PI: **HARTY, RONALD N**	Title: Development of Small Molecule Therapeutics Targeting Hemorrhagic Fever Viruses	
Received: 09/08/2020	Opportunity: PA-20-265	Council: 01/2021
Competition ID: FORMS-F	FOA Title: PHS 2020-2 Omnibus Solicitation of the NIH for Small Business Technology Transfer Grant Applications (Parent STTR [R41/R42] Clinical Trial Not Allowed)	
2R42AI138630-03	Dual:	Accession Number: 4489979
IPF: 10020652	Organization: FOX CHASE CHEMICAL DIVERSITY CENTER, INC	
Former Number: 3R41AI138630-02	Department: Pathobiology and Microbiology	
IRG/SRG: ZRG1 BCMB-G (10)B	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 3: [redacted] Year 4: [redacted] Year 5: [redacted]	Animals: Y Humans: Y Clinical Trial: N Current HS Code: 30 HESC: N HFT: N	New Investigator: Early Stage Investigator:
Senior/Key Personnel:	*Organization:*	*Role Category:*
Ronald Harty Ph.D	University of Pennsylvania	PD/PI
Jay Wrobel Ph.D	Fox Chase Chemical Diversity Center, Inc.	Co-Investigator
Olena Shtanko	Texas Biomedical Research Institute	Co-Investigator
John Kulp Ph.D	Fox Chase Chemical Diversity Center, Inc. Department: Divisi	Co-Investigator
Allen Reitz Ph.D	Fox Chase Chemical Diversity Center	Co-Investigator

OMB Number: 4040-0010
Expiration Date: 12/31/2022

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	**State Application Identifier**

1. TYPE OF SUBMISSION*

❍ Pre-application ❍ Application ● Changed/Corrected Application

4.a. Federal Identifier
R41AI138630

b. Agency Routing Number

2. DATE SUBMITTED | **Application Identifier**

c. Previous Grants.gov Tracking Number
GRANT13199627

5. APPLICANT INFORMATION **Organizational DUNS*:** [redacted]

Legal Name*: Fox Chase Chemical Diversity Center Inc
Department:
Division:
Street1*: Pennsylvania Biotechnology Center
Street2: [redacted]
City*: Doylestown
County: Bucks
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 18902-8400

Person to be contacted on matters involving this application
Prefix: First Name*: Allen Middle Name: Last Name*: Reitz Suffix: Ph.D
Position/Title: Chief Executive Officer
Street1*: Pennsylvania Biotechnology Center
Street2: [redacted]
City*: Doylestown
County: Bucks
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 18902-8400
Phone Number*: [redacted] Fax Number: [redacted] Email: [redacted]

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** [redacted]

7. TYPE OF APPLICANT* R: Small Business

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

❍ New ❍ Resubmission
● Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍ Yes ● No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Development of Small Molecule Therapeutics Targeting Hemorrhagic Fever Viruses

12. PROPOSED PROJECT
Start Date* Ending Date*
04/01/2021 03/31/2024

13. CONGRESSIONAL DISTRICTS OF APPLICANT
PA-001

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Ronald Middle Name: N. Last Name*: Harty Suffix: Ph.D

Position/Title: Professor
Organization Name*: University of Pennsylvania
Department: Pathobiology and Microbiology
Division: School of Veterinary Medicine
Street1*: [redacted]
Street2: [redacted]
City*: Philadelphia
County:
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 19104-0000
Phone Number*: [redacted] Fax Number: [redacted] Email*: [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$[redacted]
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$[redacted]
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ❍ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

❍ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Kathleen Middle Name: Last Name*: Czupich Suffix:

Position/Title*: Chief Financial Officer
Organization Name*: Fox Chase Chemical Diversity Center, Inc.
Department:
Division:
Street1*: [redacted]
Street2: [redacted]
City*: Doylestown
County: Bucks
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 18902-8400
Phone Number*: [redacted] Fax Number: [redacted] Email*: [redacted]

Signature of Authorized Representative*

[redacted]

Date Signed*

09/08/2020

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:

424 R&R and PHS-398 Specific
Table Of Contents

OMB Number: 4040-0010
Expiration Date: 12/31/2022

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Fox Chase Chemical Diversity Center, Inc.
Duns Number: [redacted]
Street1*: Pennsylvania Biotechnology Center
Street2: [redacted]
City*: Doylestown
County: Bucks
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: PA-001

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: University of Pennsylvania
DUNS Number: [redacted]
Street1*: [redacted]
Street2:
City*: Philadelphia
County:
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 19104-6205
Project/Performance Site Congressional District*: PA-002

Funding Opportunity Number: PA-20-265. Received Date: 2020-09-08T12:43:45.000-04:00

Project/Performance Site Location 2

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	Texas Biomedical Research Institute
DUNS Number:	[redacted]
Street1*:	8715 W. Military Dr.
Street2:	
City*	San Antonio
County:	
State*:	TX: Texas
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	78227-5302
Project/Performance Site Congressional District*:	TX-020

Additional Location(s) File Name:

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ● **Yes** ❍ **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ● No

If YES, check appropriate exemption number: __ 1 __ 2 __ 3 __ 4 __ 5 __ 6 __ 7 ___ 8

If NO, is the IRB review Pending? ● **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number none

2. Are Vertebrate Animals Used?* ● **Yes** ❍ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number none

3. Is proprietary/privileged information included in the application?* ● **Yes** ❍ **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Summary_Harty_Final.pdf
8. Project Narrative*	Narrative_Harty_Final.pdf
9. Bibliography & References Cited	References-Harty_Final_2.pdf
10.Facilities & Other Resources	Facilities_FCCDC.9_combined.pdf
11.Equipment	Equipment_FCCDC.2_combined.pdf

Funding Opportunity Number: PA-20-265. Received Date: 2020-09-08T12:43:45.000-04:00

Summary: The ultimate goal of this Phase II application is to develop novel small molecule, broad-spectrum therapeutics against viral infections caused by filoviruses, arenaviruses, and other viruses that depend on the PPxY L-domain motif for egress and spread of infection. Some of these viruses, including Ebola (EBOV), Marburg (MARV), and Lassa fever (LAFV) viruses, are highly pathogenic and classified as Category A bioterror pathogens. We and others have determined that efficient budding of these emerging human pathogens depends on the subversion of host proteins, such as neural precursor cell expressed developmentally down-regulated protein 4 (Nedd4), by PPxY L-domains in the matrix proteins of these RNA viruses. The identification and development of small molecule inhibitors that interfere with these virus-host interactions should effectively block virus egress, disease progression, and transmission. In these efforts we have discovered several chemical series of small molecule inhibitors of the host Nedd4/virus PPxY complex important for viral egress which led to one analog possessing proof of concept in vivo activity in a Marburg virus challenged mouse model. As FDA approved therapeutic agents for the treatment of these most of these viral infections are not available, our identification of virus-host inhibitors that may prevent virus spread will fill a significant unmet need. Moreover, these inhibitors will be broad-spectrum, and therefore will likely be effective against newly emerging viruses as well as viral variants. As described below, we will use a rigorous multifaceted approach to identify, develop, and validate PPxY budding inhibitors identified in Phase I as potent, broad-spectrum antivirals. The goal of this Phase II STTR grant application is to optimize our lead inhibitors of VP40 PPxY-Nedd4 interactions to generate full-fledged predevelopment drug candidates ready for IND directed studies. This will be accomplished by combining the pharmaceutical and medicinal chemistry expertise of the scientists at the Fox Chase Chemical Diversity Center, Inc. (FCCDC) with the expertise and experience in the experimental aspects of antiviral therapy of the Harty Lab at the University of Pennsylvania. We will realize this goal by optimizing our existing series of inhibitors, exemplified by in vivo active FC-10696, for improved potency and oral drug properties (Aim 1), evaluating new compounds based on two potent series for their ability to specifically inhibit PPxY-Nedd4 interactions and subsequent VLP and surrogate virus egress (Aim 2), identifying compounds having suitable drug properties and selectivity using *in vitro* and *in vivo* ADMET evaluation (Aim 3), and evaluating compounds for their antiviral efficacy against authentic BSL-4 viruses *in vitro* and *in vivo* (Aim 4).

Narrative:

There is an urgent need to develop antiviral therapy against emerging human RNA viruses that represent potential agents of bioterrorism (Ebola, Marburg, and Lassa fever). We have discovered small molecule compounds that disrupt budding and spread of live viruses; a process that is critical for virus dissemination and disease progression. Here, the Harty group at the University of Pennsylvania, experts in the antiviral technology of this proposal, have partnered with the small business Fox Chase Chemical Diversity Center, Inc. to further develop these broad spectrum antiviral budding inhibitors by using medicinal chemistry, live virus budding assays, and live cell imaging techniques.

Resources – Laboratories of Fox Chase Chemical Diversity Center, Inc.

Fox Chase Chemical Diversity Center, Inc. (FCCDC) is located in a 112,000 ft^2 facility known as the Pennsylvania Biotechnology Center (PA Biotech Center) at [redacted] Doylestown, PA. The Center is a recently renovated, $14 million, non-profit business incubator, funded in part by a grant from the Commonwealth of Pennsylvania, whose mission is to nurture entrepreneurship and biotechnology in the region. It is home to ~30 biotechnology companies, and the non-profit Blumberg Institute. The FCCDC has 1,600 ft^2 of laboratory space within the facility including six chemistry fume hoods and one BSL2 incubator, and 2,400 ft^2 of office space. FCCDC also conducts laboratory operations at a second nearby facility at 3700 Horizon Dr., King of Prussia, PA, the former Bachem site, where it leases 6,600 ft^2 of combined lab and office space including 11 chemistry fume hoods.

