



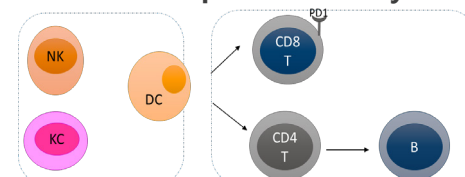
Other viruses

- Zika virus (PRVABC59)
 - Cell-based CPE and plaque assays
 - AG129 mouse model (subcontract lab)
- Dengue virus
 - Type 2 cell-based CPE assay

Immunology capabilities

- Assays for innate immunity pathways: TLRs and other assays
- In vitro and ex vivo immunological assays
 - ADCC, CDC, ADCP, ADE and NK cell cytotoxicity, etc.
 - Isolation of monocytes, lymphocytes from mouse spleen, lung, liver and lymph nodes
 - Isolation of PBMC from mouse and human blood
- FCM platform
 - Immune cell subtyping
 - Intracellular cytokine staining (ICS)
 - Tetramer and pentamer staining
 - Cell sorting
- ELISpot platform
 - T cell ELISpot
 - B cell ELISpot
 - Cultured ELISpot (in vitro stimulation)
- Biomarkers
 - Cytokines and other biomarkers by Luminex, MSD, ELISA, CBA and RT-qPCR

Innate and adaptive immune systems



Viral vector (VV) capabilities

- Various viral vectors for cloning target genes
- Preparation of endo-free plasmid DNA in various scales
- Package and production of recombinant viruses
- Purification of recombinant viruses with density gradient based ultracentrifugation
- Titration of virus using qPCR, plaque assay, TCID50 and commercial kits
- Analysis of protein impurities of the final products
- Determination of the bioactivity of the final recombinant virus products
- Assessment of empty capsid by EM
- Animal PK/PD studies
- PK/PD sample analysis: virology assays, ELISpot, FACS, ELISA, MSD, Luminex, RNASeq, qPCR, etc.

Viral vector platform: AAV

- Viral vector construction
 - Sub-clone gene of interest (GOI) into different serotype pAAV cis-plasmid DNA constructs
 - Optimization of viral vectors including promoter, codon optimization and serotype assessment
 - Preparation of endo-free pAAV cis-plasmid and complimentary plasmid DNA in various scales
- Virus package
 - Package and harvest of AAV preparations with adherent HEK293T or suspension 293F cells
 - Purification of AAV with density gradient based ultracentrifugation
- POC
 - AAV capsid purity
 - Vector genome titer (qPCR/ddPCR), AAV titration (TCID50), capsid titration (ELISA)
 - AAV serotype analysis with western blot or PCR
 - Bioactivity analysis of transgene at gene and protein levels
 - HCP/HCD analysis
 - Assessment of empty capsid by EM
 - Animal studies: virus biodistribution and shedding, transgene expression

Viral vector platform: oncolytic virus

