# Leveraging the UTHSC Regional Biocontainment Laboratory (RBL) To Augment *your* Infectious Disease Research

11<sup>th</sup> Annual SEASR Meeting, Nashville, TN June 12-14th, 2024



**Colleen Jonsson, PhD, Director** 

Liz Fitzpatrick, PhD, Associate Director

Shannon Taylor, Scientific Director

The *Mission* of the RBL is to Provide Facilities for Faculty Research Programs and For Fee-for Service Activities for Internal and External Users





Objective 1. To provide a Facility Management, Maintenance and Operation Core that will integrate preventive maintenance of mechanical and vivarium infrastructure and scientific / biosafety equipment necessary for BSL-3 and ABSL-3 containment.



Objective 2. To provide a BIOSAFETY Practices Core that will integrate the management of operations and training for BSL-3 pathogens of which some are select agents, promotes communication and harmonize compliance, security, individual competency, and practices across our RBL Staff and our Users. We also support BSL-2 research.



**Objective 3. To facilitate and advance research within the Biocontainment Research Support Service(s) Core** 

# **Outline of Presentation**

Overview of Operations, Facilities, Scientific Equipment
Examples of Ongoing Scientific Reseach

# **Facility Management, Maintenance and Operation Core**







## Vivarium Team



# Provide support for PMs and certifications of mechanical and vivarium infrastructure and scientific equipment



Annual preventative maintenance and calibration of critical scientific instruments

Certification and replacement of HEPA filters for Allentown caging system

Annual certification of biosafety cabinets

Annual certification of Phoenix controllers

Certification of boilers, chillers, HVAC (supported by UTHSC)

### **Biosafety Practices Core**



Ying Kong, PhD Team Leader Department Microbiology, Immunology, Biochemistry



Tim Barton, MS, CIH Director of Research Safety Affairs Responsible Official

Ramesh Ray, PhD UTHSC BSO Research Safety Affairs ARO



Jennifer Tate, PhD Research Safety Affairs RBL BSO

Provide a dedicated person to provide advanced hands-on training for specialized equipment and advanced protocols

BSL-3 Practices Core Leader:

- ✓ >20-yr experience in BSL-3 & ABSL-3
- ✓ Have been a UTHSC RBL user since 2013
- ✓ Joined RBL to support research services since COVID19
  - Oversees SOP renew development
     of new SOPs
  - Provides hands-on training for specialized equipment
  - Supervises samples transporting out of the RBL after inactivation



**IVOS II Sperm Analyzer** 



## Provide support services for the BSL-3 practices

Dr. Jennifer Tate RBL Biosafety Officer:

**RBL BSO Onboarding Progress** 





# **Comprehensive Research Facilities & Services**



### **Science Core**



Shannon Taylor, PhD Scientific Director



Bernd Meibohm, PhD



Dong Yang, PhD Program Manager



Marjo Willem, PhD



Anjali, PhD



Kelli Brown, PhD



Yuting Zheng, BS Jyothi Parvathareddy



Yi Xue, PhD

Provide support to the Global Research Community for biodefense, emerging infectious diseases, and pandemic preparedness and response.

#### 16 Internal Users from 8 departments:

- ✓ Clinical Pharmacy & Translational Science: 3
- ✓ Medicine-Nephrology: 1
- Microbiology, Immunology & Biochemistry: 5
- ✓ Pediatrics: 3
- ✓ Pharmaceutical Sciences: 1
- ✓ Physical Therapy: 1
- ✓ Physiology: 2
- ✓ Neuroanatomy: 2

#### EMORY arrowhead GHDDI 药物研发中心 redueen RedHill **RUTGERS** Biopharma. Esperovax Therapeutics St. Jude Children's Research Hospital FI N Finding cures. Saving children. UNIVERSITY OF MAURITIUS

**Selected External Users** 

### Small Animal Facility

Mice Hamsters Rattus Ferrets Cotton Rats Guinea Pigs









# Comprehensive Animal Services



Dong Yang, PhD Program Manager



## Animal Model Development



hACE2 COVID-19 Mouse Model



# **Clinical Chemistry and Hematology**

Campus-Wide Services For all Sample Types, i.e. BSL2 and BSL3



#### X•pedite<sup>™</sup> HEM<sup>3</sup>

(1) 20 parameters and differentiation of leukocytes into 3 sub populations for comprehensive analysis.

(2) minimal sample volume of only 9.8  $\mu$ L whole blood.

(3) capabilities of testing broad species with 7 pre-installed and 4 customizable animal profiles.