Offices. The main FCCDC offices are located within the PA Biotech Center. They are on the same floor as the laboratories and with dedicated administrative assistance available within a reception area. The offices are equipped with a computer, conferencing-capable phone system to facilitate collaborative discussions, and locking file cabinets for storage of confidential study information. The office suite includes two conferences rooms, one of which is set-up for computer based presentations. FCCDC staff are fully supported by an associated administrative infrastructure (copy machines, fax, reception, video conferencing, etc.) A computer network connected by T1 lines for scientific writing/editing, data analysis, and graphics/illustration is available at the PA Biotech Center.

FCCDC laboratories have the following equipment that will be specifically used for supporting the work described in this grant application: two 300-MHz ^{1}H NMRs with multinuclear probe capability (^{13}C, ^{31}P, ^{18}F), four LC/MSs including three Micromass ZQ mass spectrometers with Waters 2695 HPLC with diode array detectors, multiple ISCO and Biotage personal chromatography purification equipment, multiple Gilson 215 semi-preparative HPLC systems, Biotage Initiator microwave, two Parr hydrogenators, 18 rotary evaporators, Genevac EZ2 Evaporation System, Mettler microbalances, JChem temperature controllers, Innova platform shaker, miscellaneous smaller glassware, PCs, printers, and related equipment, BSL2 incubators for cell culture, laminar flow biosafety cabinets, multiple pipettors, common refridgerators and freezers.

Chemical compound information is stored within an MS Excel workbook and is accessible via Instant JChem which is also used to calculate biophysical properties such as LogP/D and tPSA (www.chemaxon.com). FCCDC has a companylicense for the structure and literature searching tool SciFinder-n, and free on-site access via the Pennsylvania Drug Discovery Institute to >30,000 reagents and starting materials (including >800 boronic acids) that are binned in functionality appropriate trays and can be searched electronically.

Records Retention. All FCCDC final compound samples are stored in a locked repository. All laboratory notebooks are signed daily and witnessed on a monthly basis. We observe standard industry practices in the area of confidentiality. Laboratory notebooks are kept under lock and key in the evenings and weekends. NMR data is stored on a primary server which is backed up to a second server. We have a license to ACD software, allowing us to print out coupling constants, multiplicities and chemical shifts of NMR spectra quickly, which is very useful when writing patent applications or manuscripts for publication.

Collaborative Scientific Environment. Fox Chase Chemical Diversity Center, Inc. holds regular and periodicgeneral research review and specific project review meetings. Project meetings would be held with the relevant research staff at our academic partner either in person, by go-to-meeting web review, or by phone. The PA Biotech Center and the non-profit Pennsylvania Drug Discovery Institute hosts scientific seminars on approximately a monthly basis, including eminent researchers in the area of drug discovery. Speakers have included Bill Greenlee, Magid Abou-Gharbia, Donna Huryn, and Dan Skronovsky.

Facilities and Resources

Harty, Ronald N.

Laboratory: 1,008 sq. ft. of BSL-2 laboratory space including a tissue culture area. The laboratory can accommodate up to 7 people and is equipped with one 6-ft and one 4-ft biohazard hoods for cell culture and virus experiments, three tissue culture incubators, two ultra low freezers, two 37° C floor shaker/incubators, two Beckman L90 ultracentrifuges, two Beckman Avanti J-E centrifuge, and several refrigerators/freezers. An additional shared equipment suite and darkroom are available on the floor.

Animal: N/A

Clinical: N/A

Computer: I have a Mac Desktop, a MacBook Pro laptop, and an HP Laserjet M1212 laser printer/scanner/fax in my office. The laboratory is equipped with two Dell Optiplex GX260 systems and a Mac Desktop with all necessary software along with two laser printer/scanner/fax units. All computers have internet and email access.

Office: I have a 92 sq. ft. office within my research lab.

Other: Secretarial services are provided by the Department of Pathobiology. Building facilities, information technology (IT), and glassware services are located in the building, along with a meeting/conference room.

Scientific Environment: The laboratory is within the School of Veterinary Medicine at the University of Pennsylvania. There are a plethora of active virology, immunology, molecular biology, biochemistry and parasitology laboratories within the School of Veterinary Medicine and University wide with an abundance of both pre- and postdoctoral trainees.

Biohazards: The work in my laboratory involves studies approved for BSL-2 conditions.

Penn Vet Imaging Facility**:** The Veterinary School Core Imaging Facility houses a two-photon/confocal (4 laser) spectral imaging Leica SP5 system on a DM6000 microscope with tandem scanner, five internal spectral detectors, and four external non-descanned detectors and motorized XY stage. This system is optimized for multicolor intravital imaging. The core also has a Yokagawa CSU X1 spinning disk confocal head on a Leica DMI4000 microscope and an LMM5 dual laser excitation source, a Hamamatsu 512 EM camera, and an ASI motorized and computer controlled XYZ stage. This instrument is housed within an environmental (temperature and CO2 controlled) chamber and is ideal for long term imaging of live cells. We just acquired a new Leica SP5 spectral imaging microscope on an inverted stand (DMI6000) that is equipped with 3 continuous wave lasers, 3 pulsed lasers and a Leica/Picoquant Fluorescence Lifetime Imaging (FLIM) system. This instrument is optimized for live cell imaging of cells and specifically for real time measurements of protein-protein interactions. This instrument is the only one of its kind at the University of Pennsylvania and is ideally configured for the FLIM measurement of virus-host interactions. The core also has several software packages for image analysis including Metamorph from Molecular Devices, Volocity from Improvision, and the LAS-AF suite from Leica.

FACILITIES AND OTHER RESOURCES

Texas Biomedical Research Institute

ENVIRONMENT: Texas Biomedical Research Institute (Texas Biomed) was founded as a private, not-for-profit research institution in 1941 by Thomas B. Slick Jr., with a goal to unravel the mysteries of disease through innovative thinking, creative problem solving, and cutting edge technologies. Located on a 200-acre campus on the northwest side of San Antonio, Texas, our scientists work each and every day to improve human health by being a world leader in infectious disease research. Texas Biomed partners with hundreds of researchers and institutions, targeting advances in the fight against infectious diseases impacting our communities around the world, including AIDS, TB, hepatitis, malaria, hemorrhagic fever viruses, parasitic infections and many other infectious diseases. We also study co-morbidities of infection and vulnerable populations, such as the very young and the elderly and those suffering from cardiovascular diseases and metabolic disorders.

Texas Biomed is staffed by more than 350 employees including a multidisciplinary team of approximately 65+ doctoral-level scientists – physiologists, geneticists, virologists, immunologists, molecular biologists, cell biologists, physicians and veterinarians – who lead more than 200 major research projects. Texas Biomed also supports 150 technical staff and an administrative support staff of more than 100 professionals.

Texas Biomed recently underwent a strategic planning process and defined its focus as an institute that studies infectious diseases and conditions that make an individual susceptible to infectious diseases (aging, metabolic syndrome, etc.). The Institute developed three new programmatic areas: Host-Pathogen Interactions is the study of the basic biology of infection in humans and animals – the immediate intersection of host and pathogen. Disease Intervention & Prevention is the development of diagnostics, treatments and vaccines to reduce the severity of/or cure infection. Population Health is the identification of correlates of disease susceptibility or resistance to infectious diseases on a population level. Each program has two faculty leads who focus on the academic development of the programs and their themes. Administrative responsibilities are supported through the Office of the Vice President for Research, unencumbering scientists to pursue the academic mission of Texas Biomed.

Texas Biomed supports a culture of learning and development. The Institute holds a weekly seminar that hosts an external guest approximately monthly, and internal presentations from faculty and trainees at all other times. Attendance at seminar ranges between 50 and 80 faculty and research staff. Administrative staff are also frequently in attendance to learn about the latest research at Texas Biomed. Texas Biomed supports a monthly journal club that rotates across the three programmatic areas that typically has an attendance of 20-40 researchers. Individual laboratories also hold their own journal clubs, frequently shared across multiple scientific groups. The Vice President for Research runs a monthly training course for Staff Scientists on how to manage a laboratory, and a monthly training course for a subset of Staff Scientists on how to write a grant. Staff Scientists at Texas Biomed are the equivalent of Research Faculty. Human Resources provide training through a Lunch & Learn lecture series and individualized training programs on management and leadership. Communication at Texas Biomed is facilitated by a monthly institute-wide newsletter and video blogs.

Texas Biomed is part of a city wide, 4-institute collaborative (with University of Texas Health Science Center, University of Texas San Antonio, and Southwest Research Institute) that works synergistically to increase the biomedical reputation of the city of San Antonio. Initiatives include the San Antonio Vaccine Development Center and the Precision Therapeutics Program, which are supported by and foster collaboration across the four institutions. Administrative collaboration includes negotiated rates for use of each institutions cores, participation in graduate and training programs, and input on faculty search committees including consideration for dual hire appointments.

RESEARCH FACILITIES: Our faculty and staff scientists conduct their research amid 550,000 square feet (sq. ft.) of space that includes laboratories and core facilities, offices, auditorium, animal hospital, library, and animal facilities, including a six-acre corral. Research laboratories are spaced across three main buildings for a total of 55,361 sq. ft. of space.

Moorman (building 35; 31,954 sq. ft.; where Shtanko lab is located): The Moorman building has 12 laboratories totaling 9,459 sq. ft. for *in vitro* BSL2 studies. Each lab has a dedicated technician office. Faculty offices (approx. 300 sq. ft.) are peripheral to the laboratories and directly adjacent to offices and cubicles for staff and students. The Moorman can support between 6-12 faculty, depending on space requirements. The Moorman also supports approximately 1,200 square feet of select agent BSL3 laboratory, and a 2,100 sq. ft. of ABSL4 suite laboratory (1,200 operational, remaining space is mechanical and annex space).

Dr. Shtanko has 800 sq. ft. of lab space which is outfitted with common equipment necessary for studies in cell biology, molecular biology, and immunology. She also has private office space located in the same building within 80 feet of the lab.

Earl Slick (building 71; 59,556 square feet): The Earl Slick building has 14 laboratories totaling 12,298 sq. ft. for *in vitro* BSL2 studies, 1,099 square feet for BSL2+ studies (for HIV), 1,840 sq. ft. of BSL3 prep space, and 10,500 sq. ft. of office space. Faculty offices (approx. 250 sq. ft.) and offices for support staff and trainees are in close proximity to associated laboratory space. Earl Slick can support between 7-14 faculty, depending on space requirements. Laboratories for individual faculty are outfitted with common equipment necessary for studies in cell biology, molecular biology, and immunology.

There is a dedicated blood draw room immediately next to the BSL2 lab space, to ensure privacy during phlebotomy procedures. This room is equipped with a refrigerator for juice boxes for donors and a phlebotomy chair with upholstered arms and foot rest, and can recline to the Trendelenburg position. Only trained individuals will serve as phlebotomists.

Urschel-Slick (building 12; 57,875 sq. ft.): The Urschel-Slick building has 16 laboratories totaling 11,995 sq. ft. for BSL2 studies, 7,500 sq. ft. for BSL3 studies, including 1,840 sq. ft. of BSL3 prep space, and 10,500 sq. ft. of office space. Faculty offices (approx. 250 sq. ft.) and offices for support staff and trainees are in close proximity to associated laboratory space. Urschel-Slick can support between 8-16 faculty, depending on space requirements. Urschel-Slick is the location of a recently renovated 7,500 sq. ft. ABSL3 and rodent vivarium.

Ledford (building 38; located 4,876 sq. ft.): The Ledford building is a free-standing building with 2,579 sq. ft. of laboratory space and 1,305 sq. ft. of adjacent office space. The Ledford building houses the institute Central Core Facility and is no more than 3 minutes walking distance from any research space at Texas Biomed. The building is keycard access restricted and monitored. Core staff are located within the building to provide support for any instrument. The Ledford building has a darkroom for individual light sensitive equipment.

UT Health San Antonio (10 min drive) has the following cores available to the PI: Mass Spectrometry, Bioanalytics & Single cell, Biobanking & Genome Analysis, Biomolecular NMR, Flow Cytometry, Macromolecular interactions, Microt CT, Electron Microscopy, Optical Imaging, and X-ray Crystallography (see http://research.uthscsa.edu/RCL/cores.shtml). For detailed UT Health San Antonio Core Resources, see: http://research.uthscsa.edu/RCL/Resourcepages.shtml

CLINICAL: Human subject studies are reviewed by UT Health San Antonio Institutional Review Board through a joinder agreement. UT Health is located approximately a 15-minute drive from Texas Biomed and is affiliated with University Hospital, Audie L. Murphy VA Hospital, Robert B. Green Campus, and the County Public Health Hospital.

ANIMAL: Texas Biomed has an independent Institutional Animal Care and Use Program which is responsible for monitoring vertebrate animal research to be compliant with USDA and AAALAC standards, and adheres to the Animal Welfare Act, Public Health Service Policy on Humane Care and Use of Laboratory Animals, and the *Guide for the Care and Use of Laboratory Animals*. Animal Welfare Assurance #A308201. Texas Biomed has been accredited by AAALAC continuously since 1973 (AAALAC File #000246).

A dedicated rodent vivarium is located in the Slick-Urschel building with current capacity to house rodents on ventilated racks or in static caging. Space is available for expansion and to accommodate additional species. The vivarium is supported by a dedicated supervisor and staff, and veterinary care. The vivarium staff also support rodent studies at ABSL3.

COMPUTER: In addition to personal computers, there are 57 desktop and 31 laptop computers in addition to printers and fax machines available within the Moorman and adjacent buildings, and the Library to be used for instrument operation, data management, word processing, graphics and manuscript preparation. These computers are connected to the central network and are protected by individual UPS devices. There is open access through the Internet to library resources at Texas Biomed.

Scientists can use the institutional accounts of the Slack discussion platform and the Dropbox file share service to increase communication and data sharing internally and externally with collaborators. On servers, scientists have access to a wide variety of software and programming and scripting languages such as FORTRAN, C, C++, Java, Tcl, Perl, Python, Ruby, bash, R, etc. Statistical and mathematical packages available include GraphPad, Maple, S-PLUS, and R. Software available for genetic analysis includes PAP, S.A.G.E., MENDEL, FISHER, LINKAGE, CRI-MAP, PREST, Merlin, GeneHunter, SimWalk2, Loki, PLINK, GATK, vcftools, Ingenuity Pathway Analysis, kinship2, and SOLAR. Specific software or packages can be installed on demand.

A dedicated staff of 9 persons led by the Vice President for Information Technology maintains and updates current systems and assists scientists in specific requests (installation of software, libraries and packages, help in debugging failed compilation). A ticket system is in place to efficiently address issues. The technical staff has expertise in database management in both SQL-based software and the PEDSYS database system, developed in-house for management and analysis of genetic, demographic, epidemiological and laboratory data.

Data and System Security

We have implemented extensive security measures to protect our computers and data files against unauthorized access. Our security measures are compliant with IRB requirements for human data. Our internet connection is a 100 Mb/s Ethernet connection, using a leased router managed by our internet provider, and protected by our Palo Alto Networks next generation security appliances, in a redundant configuration.

Users connect to the system over the network using either SSH/SFTP or VPN connections. Access to network is removed on or before the day that an individual's employment is terminated, unless prior arrangements have been made. Access logs are checked periodically for repeated login failures which might suggest an attempt to break into our system. Countermeasures are taken if evidence of such an attempt is found. Access to central files containing research, clinical, support, or administrative data is restricted to a list of approved users. Separate lists are maintained for each research or administrative unit (laboratory, project, etc.).

Disk-based daily incremental and monthly full backups of software and data are performed using the Veritas NetBackup system. Long-term data archiving is provided by a single six drives, 113-slot 800 GB tape library, and four 25-slot 400 GB tape libraries.

REDCap (Research Electronic Data Capture) is available for sharing and managing data across computer platforms and between research institutes. REDCap is a secure, web-based application for building and managing online surveys and databases. REDCap provides automated export procedures for seamless data downloads to Excel and common statistical packages (SPSS, SAS, Stata, R), as well as a built-in project calendar, a scheduling module, ad hoc reporting tools, and advanced features, such as branching logic, file uploading, and calculated fields.

BIOSAFETY:

ABSL4 laboratory

Texas Biomed is home to the only operational ABSL4 lab owned by a private institution. The ABSL4 lab supports basic research as well as vaccine and therapeutics testing of agents with no cure. This unique resource has supported the research of faculty in Texas Biomed in emerging diseases and bioterror agents including the hemorrhagic fever viruses Ebola virus and Marburg virus.

This full-suit lab is CDC and USDA certified for work on human and animal pathogens. Access to the ABSL4 area is secured via three successive magnetic card/keypad controlled airlock passages: The first is located at the entry foyer to the BSL3 suite, the second at the entry foyer to the outer changer room, and the third at the entry foyer to the ABSL4 area. The ABSL4 area is equipped with an entry and exit decontamination airlock and an emergency exit/decontamination chamber. The latter also serves as an equipment decontamination

chamber to allow for the repair, removal and/or replacement of faulty equipment without requiring a complete laboratory decontamination process. All supply and exhaust air for the ABSL4 passes through dual (and tandem) HEPA filters.

Critical parameters (air supply and exhaust flow rates, pressure differentials, normal and emergency breathing air, decontamination systems, etc.) are continuously monitored. Personnel within the lab communicate, and are actively monitored by outside personnel, through a scrambled UHF communication system worn inside the laboratory suit. The 500 sq. ft. ABSL4 laboratory staging area includes an air locked suit change room, two inner change rooms with pass-through showers, and an outer “clean” change room equipped with sinks, lockers, toilets, etc. Critical mechanical support devices are located in an attached 3,000 sq. ft. mechanical support complex. All critical equipment and devices (valves, etc.) are redundant, including duplicate air-supply and exhaust systems, decontamination shower systems, as well as the compressors, pumps, valves, heater cores, etc. that comprise the liquid waste decontamination system. All critical mechanical devices operate on a system with automatic emergency electricity backup generators. The ABSL4 area (including mechanical support rooms) is equipped with an elaborate intrusion detection system that includes electronic door and roof hatch sensors, motion detectors, etc.

The laboratory contains 3 x class IIB biological safety hoods, 4 x water-jacketed CO2 incubators, low-, high- and ultra-speed centrifuges equipped with both analytical and preparative scale rotors, a microfuge, 2 x 4°C refrigerators, 2 x liquid nitrogen storage systems, and 3 x -80°C freezers. The ABSL4 also has an optical ELISA plate reader, MAGPIX multiplexing 96-well format unit, luminometer, Innova 4000 environmental shaker, microplate shaker, BioFlo III fermenters with chillers, Evos fluorescent microscope, pass-through autoclave, dunk tank, facsimile machine and networked computers.

Texas Biomed maintains an experienced and trained staff of scientists, veterinarians, research technicians and veterinary technicians available to perform studies at high biocontainment. These individuals have demonstrated proficiency at conducting *in vitro* and animal studies with the agent identified in the proposal. The ABSL4 Operations and Safety Manuals specify policies, procedures, and standard operating procedures (SOPs) for the safe handling of biological materials in biosafety laboratories. The policies, procedures, and SOPs comply with applicable Federal, State, and municipal regulations and with the guidelines “Biosafety in Microbiological and Biomedical Laboratories” issued by the CDC and the National Institute of Health (NIH). Employees are trained from these manuals on each facilities mechanical systems, biosafety, biocontainment and security. Employees are also trained according to project specific and SOPs. These procedures apply to all that use, generate, store, or dispose of potentially infectious materials in Texas Biomed biosafety laboratories and to persons who must enter these laboratories to perform services.