Respons®910 VET (1) samples of serum, plasma and whole blood. (2) low sample volume 2-30uL.

(3) capacity of running 31 assays and 6 calculated tests

# Host Gene Responses











MiSeq Cytogenomics DNA-Protein Interaction Analysis Gene Expression Analysis Genotyping Inherited Disease QuantStudio<sup>™</sup> 6 SYBR®, TaqMan® 96/384 FAM<sup>™</sup>, VIC<sup>™</sup>, ABY and JUN dyes, up to 4 targets in a single reaction.

#### MAGPIX®

QUANTI-GENE-up to 50 analytes per sample

The dynamic range is >3.5 logs with expected read time for a 96-well plate in < 60 min (up to 4,800 tests/hour). CHROMIUM X Assay Single Cell Gene Expression LT Single Cell Gene Expression

# Immune Responses









CYTEK Aurora Spectral Cytometer

3 Lasers: Violet (405nm) Blue (488 nm) and Red (640nm) Detection of up to 24 colors: Violet laser – 11; Blue laser – 9; and Red laser – 4 colors CTL Immunospot<sup>®</sup> S6 Universal Analyzer

single- and dual-color enzymatic ELISPOT and 3 fluorochromes Miltenyi MACSQuant<sup>®</sup> Tyto<sup>®</sup> Cell Sorter

3 lasers, 10 parameter cell sorting

# <u>Immunology</u>





# Implementing Provantis to provide good laboratory practices (GLP) in one suite of the ABSL-3 over next 3 years





Training Emulate Envision CQ1 Apex microCT **ECHO** IVIS Agilent MiSeq Cytek Tyto QPCR Pathology



Provide training of staff and users on equipment through hands-on workshops

### NIH G20 funded instrumentation

- Improved in vitro models to drive innovation in and evaluations of next generation antivirals using the human based Organ-Chips
  - Emulate
- Improved in-life end points for basic, discovery and preclinical animal models
  - Molecubes MicroCT
  - Vevo 3100 ECHO

- New capabilities for in vitro assays for drug discovery and mechanism of action
  - Olympus APX100 Digital Imaging System (Live or fixed)
  - CellVoyager CQ1 Benchtop High-Content Analysis System (Live or fixed)
- Improved after-life end points for basic, discovery and preclinical animal models
  - SCIEX Triple Quad 6500+ LCsystem
  - Cytek® Aurora Spectral Cytometer
  - BLAZE Light Sheet Microscope

Center of Excellence for Encephalitic Alphavirus Therapeutics



#### NIH U19 AI142762-01

VEEVIRUS - WEEVIRUS - EEEVIRUS





### THE UNIVERSITY OF TENNESSEE HEALTH SCIENCE CENTER.

Dr. Colleen B. Jonsson In Vivo Antiviral Resistance Murine Efficacy Studies Dr. Bernd Meibohm Pharmacokinetics, PK-PD Dr. Elizabeth Fitzpatrick Immune Response





**Dr. Dong Hoon Chung** Antiviral Screening Mechanistic Studies



Regulatory Support





*Dr. Jennifer E. Golden* Synthetic and Medicinal Chemistry *Dr. Ed Elder* Formulation





Dr. Shannan Rossi NHP Efficacy Studies Viral Seed Stocks Dr. Scott Weaver Subject Matter Expert

# ACKNOWLEDGEMENTS





#### VEEVIRUS - WEEVIRUS - EEEVIRUS

#### **Funding**

USAMRAA W81XWH-08-2-002 Jonsson and Schmaljohn (PI) USAMRAA W81XWH-10-2-0064 Jonsson and Schmaljohn (PI)

NIH MLI (MLPCN, HTS and Hit Development): NIH U54 HG005034 Jonsson (PI) NIH U54HG005031 Aube (PI) NIH 1R03MH087448 Chung (PI) NIH R01 AI118814 Jonsson, Chung, Golden (MPI) NIH U19 AI142762-01, CETR

#### Jonsson lab

Evan Williams, Ph.D. Walter Reichard Yi Xue, Ph.D. Alex Machado, Ph.D. Jasper Lee, Ph.D. \* Haley Writt\* Ernestine Hayes, DVM\*

#### <u>RBL</u>

Dong Yang, Ph.D. Lillian Zalduondo Jyothi Pavarthareddy Yuting Zheng Stacey Barnett Ying Kong, Ph.D. Crystal Murphy