Select agent use, transfer or possession is forbidden without the permission of the Responsible Official (RO) and until the required forms filed and written approval received from the CDC Select Agent Program. Upon approval, ABSL4 investigators desiring to work on a BSL4 project must also submit an application to the Biohazard and Safety Committee for approval. The Committee is responsible for evaluating the facility, equipment, and staff capabilities to perform work in a safe manner.

Infectious cultures and inventory stocks are stored inside the ABSL4 laboratory in locked refrigerators and freezers. An electronic Inventory (eInventory) system is used to document usage of infectious stocks. All infectious material stored in refrigerators or freezers is properly labeled and stored in containers capable of withstanding thermal shock of freezing and thawing. Each container is labeled with the identity of the infectious agent, the date of the preparation, and a barcode that links the material to the more inclusive information contained in the inventory database.

When work is completed, all infectious cultures are removed from workbenches and cabinets and stored in a designated refrigerator or freezer. Materials to be discarded are placed in a sealable container filled with a suitable disinfectant. The container is placed in a discard pan containing the disinfectant. Discard pans are placed on a cart and transported to the autoclave. Labware containing infectious liquids are stored and transported in leak-proof containers large enough to contain the fluid in case of leakage.

Biocontainment Program

Texas Biomed has established an institution-wide comprehensive BSL3/BSL4 and CDC certified Select Agent BSL3 facilities program. The development of a centralized program for the campus ensures that 1) the growth and development of BSL4/BSL3/BSL2 facilities will remain well-coordinated both scientifically and administratively at all levels of the program; 2) spaces will be developed in the most effective manner based on an accurate assessment of need and cost; 3) technical support will be coordinated among the facilities; 4) training will be optimized, including sending key personnel to important national meetings for training and education on an ongoing basis, a critical need considering the rapidly changing landscape regarding security requirements and controls; and 5) assurance that broad expertise on campus is represented in BSL4/3/2 discussions regarding a) work with bacteria, viruses, toxins, etc; b) entry criteria for users, including the CDC/FBI program; c) exposure protocols for users; d) proper use and maintenance of animals; e) user issues and concerns; and f) infrastructure issues and concerns.

Institutional BSL3/4 compliance oversight is provided by Environmental Health and Safety (EHS). EHS provides the following services for the entire institute operation: radiation safety, environmental compliance, occupational health and safety, research safety and biosafety. Texas Biomed has a program director for the BSL3/BSL4/Biosecurity program, a Responsible Official and alternate for the Select Agent Program and a Biohazardous and Safety Committee (BSC).

Complementing and interacting with the regulatory and compliance arm of the BSL3/BSL4/Biosecurity activities on campus is the centralized research oversight structure. The Biocontainment Program is managed by a BSL3 Director, BSL4 Director, and two biocontainment program coordinators. These staff work closely with the Director of Maximum Containment Contract research. Program operations include an institute-wide Biosafety-3/4 Advisory Committee (BAC) that consists of the EHS Director, BSL3 Director, BSL4 Director/IBC Chair, and coordinators. The VP for Research/RO attends the meeting monthly. The charge of the BAC is to develop and review institution BSL3/4 rules and regulations, develop and refine BSL3/4 user entry requirements for certification, evaluate the use and efficiency of campus BSL3/4 facilities, develop and refine the BSL3/4 medical surveillance program, handle user issues as they arise, and provide oversight for the operation of the facilities. A complete Texas Biomed BSL3/Select agent/BSL4 education and entry program for users is in place. Texas Biomed users meet as a group (BUG) with leadership on a monthly basis.

Access to BSL3/4 facilities is granted only when personnel receive thorough biosafety and biosecurity training and proper on-site training. Training materials are reviewed by the Responsible Officer (RO) and/or Alternative Responsible Officer (ARO). Biosafety and biosecurity training emphasizes the facility design and system in place as well as the current rules and regulations users must follow. Refresher training is provided on an annual basis and time sensitive issues are discussed in the monthly BSL3/4 user group (BUG) meeting. On-site training is focused on demonstrating proper application of personal protective equipment (PPE), facility features, and proper usage of equipment. Junior scientists must be accompanied and supervised by senior scientists who are experienced in BSL3/4-related techniques to ensure proper handling of infectious materials. The BSL3/4 program at Texas Biomed ensures that all BSL3/4 users are fully trained and supported by operational staff, relieving some of the burden of training and oversight from the PI.

Select Agent Research

Texas Biomed is a select agent registered entity with Health and Human Services, Centers for Disease Control and Prevention and U.S. Department of Agriculture, Animal Plant Health Inspection Service, National Select Agent Program. The Institute has been inspected by the CDC National Select Agent Program for use of HHS Select Agents and Toxins, Overlap Select Agents and Toxins and USDA Select Agents and Toxins. The registration number is C20171120 1976.

OTHER INSTITUTIONAL RESOURCES:

Biology Core instrumentation and services

The Biology Core, housed in the Central Core facility, is operated by a Director and two PhD level Managers.

Microscopy: The Biology Core operates a Zeiss 3-Channel Confocal System Microscope LLC LSM800 and a Nikon C2+ Confocal Module microscope. Microscopes are each located within 2 separate buildings (Earl Slick and Moorman) to be in close proximity to the most frequent users.

Microscopy in Moorman building is performed using a Nikon Ti Eclipse microscope housed in a separate, dedicated microscopy room. The Nikon Ti Eclipse microscope is necessary for high resolution and high-speed image acquisition of cells and fluorescently labeled virus-like particles. It is fitted for both high speed widefield image capture as well as confocal capabilities using the Nikon C2+ scan head with high sensitivity photomultipliers. The microscope has automated image acquisition ability with electronic XYZ piezo stage and Nikon elements software suitable for high content screening of drug libraries, siRNA and expression plasmids. Objective lenses are of the highest quality including oil immersion 40x NA 1.30 lens, 100x oil immersion lambda NA 1.45 lens as well as conventional air 4x, 10x, 40x, and 60x lenses. The scope is fitted with a heated, CO_2 fed, incubator stage for live cell work. The camera for widefield imaging is an ANDOR iXon3 EMCCD camera, which provides rapid, highly sensitive acquisition of 14-bit and 16-bit images with high linear sensitivity. Software is a key part of quantitative image analysis. AutoQuant X3 software provides image deconvolution and interfaces with Imaris (Bitplane) to identify and measure image features, including measuring the distance between features in 3D. CellProfiler software is used to count and measure object intensity in multiple images and interfaces with FCSExpress to perform image cytometry, which allows gating of cell populations like FACS. The latter allows analysis of cell features after gating which is an advantage. ImageJ processing software is also available.

BD FACSymphony: Available since September of 2019, the 5-laser BD FACSymphony cytometer is capable of 28 color acquisition, and it is equipped with a High Throughput Sampler. This advanced instrument features an ultra-quiet VPX electronics system that supports up to 50 high-performance photomultiplier tubes (PMTs) and improves detection sensitivity to enable the identification and analysis of rare cell types and events. The version at the Core is equipped with a 355 nm (UV), 405 nm (violet), 488 nm (blue), 561 nm (yellow-green) and 640 nm (red) lasers. When analyzing panels designed with BD Horizon Brilliant dyes, this platform helps to overcome research challenges, such as collecting maximal information from a precious sample, and increases lab throughput with broad phenotyping panels that combine multiple cell-line-specific panels. This highly customizable platform can be configured so that you can select from multiple laser wavelengths and power ratings, and choose the positions of decagon detection arrays to address the requirements of your specific research application.

Beckman Coulter Cyan ADP: The CyAn™ LX Flow Cytometer provides three excitation lines, eleven parameters, complete compensation, and high-speed acquisition, in a bench-top configuration. The 405 nm, 488 nm, and 633 nm lasers are fixed and alignment-free, with spatially separated beams for minimal optical crosstalk. Spatially separated optical apertures, high-performance solid-state detectors, and an obscuration bar minimize unwanted laser radiation. User-interchangeable filters provide experimental flexibility. Eleven acquisition channels (including forward scatter, side scatter, and 9 colors) provide parallel processing of pulse height, pulse length, area, and log for each parameter.

BD Accuri C6 Plus: The 2-laser BD Accuri C6 plus cytometer is capable of 4-color acquisition. The BD Accuri is dedicated to BSL2+ HIV studies. The BD Accuri C6 Plus is equipped with a blue and red laser, two light scatter detectors, and four fluorescence detectors with optical filters optimized for the detection of many popular fluorochromes.

BD FACSAria cell sorter: The 4-laser FACSAria II sorter is capable of 18-color, 4-way sorting and has an automated cell deposition unit for 6, 12, 24, 48 and 96 well sorting. The BD FACSAria™ cell sorter is a fixed-alignment cuvette flow cell that can detect up to 18 colors, for a total of 20 parameters. Cells passing through the cuvette flow cell are excited by four solid state lasers at 407-nm, 488-nm, 561-nm, and 633-nm. Advanced digital electronics improve sort performance and provide significant advantages in instrument ease-of-use. This cell sorter is contained within a Baxter High Containment Biosafety cabinet within one of the BSL3 laboratories.

BDInflux cell sorter: The 5-laser BDInflux cell sorter is capable of 6-way sorting. The BDInflux is primarily dedicated to single cell sorting of malaria parasites and sorting of pluripotent stem cells.

Luminex 100 and 200: The Luminex with xMAP (multi-analyte platform) technology systems measure analytes (cytokines, chemokines, etc.) on a multiplex platform. These instruments are operated with dedicated computers running Luminex Xponent software; analysis of reactivity is done with Masterplex QT software.

Molecular Services Core instrumentation and services

The Molecular Services Core (MSC) is located in the Central Core Facility and is operated by a Director, a full-time manager, and technical support staff. The MSC is dedicated to assist researchers in the development and implementation of state-of-the-art molecular approaches in the support of their research. The MSC has an active Users' Group that periodically reviews available services, technology, and operations. The MSC is equipped with instrumentation to conduct most genomic and gene expression applications, and MSC personnel are trained in numerous technical and bioinformatic approaches.

Equipment: The MSC provides access to an Illumina iSeq 100 and Illumina MiSeq sequencer for low- to mid-output NGS applications. Fully automated sequencing sample preparations of 8-96 samples are supported with a Sciclone G3 NGSx automated liquid handling system. A 10X Genomics Chromium controller and Fluidigm C1 are available for single cell NGS applications. Additional instrumentation includes a Sage PippinHT for automated size selection and PCR purification, an Agilent 2100 Bioanalyzer and 4200 TapeStation for determining RNA, DNA, and protein integrity, and a Nanodrop spectrophotometer and Invitrogen Qubit Fluorometer for DNA, RNA and protein sample quantitation. Multiple additional thermal cyclers are available to support these instruments.

The MSC also provides access to an Illumina iScan with autoloader to allow 24 h service without user intervention for high-throughput genotyping and gene expression analyses (allowing the processing of up to 96 multi-sample BeadChips per day). The iScan System supports rapid, sensitive, and accurate imaging of Illumina's array-based genetic analysis products. A Tecan Freedom Evo liquid handling platforms is also available for automation of all Illumina protocols.

Multiple PCR machines (996- and 384-well blocks), two AB QuanStudio 5 and one QuantStudio 6 Real Time PCR Systems and an AB QuantStudio 3D Digital PCR System are available to support real-time PCR, TaqMan genotyping, gene expression, absolute quantification, and rare allele detection assays. The Core Facility also has a TECAN GENios multimode plate reader with fluorescence, absorbance and glow luminescence modes and a high-performance Tecan plate washer for both 96- and 384-well plates.

Additionally, a BioRad Chemidoc gel documentation system with UV and chemiluminescence capabilities is available in an electrophoresis clean room. Two Beckman ultracentrifuges are available as well.

A dedicated computer server for sequence analysis is available and consists of a Dell PowerEdge R830 server with two Xenon E5-4640 2.1GHz processors (48 cores), 32TB of useable space, and 256 GB RAM. Partek Flow Genomic Analysis Software is supports start-to-finish analysis for next generation sequencing data applications.

Services: Services provided by the MSC facility include RNA and DNA isolation, quality determination, and quantitation from a variety of sample sources including blood, tissues, cells, and body fluids, sequencing library preparations supporting whole genome, transcriptome, exome, smRNA, ChIP, and targeted sequencing applications, onsite low and mid-output next generation sequencing services, and standard sequencing data analysis and bioinformatics for DNA, RNA, and smRNA sequencing.

Pathology

The pathology facility has the following rooms in Building 10: necropsy (288 sq. ft.), hematology (162 sq. ft.), chemistry (160 sq. ft.), tissue processing (162 sq. ft.), slide processing and administration (224 sq. ft.), refrigerator/freezer room (148 sq. ft.), storage room (132 sq. ft.), multi-use area (bacterial plating, automated immunohistochemistry, and additional clinical pathology microscopy station - 288 sq. ft.), four offices for the pathologists and technicians, and a room that houses the stereology microscope and visiting foreign and

American veterinarians and students. It also has a 332 sq. ft. climate controlled slide, block and wet tissue storage room in the quarantine building and a 216 sq. ft. walk in refrigerator located outside and adjacent to the necropsy room.

The necropsy facility is organized to safely handle any necropsy (BSL2) or tissue share project. It has a downdraft stainless steel table, a mobile MOPEC HEPA filtered bone saw, and protective clothing for handling hazardous necropsies. Tissue samples can be collected in formalin, paraformaldehyde, or other fixatives as required by a given research project. Tissues can also be collected in various media, OCT embedded for frozen sectioning, snap frozen, or collected fresh. Perfusion fixation of tissues is also available. A Nikon D200 digital camera is used in the necropsy suite to record lesions. Digital gross and microscopic images are stored on the pathology server and are searchable by either pathology accession number or animal ID. The pathologists also provide on-site necropsy support for nonhuman primate studies in the BSL3 and BSL4 containment areas.

The histology laboratory provides tissue processing, embedding, paraffin and frozen tissue sectioning, routine and special staining, and immunohistochemistry procedures. The laboratory is equipped with a Tissue-Tek processor and embedding center as well as automatic stainers for routine H&E and immunohistochemistry staining. The lab is also equipped with an automated coverslipper, centrifuge, cytospin, cryostat, oven, microscope, dissecting microscope, two microtomes with automatic features, refrigerators, scales, a safe for GLP and animal rule specimens, and other ancillary equipment. A Nikon DXM1200C camera on a Nikon 801 microscope and an Olympus SC100 camera on an Olympus BX50 microscope are used to obtain images from stained slides. The histology laboratory has developed immunohistochemistry procedures for several antigens/pathogens, including filovirus, herpesvirus papio, baboon reovirus, alphavirus, flavivirus, amyloid beta- 42, herpes simplex virus, as well as antibody purification. Available antibodies include cluster of differentiation markers (CD) CD68 (KP1), CD68 (3F103), CD20, CD3, S-100, Ki-67, NeuN, Nurr1 (2 antibodies), HSV1, progesterone receptor, estrogen receptor, Von Willebrand Factor, and TUNEL assay for apoptosis. Other IHC procedures are developed as needed.

EQUIPMENT (all onsite) – Fox Chase Chemical Diversity Center Inc

NMR: Two Varian Mercury Plus 300-MHz Inova NMRs with multinuclear capability (^{1}H, ^{13}C, ^{31}P, ^{18}F).

HPLC/MS:
- Four Micromass ZQ Mass Specs. with Waters 2695 HPLC with 996 diode array detector.

Chromatography:
- Three Gilson 215 semi-prep HPLC systems, multi-wavelength, automated fraction collection.
- Teledyne Isco CombiFlashRf automated chromatography system.
- Three Isco Combiflash Sg 100c personal chromatography systems.

Evaporation:
- Genevac EZ2 Evaporation System.
- FTS Systems Flexi-dry Lyophilyzer.
- 18 Rotavapors with vacuum pumps (Buchi, Heidolph).
- 2 VWR Sheldon 1400E Vacuum Ovens with Edwards oil pumps.

Hydrogenation: Two Parr 3911 Shaker hydrogenators.

Reaction:
- Biotage Initiator microwave synthesizer with 60 position autosampler.
- Innova platform shaker, 2000.
- 4 J-Kem Gemini-2 dual temperature controllers with teflon-coated thermocouples.
- 24Ika Magnetic Stirrer/Hotplates.

BL2 Biological and Tissue Culture Labs:
- Baker SG400 and Labconco A2 Class II biological safety cabinets.
- ABI Prism 7000 Sequence Detection System.
- New Brunswick Environmental incubator shaker and incubator.
- Biotek Synergy 2 Multi-Detection Microplate Reader.
- Molecular Devices SpectraMax 190 microplate reader.
- TitertekMultidrop.
- LabomediVU 1500 Microscope.
- Nikon Eclipse (TE 2000) microscope interfaced with image analysis software
- Fisher and VWR Carbon dioxide incubators.

Computational Chemistry:
- The PA Biotechnology Center has 600Mbs Internet access, more than sufficient.
- Two AMD Threadripper 2950X 16 Core Workstations: Both workstations have 64GB of DDR4 RAM, 1x Nvidia GeForce RTX 2070 GPU, and 1TB Samsung 970 Evo NVMe SSD.
- One GPU server with 2x Intel Xeon Gold 5218 16 core/32 thread 2.3GHz, 96GB of DDR4 RAM, with 5 GPUs (2x Nvidia Titan RTX, 2x Nvidia Titan V, 1x Nvidia 2080Ti), 1x 512GB Samsung 970 PRO NVMe SSD boot drive, and 2x 2TB Samsung 860 EVO SSDsTwo Dell Precision dual-processor Xeon 5680 workstations with Nvidia 470GTX graphics cards;
- Schrodinger's Small Molecule Drug Discovery Suite (all modules);
- GROMACS (molecular dynamics with customized scripts for automation);

Storage, Miscellaneous:
- Two (2) Justrite 45 Gallon Flammable Safety Cabinets.
- Four (4) Refrigerator/Freezers, Frigidaire, GE, Revco.
- SciFinder-n, latest, most powerful search engine for chemistry

Reagents and starting materials collection available onsite: >30,000 members including >1,000 boronic acids, bar-coded, searchable electronic database.

Major Equipment

Harty, Ronald N.

Major equipment in my laboratory and the adjacent equipment suite includes: 2 Beckman L90 ultracentrifuges, 2 Beckman J21 centrifuges with appropriate rotors, DNA and protein electrophoresis systems, 4 heated water baths, 3 CO_2 cell culture incubators, 2 cell culture bio-cabinets, 2 bacterial shaker/incubators, a fume hood, 3 -85°C ultralow and 3 -20°C freezers, 2 refrigerators, a Savant speedvac concentrator, 2 PCR units, 2 rotator platforms, 3 microfuges, a gel dryer, 3 liquid nitrogen tanks, an inverted Olympus microscope, phosphoimager and related software, STORM scanner, Biotek Synergy microplate reader, X-ray film developer and darkroom.