# Goal of CETR: Antiviral Treatment for Human Infections of New World, Neurotropic Alphaviruses



- non-specific febrile illness
- aseptic meningitis can progress to nausea and vomiting to somnolence (drowsiness)
- encephalitis inflammation and/or
  infection of the brain
  (versus meninges)
  leading to seizures,
  paralysis confusion,
  coma



#### 348,140 national repository (MLI) compounds

VEEV TC83 cytopathic effect assay (CPE), EC<sub>50</sub>

mammalian cytotoxicity, CC<sub>50</sub>

- ✓ feasibility assessment
- ✓ promiscuity analysis
- ✓ physiochemical properties

resynthesi

**CPE** confirmation

viral titer reduction

- ✓ activity profile
- ✓ inherent SAR

CPE EC<sub>50</sub> = 0.78  $\mu$ M CC<sub>50</sub> > 50  $\mu$ M titer reduction (5  $\mu$ M) = 63-fold molecular weight = 411.43 g/mol CLogP = 3.4 tPSA = 90.96 Å<sup>2</sup> PBS solubility = 122  $\mu$ g/mL

quinazolinone hit

PLOS Pathog. 2014, 10(6), e1004213.

# Prioritized Hit (905) from VEEV (TC83) HTS (BSL2)

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### **BDGR-49**



#### $\equiv$ Science $\triangleleft$

A brain-penetrant alphavirus RNA synthesis inhibitor is therapeutic in encephalitic mice

XUFENG CAO, DONG YANG, ET AL.

A brain penetrant, encephalitic alphavirus RNA synthesis inhibitor, BDGR-49, exhibits prophylactic and therapeutic efficacy in...

Cao, 2023 PMID: 37043558

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# Pathology and Immunohistochemistry





**hippocampus at 6 dpi.** VEEV TC-83-infected pyramidal cells in pyramidal layer, neurons in stratum lacunosum-moleculae layer and neurons in granule layer: molecular layer and polymorph layer of dentate gyrus.

Arrow shows infected oligodendrocytes in corpus callosum and fiber tracts.

# VEEV TC-83-Vero 76 Evolves Dominant Resistance Profile to ML336 Through a Variety of Trajectories Lee et al. (2020) PMID: 32878897



Emulate Organ on a Chip, Walter Reichard, Graduate Student, Department of Microbiology, Immunology, and Biochemistry

5 Porous Membrane **Endothelial Cells** Bottom Channel 8



Neurons

Microglia Astrocytes

Pericytes Top Channel

7

8

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## Prophylactic Treatment & Pharmacodynamic Studies of EEEV Infection (NIH AVIDD)

2023.056A Survival study of BDGR-251 IP BID -2hr prophylactic treatment against EEEV V105



24 mg/kg (24 mg/kg Q24h)





GOAL

Ashish Srivastava, PhD, UTHSC, College of Pharmacy

Anjali, PhD, UTHSC, RBL

✓ PK/PD model based analysis of viral dynamics and

251 to define dosing regimen for EEEV.

their modulation with the antiviral activity of BDGR-



# Molecubes MicroCT

Trainings: 3 online (1/18/23, 5/22/23, and 10/12/23) and 2 in person, 2022, 4/5/24









Manual Ma

Amber M. Smith, Ph.D. Principal Investigator Associate Professor in the Department of Pediatrics & Institute for the Study of Host-Pathogen Systems at the University of Tennessee Health Science Center





Using the VEVO ECHO to probe the function of the heart during infection

Dr. Ravi, Department of Surgery, University of Connecticut Health, Farmington, CT, USA







Vero E6 cell lines are incubated with experimental compounds and introduced to SARS-CoV-2 Propagation results in virus mediated cell death

Active compounds inhibit viral induced cell death without compromised cell viability Surviving cells can be quantified via luminescence Hits are reported as % activity compared to no virus (cells) and cells plus virus

SARS Coronavirus - Severson et al. 2007. J. Biomol. Screening 12:33-4 Influenza Virus - Severson et al 2008 J. Biomol. Screening West Nile Virus- Chung et al. Molecules. 2010 3: 1690–1704 Respiratory Syncytial Virus -Chung et al 2013 Virol J **10**, 19 Venezuelan Equine Encephalitis Virus-Chung et al PLoS Pathog. 2014 10: e1004213

Average infection rate:

> 92.72 % Control

73.37 % 0.25 μM

50.5 % 0.5 μM

9.65 % 0.25 μM













Olympus APX Benchtop Fluorescence Microscope (Live or Fixed)



# Thank you!

# **RBL** Funding

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