MAJOR EQUIPMENT

Texas Biomedical Research Institute

Apart from the equipment located in Texas Biomed Core Facilities, Dr. Shtanko also has access to the following equipment:

Shtanko Lab

Class IIA 4 feet Biosafety cabinets (1)
Tissue culture CO2 incubators (1)
370 Incubators for bacterial work (1)
Inverted microscopes (1)
Refrigerated microcentrifuge (1)
Microcentrifuge (1)
Water baths (2)
Heat blocks (2)
Rockers (2)
Neon transfection system (1)
Geni syngene gel imaging system (1)
Viaflo Assist Integra system (1)
Impulse Sealer (1)
Dryfast Welch 2014B-01 (1)
Isotemp FisherScientific 205 (1)
Multidrop Combi SMART dispenser (1)
Milli-Q water purification system (1)
Agarose/PAGE gel apparatus and power packs (2)
Countess Invitrogen C10281 (1)
Vortexes (2)
Thermoscientific legend XTR centrifuge (1)
-20°C freezers (1)
-80°C freezers (1)
4°C refrigerators (1)
Liquid nitrogen storage system (1)
Biophotometer Eppendorf 6132 (1)
Uninterrupted power supply (UPS) unit (2)
iBlot 2 gel transfer device (1)
Balances (2)

ABSL4 Facility

Class IIB 6 feet Biosafety cabinets (3)
Tissue culture CO2 incubators (4)
Hydro Peroxide vapor decontamination systems (2)
Beckman ultracentrifuge Optima (1)
Refrigerated Benchtop fuge Eppendorf 5810R (1)
Microcentrifuge (1)
Multidrop Combi SMART dispenser (1)
Milli-Q water purification system (1)
Select agent tracking phase I and II (2)
Select agent geneport system (1)
Water baths (2)
Heat blocks (7)
Rockers (2)
Optical ELISA plate reader (1)
Luminometer microplate (1)
Innova 4000 environmental shaker (1) Piccolo
Evos fluorescent microscope (1)
BioFlo III fermenters with chillers (2)
Xpress Analyzer (2)
Abaxis VetScan VS2 (3)
Ventilated rabbit cage unit (2)
One Cage 2100 ventilated racks and cages (2)
bioBUBBLE containment enclosure (4)
TissueLyser II and accessories Qiagen (2)
Procyte hematology (1)
DSI telemetry system large animal (2)
NanoDrop One Microvolume UV-Vis Spectrophotometer (1)
-80°C freezers (3)
CytoSMART LUX system (2)
4°C refrigerators (2)
Liquid nitrogen storage system (2)
MAGPIX 96-well unit (1)

Moorman Research Building (where Dr. Shtanko lab is located)

Laminar flow hoods (17)
Tissue culture CO2 incubators (18)
370 Incubators for bacterial work (3)
Nikon Ti Eclipse confocal microscope system (1)
Virus Counter 2100 (1)
Cherry Biotech microscope system (1)
NanoDrop One spectrophotometer (2)
Inverted microscopes (7)
Refrigerated microcentrifuges (5)
Microcentrifuges (25)
Benchtop medium speed centrifuges (14)
Beckman ultracentrifuge Optima L100K (2)
FACSAria flow cytometer (1)
Milli-Q water purification systems (2)
Countess Invitrogen C10281 (4)
Touch thermal cycler C1000 (1)

Cold room (1)
Dark room (1)
BSL2 labs (12)
BSL3 labs (3)
ABSL4 labs (1)
4°C refrigerators (14)
Liquid nitrogen storage units (7)
-20°C freezers (12)
-80°C freezers (10)
pH meters (4)
QStudio 6 Fix-Realtime PCR system (1)
KrosFlo Research II TFF system (1)
Image processing station for Nikon C2+ confocal module microscope (1)
Odyssey SA IR imaging system (1)
Illumina MiSeq System (1)
Eppendorf epMotion M5073 automated DNA/RNA purification system (1)
BenchPro plasmid processing station (1)
HM Hematology analyzer (1)
MagNa Lyser with rotors (1)
Abaxis VetScan VS2 (5)
Aero3G Biaera aerosol control platform (1)
Aerodynamic particle sizer spectrometer 3321TSI (1)
Aerosol Diluter 3302A TSI (1)
Primate telemetry repeater (1)
VS Blood Chemistry analyzer (1)
iMark Absorbance microplate reader (2)
Luminometer microplate readers (4)
TECAN Genios multimode plate reader (1)

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Ronald Middle Name N. Last Name*: Harty Suffix: Ph.D

Position/Title*: Professor
Organization Name*: University of Pennsylvania
Department: Pathobiology and Microbiology
Division: School of Veterinary Medicine
Street1*: [redacted]
Street2:
City*: Philadelphia
County:
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 19104-0000

Phone Number*: [redacted] Fax Number: [redacted]

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: 1991

Attach Biographical Sketch*: File Name: Harty_Bio.pdf

Attach Current & Pending Support: File Name:

Tracking Number: GRANT13199760 Funding Opportunity Number: PA-20-265 . Received Date: 2020-09-08T12:43:45.000-04:00

PROFILE - Senior/Key Person

Prefix: First Name*: Jay Middle Name Edward Last Name*: Wrobel Suffix: Ph.D

Position/Title*: Vice President, Academic Relations
Organization Name*: Fox Chase Chemical Diversity Center, Inc.
Department:
Division:
Street1*: Pennsylvania Biotechnology Center
Street2: [redacted]
City*: Doylestown
County:
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 18902-8400

Phone Number*: [redacted] Fax Number: [redacted]

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PhD Degree Year: 1983

Attach Biographical Sketch*: File Name: Wrobel_biosketch_for_Harty.4.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: Olena Middle Name Last Name*: Shtanko Suffix:

Position/Title*: Staff Scientist I
Organization Name*: Texas Biomedical Research Institute
Department: Virology and Immunology
Division:
Street1*: [redacted]
Street2:
City*: San Antonio
County:
State*: TX: Texas
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 782270000

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PHD Degree Year: 2010

Attach Biographical Sketch*: File Name: 2._Bio_Shtanko_20-240_-_final.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: John Middle Name Last Name*: Kulp Suffix: Ph.D

Position/Title*: Director of Computational Chemistry
Organization Name*: Fox Chase Chemical Diversity Center, Inc. Department: Divisi
Department:
Division:
Street1*: Pennsylvania Biotechnology Center
Street2: [redacted]
City*: Doylestown
County:
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 18901-8400

Phone Number*: [redacted] Fax Number: [redacted]

E-Mail*: [redacted]

Credential, e.g., agency login:

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PhD Degree Year: 2006

Attach Biographical Sketch*: File Name: Kulp_biosketch_for_Harty.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: Allen Middle Name Bernard Last Name*: Reitz Suffix: Ph.D

Position/Title*: CEO
Organization Name*: Fox Chase Chemical Diversity Center
Department:
Division:
Street1*: [redacted]
Street2:
City*: Doylestown
County:
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 189020000

Phone Number*: [redacted] Fax Number: [redacted]

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PHD Degree Year: 1982

Attach Biographical Sketch*: File Name: Reitz_Biosketch_for_Harty.2.pdf

Attach Current & Pending Support: File Name:

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Harty, Ronald N.

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Professor of Microbiology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Lowell, Lowell, MA	B.S.	06/1985	Biology
Louisiana State University Med. Ctr., Shreveport	Ph.D.	08/1991	Virology
Louisiana State University Med. Ctr., Shreveport	Postdoctoral	01/1993	Virology
Mount Sinai School of Medicine, New York, NY	Postdoctoral	08/1998	Virology

A. Personal Statement

The overall goal of my research on negative-sense RNA viruses is to understand how these viruses assemble and bud from cells, and how host protein recruitment and virus-host interactions contribute to these late stages of virus replication. My current research program is focused on understanding the molecular mechanisms of filovirus (Ebola and Marburg viruses) and arenavirus (Lassa fever) assembly/budding, so that we can identify broad-spectrum inhibitors of virus-host interactions and validate their efficacy in blocking virus budding. Indeed, through a highly collaborative and productive effort in partnership with Dr. Jay Wrobel's team at Fox Chase Chemical Diversity Center, and Dr. Olena Shtanko at the BSL-4 laboratory at Texas Biomedical Research Institute, we have identified lead candidate budding inhibitors with potential broad-spectrum activity. In Phase 2, we will rapidly advance and develop these lead candidate therapeutics for NIAID Category A pathogens toward clinical trials. Overall, my expertise, experience, and demonstrated record of successful and productive research in the areas of RNA virus-host interactions and virus egress/transmission has prepared me to lead the proposed collaborative experiments described in this proposal.

1. **Harty, R. N.**, Brown, M. E., Wang, G., Huibregtse, J., and Hayes, F. P. 2000. A PPxY- motif within the VP40 protein of Ebola virus interacts physically and functionally with a ubiquitin ligase: Implications for filovirus budding. *Proc. Natl. Acad. Sci. U.S.A*. 97(25):13871-13876. PMCID: PMC17668.
2. Han, Z., Madara, J.J., Herbert, A., Prugar, L.I., Ruthel, G., Lu, J., Liu, Y., Liu, W., Liu, X., Wrobel, J.E., Reitz, A.B., Dye, J.M., ***Harty, R.N**., and *Freedman, B.D. (*co-corresponding authors). 2015. Calcium regulation of hemorrhagic fever virus budding: Mechanistic implications for host-oriented therapeutic intervention. PLoS Pathog, 11(10):e1005220. PMCID: PMC4634230.
3. Liang, J., Sagum, C.A., Bedford, M.T., Sidhu, S.S., Sudol, M., Han, Z., and **Harty, R.N**. 2017. Chaperone-mediated autophagy protein BAG3 negatively regulates Ebola and Marburg VP40-mediated egress. PLoS Pathog, Jan. 11;13(1):e1006132. PMCID: PMC5226679.
4. Han, Z., Dash, S., Sagum, C.A., Ruthel, G., Jaladanki, C.K., Berry, C.T., Schwoerer, M.P., Harty, N.M., Freedman, B.D., Bedford, M.T., Fan, H., Sidhu, S.S., Sudol, M., Shtanko, O., and Harty, R.N. 2020. Modular mimicry and engagement of the Hippo pathway by Marburg virus VP40: Implication for filovirus biology and budding. PLoS Pathog., Jan.6;16(1):e1008231. PMCID: PMC6977764.

B. Positions and Honors

1998-2004	Assistant Professor, Dept. of Pathobiology, School of Veterinary Medicine, University of Pennsylvania
2004-2015	Associate Professor, Dept. of Pathobiology, School of Veterinary Medicine, University of Pennsylvania
2014-present	Head, Laboratory of Infectious Diseases & Immunology, Dept. of Pathobiology, School of Veterinary Medicine, University of Pennsylvania
2015-present	Professor, Dept. of Pathobiology, School of Veterinary Medicine, University of Pennsylvania

Other Experience and Professional Memberships

1987–present	Member, American Society for Microbiology and American Society for Virology
2001	Pfizer Award for Excellence in Research, University of Pennsylvania
2002	Dean's Award for Leadership in Basic Science Education, University of Pennsylvania
2003-2007	Member, Special Emphasis Panel, Biodefense and Emerging Pathogens, NIAID, NIH.
2004-2005	Chair of the Filovirus Workshop Sessions at the American Society for Virology Meetings at McGill University, Montreal, Canada and at Penn State University, University Park, PA
2004-present	Member – Editorial Board, Journal of Virology; Ad Hoc Reviewer for Virology, Traffic, Molecular Cell, Vaccine, PNAS, PloS Pathogens, Cell. Micro., and J. of Gen. Virol.
2005	Member - Drug Discovery and Mechanisms of Antimicrobial Resistance Special Emphasis Panel Study Section [ZRG1 DDR (01) Q], Infectious Diseases and Microbiology IRG, NIH.
2005-2010	Member - Special Emphasis Panel, ZRG1 F13 NRSA Fellowships, NIH.
2008-2009	Chair - Special Emphasis Panel, ZRG1 F13 NRSA Fellowships, NIH.
2014	Ad Hoc Reviewer – NIH/NIAID Topics in Virology Study Section, ZRG1 IDM-W (applications having conflicts with standing VIR-A and VIR-B study sections)
2014	Ad Hoc Reviewer – NIH/NIAID, Pathogenic Eukaryotes (PTHE) Study Section
2015	Ad Hoc Reviewer – NIH/NIAID, Drug Discovery and Mechanisms of Antimicrobial Resistance (DDR) Study Section ZRG1 IDM-T (82)
2015	Ad Hoc Reviewer – NIH, Special Emphasis Panel ZRG1 AARR-D (02) M
2016	Chair – NIAID, Special Emphasis Panel to review an Ebola P01 application.
2017	Ad Hoc Reviewer – NIH/NIAID VIR-B study section
2018	Ad Hoc Reviewer – NIH R13 (Support for Scientific Conferences) Review Panel
2019	Reviewer – FY19 Congressionally Directed Medical Research Programs (CDMRP), Peer Reviewed Medical Research Program (PRMRP)

C. Contributions to Science

1. <u>Viral L-domain/host interactions regulate virus egress and spread</u>. Viruses have developed unique and complex molecular mechanisms to ensure efficient egress of mature virions from infected cells. I focused my early studies on unraveling the intricate roles of both viral and host proteins in this process, and particularly the specific recruitment of host factors to promote efficient budding of infectious virus. A better understanding of these virus-host interactions and the mechanisms of virus budding not only will provide fundamental insights into the functions of both viral and host proteins, but also will lead to the emergence of novel strategies to inhibit virion egress and spread. The Late (L) budding domain motifs (PTAP and/or PPxY) are highly conserved in the matrix proteins of a wide array of RNA viruses (*e.g*. filoviruses, arenaviruses, rhabdoviruses, paramyxoviruses, henipaviruses, and retroviruses) and represent attractive and novel targets for the development of therapeutics having broad-spectrum antiviral activity. My lab was first to demonstrate that the Ebola virus VP40 protein plays a central and sufficient role in virion assembly and egress, due in part to the presence of overlapping PTAP and PPEY L-domains. We went on to show that independent expression of VP40 led to the production and egress of virus-like particles (VLPs) that accurately mimic budding of live infectious virus. We used VP40 VLPs and genetically engineered VSV recombinants that expressed WT and mutant L-domain motifs from Ebola virus VP40, to show that budding is dependent on viral L-domain mediated recruitment of host proteins associated with the ESCRT pathway (Tsg101 and Nedd4) for complete virus-cell fission or separation. Most recently, we have identified for the first time a third functional L-domain motif ($YPx_{(n)}L$) within Ebola virus VP40 that serves as an alternative L-domain motif to enhance VP40 VLP release by recruiting and interacting with host ESCRT-associated protein Alix.

a. **Harty, R. N.**, Brown, M. E., Wang, G., Huibregtse, J., and Hayes, F. P. 2000. A PPxY- motif within the

VP40 protein of Ebola virus interacts physically and functionally with a ubiquitin ligase: Implications for filovirus budding. *Proc. Natl. Acad. Sci. U.S.A*. 97(25):13871-13876. PMCID: PMC17668.

b. Licata, J. M., Han, Z., Simpson-Holley, M., Wright, N. T., Paragas, J., and **Harty, R. N**. 2003. Overlapping motifs (PTAP and PPEY) within the Ebola virus VP40 protein function independently as late budding domains: Involvement of host proteins tsg101 and vps4. J. Virol. 77(3), 1812-1819. PMCID: PMC140960

c. Han, Z., Schwoerer, M.P., Hicks, P., Liang, J., Ruthel, G., Berry, C.T., Freedman, B.D., Sagum, C.A., Bedford, M.T., Sidhu, S.S., Sudol, M., and **Harty, R.N**. 2018. Host protein BAG3 is a negative regulator of Lassa VLP egress. Diseases, Jul 13;6(3). pii: E64.

d. Han, Z., Dash, S., Sagum, C.A., Ruthel, G., Jaladanki, C.K., Berry, C.T., Schwoerer, M.P., Harty, N.M., Freedman, B.D., Bedford, M.T., Fan, H., Sidhu, S.S., Sudol, M., Shtanko, O., and Harty, R.N. 2020. Modular mimicry and engagement of the Hippo pathway by Marburg virus VP40: Implication for filovirus biology and budding. PLoS Pathog., Jan.6;16(1):e1008231. PMCID: PMC6977764.

2. Identification and development of host-oriented L-domain inhibitors of virus budding. Based in part on our steady progress in elucidating the molecular aspects and host involvement in virus budding from studies described above, and our long-term goal of developing antivirals, a current major effort in my lab is to identify, develop, and optimize small molecule compounds targeting viral L-domain/host interactions to inhibit virus egress and spread. We postulate that for viruses such as Ebolavirus, administration of such an antiviral therapeutic during an outbreak would inhibit virus dissemination and spread in infected individuals, thus slowing disease progression and allowing the individual's immune system time to mount a robust response to effectively combat and clear the infection. Indeed, there is a vital need for the advancement and development of effective and safe therapeutics against emerging, high priority pathogens such as Ebola, Marburg, Lassa fever, and Junín viruses, and since these virus-host interactions represent a common mechanism in a range of emerging RNA viruses, we predict that they represent an Achilles' heel in the life cycle of these RNA virus pathogens. Toward this end, we have ongoing, fruitful collaborations with pharmacologists and virologists at the BSL-4 labs at USAMRIID and with medicinal chemists at Fox Chase Chemical Diversity Center, which has led to the identification of two successful lead series of PTAP and PPxY budding inhibitors that exhibit on-target, broad-spectrum antiviral activity against a wide array of RNA viruses. Specifically, we demonstrated that our current lead PTAP inhibitor, compound 0013, blocked egress of both Ebola and Junín VLPs at nanomolar concentrations, and our current lead PPxY inhibitors, compounds 4 and 5, blocked egress of Ebola, Marburg, and Lassa fever VLPs. Most exciting were our findings that these compounds could also block budding of live infectious viruses, including VSV and Junín (Harty lab), rabies (in collaboration with Dr. M. Schnell, TJU), Ebola (Kikwit) and Marburg (Ci67) (in collaboration with Drs. Andrew Herbert and J. Dye, USAMRIID, unpublished data). Currently, we are using Structure Activity Relationship (SAR) in collaboration with medicinal chemists Drs. J. Wrobel and A. Reitz (FCCDC) to identify analogs with enhanced potency and low cytotoxicity, as we seek to further transition one or more full-qualified L-domain inhibitors into more detailed IND-directed pharmacokinetic, pharmacodynamic and toxicity studies.

a. Liu, Y., Lee, M. S., Olson, M. A., and **Harty, R. N**. 2011. Bimolecular complementation to visualize filovirus VP40-host complexes in live mammalian cells: toward the identification of budding inhibitors. Adv. Virol., pii: 341816. PMCID: PMC3217271.

b. Lu, J., Han, Z., Liu, Y., Liu, W., Lee, M.S., Olson, M.A., Ruthel, G., Freedman, B.D., and **Harty, R.N.** 2014. A host-oriented inhibitor of Junin Argentine hemorrhagic fever virus egress. J. Virol. 88(9):4736-4743. PMCID: PMC3993831.

c. Han, Z., Lu, J., Liu, Y., Davis, B., Lee, M.S., Olson, M.A., Ruthel, G., Freedman, B.D., Schnell, M.J., Wrobel, J., Reitz, A., and **Harty, R.N.** 2014. Small molecule probes targeting the viral PPxY-host Nedd4 interface block egress of a broad range of RNA viruses. J. Virol. 88(13):7294-7306. PMCID: PMC4054416.

d. Loughran, H.M., Han, Z., Wrobel, J.E., Decker, S.E., Ruthel, G., Freedman, B.D., **Harty, R.N**., and Reitz, A.B. 2016. Quinoxaline-based inhibitors of Ebola and Marburg VP40 Egress. Bioorg. Med Chem Lett., 26:3429-3435. PMCID: PMC4955528.

3. Calcium regulation of hemorrhagic fever virus budding. In non-excitable cells, Ca^{2+} signals are generated and maintained through a tightly regulated process termed "store-operated Ca^{2+} signaling". As ER Ca^{2+} levels decrease, Ca^{2+} dissociates from C-terminal EF hands of the ER membrane-resident protein Stromal Interaction Molecule 1 (STIM1). Ca^{2+} dissociation from STIM1 initiates its oligomerization and localization to ER regions

adjacent to the plasma membrane where it interacts with and activates Orai1 Ca^{2+} channels that control extracellular Ca^{2+} influx. While previous studies in my lab implicated Ca^{2+} broadly in control of Ebola VP40 VLP formation, we have now demonstrated for the first time that filovirus VP40 and arenavirus Z matrix proteins trigger host cell Ca^{2+} signals by activating the ER Ca^{2+} sensing protein STIM1, which then activates plasma membrane Orai1 (Ca^{2+}) channels. Furthermore, we demonstrated that VP40 control of VLP production critically depends upon Orai1-mediated Ca^{2+} entry, as suppressing the expression or inhibiting the function of STIM1 and Orai1, or pharmacologically inhibiting Ca^{2+} permeation of Orai with selective channel blockers profoundly inhibits production of VLPs. Importantly, we also established that Orai1 mediated Ca^{2+} signals regulate transmission and spread of infectious Ebola, Marburg, Lassa fever, and Junín viruses in collaboration with John Dye's group at USAMRIID. Together these results have broad and profound significance because of **1**) the insight they provide into mechanisms that control virus egress and transmission, **2**) their establishment of STIM1 and Orai1 as conserved, essential, and immutable host proteins that underlie Ca^{2+} signals critical for enveloped RNA virus egress, and **3**) their validation of Orai blockers as the first of a family of broad-spectrum, host-oriented anti-viral therapeutics. Our ongoing research is focused on **1**) defining the mechanism of virus induced calcium signaling, **2**) defining the role of calcium in both host and virus dependent steps of budding, and **3**) pursuing the development of small molecule inhibitors of the Orai1 channel as a host-oriented therapeutic approach to block egress of a wide-array of RNA viruses.

a. Han, Z and **Harty, R. N**. 2007. Influence of calcium/calmodulin on budding of Ebola VLPs: Implications for the involvement of the Ras/Raf/MEK/ERK pathway. Virus Genes, 35(3):511-520. PMID:17570046.
b. Madara, J.J., Han, Z., Ruthel, G., Freedman, B.D., and **Harty, R.N**. 2015. The multifunctional Ebola virus VP40 matrix protein is a promising therapeutic target. Future Virol., 10(5):537-546. PMCID: PMC4480923.
c. Han, Z., Madara, J.J., Herbert, A., Prugar, L.I., Ruthel, G., Lu, J., Liu, Y., Liu, W., Liu, X., Wrobel, J.E., Reitz, A.B., Dye, J.M., ***Harty, R.N**., and *Freedman, B.D. (*co-corresponding authors). 2015. Calcium regulation of hemorrhagic fever virus budding: Mechanistic implications for host-oriented therapeutic intervention. PLoS Pathogens, 11(10):e1005220. PMCID: PMC4634230.
d. Freedman, B.D. and **Harty, R.N**. 2016. Calcium and filoviruses: A budding relationship. Future Microbiol., 11:713-715.

4. Innate immune defenses of hemorrhagic fever virus infection. Innate immune responses to virus infection provide a critical first line of defense for the host against the invading pathogen. Understanding the complex interplay between the host innate immune defense mechanisms and counteraction by the filoviruses is crucial for developing novel antiviral strategies, vaccines, and therapeutics. We have been interested in the host innate immune response to filoviruses and have investigated several mechanisms of host innate immune mediated defenses involving host proteins TLR4, SOCS1, SOCS3, and ISG15. ISG15 is an interferon stimulated gene that has garnered much attention recently due to its broad-range of antiviral activity against a plethora of pathogens including DNA and RNA viruses. We demonstrated for the first time that ISG15 inhibited budding of EBOV VP40 VLPs in a PPxY L-domain dependent manner, and that such inhibition involved impairment of host Nedd4 ligase activity. We continue to investigate whether ISGylation of additional VP40 host interactors (*e.g.* IQGAP1) can adversely affect VP40 function in budding. In addition, we are interrogating the effect of ISG15 expression on inhibition of Junín virus assembly and budding *in vitro* and *in vivo* using ISG15 -/- mice (in collaboration with Dr. A. Garcia-Sastre, MSSM). These studies will reveal new host innate immune defense mechanisms that may regulate the budding processes of several high priority NIAID Category A pathogens. Moreover, this information will be critical to identify strategies (therapies and/or vaccines) designed to tip the scale in favor of the host in the battle between viral pathogens and host innate immune defenses.

a. Okumura, A., Pitha, P. M., and **Harty, R. N.** 2008. ISG15 Inhibits Ebola VP40 VLP Budding in an L-Domain Dependent Manner by Blocking Nedd4 Ligase Activity. *Proc.Natl. Acad. Sci. USA*, 105(10):3974-9. PMCID: PMC2268823.
b. Okumura, A., Pitha, P. M., Yoshimura, A., and **Harty, R. N**. 2010. Interaction between Ebola virus glycoprotein and host TLR-4 leads to induction of pro-inflammatory cytokines and SOCS1. J. Virol., 84(1):27-33. PMCID: PMC2798428.
c. Lu, J., Qu, Y., Liu, Y., Jambusaria, R., Han, Z., Ruthel, G., Freedman, B., and **Harty, R. N**. 2013. Host IQGAP1 and Ebola virus VP40 interactions facilitate VLP egress. J. Virol., 87(13): 7777-80. PMCID: PMC3700276.
d. Okumura, A., Rasmussen, A.L., Halfmann, P., Feldmann, F., Yoshimura, A., Feldmann, H., Kawaoka, Y., **Harty, R.N**., and Katze, M.G. 2015. Suppressor of cytokine signaling 3 is an inducible host factor

during Ebola virus infection and 1 regulates virus egress. J. Virol., 89(20):10399-10406. PMCID: PMC4580175.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/ronald.harty.1/bibliography/44131509/public/?sort=date&direction=ascending.

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing:

R21 AI138052 Harty 01/15/18 – 12/31/20
Modular Domains of Host Proteins Regulate Filovirus Maturation
Major Goal – To elucidate the role of host protein BAG3 in filovirus egress.
Role: PI

R21 AI129890 Freedman 12/01/17 – 11/31/20
The Membrane Repair Channel TRPML1 Regulates Ebola Virus Budding
Major Goal – To elucidate the role of host channel TRPML1 in regulating Ebola virus egress
Role: co-I

R41 AI138630 Harty 06/01/18 – 05/31/21
Small Molecule Therapeutics Targeting Hemorrhagic Fever Viruses
Major Goal – To develop PPxY inhibitors of virus budding.
Role: PI

R21 AI139392 Harty 06/08/18 – 05/31/21
Dueling PPxY Motifs of Filovirus VP40 and Host Angiomotin: Effects on Innate Immune Defenses and Tight Junction Integrity at Immune Privileged Sites.
Major Goal – To examine the effect of VP40 on intestinal epithelium using a CACO2 model.
Role: PI

[redacted] Harty 01/01/19 – 12/31/20
Major Goal – To test a unique series of budding inhibitors against Filovirus/Arenavirus infections.
Role: PI

R21 EY031465 Harty 04/01/20 – 03/31/22
Predicted Role of Ebola VP40-Host Interactions in Ocular Pathology and Persistence
Major Goal – To determine whether filovirus VP40 contributes to ocular pathology using an eye-on-a-chip model.
Role: PI

Completed:

R33 AI102104 Harty 09/01/12 – 06/30/19
Host-Oriented Therapeutics Targeting Filovirus Budding.
Major Goal – To elucidate the mechanisms and potency of PTAP L-domain inhibitors of RNA virus budding.
Role: PI, No overlap with current proposal

BIOGRAPHICAL SKETCH

NAME: Jay Wrobel

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Vice President, Academic Relations, Fox Chase Chemical Diversity Center, Inc.

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
University of Wisconsin-Milwaukee	B.S.	1978	Chemistry
Cornell University	Ph.D.	1983	Organic Chemistry

A. Personal Statement

I am extremely well suited to for the medicinal chemistry leadership of this proposal. I have 34 years of experience in pharmaceutical industry focused on medicinal chemistry and drug design on a variety of drug discovery programs. In my current position at Fox Chase Chemical Diversity Center (FCCDC), I am involved in creation, leadership and management of medicinal chemistry and drug discovery partnerships with academic and biotech researchers to advance chemical hits to viable preclinical drug candidates up to and including the IND stage.

I was formerly Senior Director of Medicinal Chemistry at Wyeth/Pfizer in Collegeville, PA where I mentored and guided the efforts of up to 31 medicinal chemists, and worked successfully with outside alliance partners in the design and synthesis of novel agents for multiple drug discovery programs (hit to Preclinical Development). I was directly involved in bringing forward nine development track candidates (Phase 0 and beyond). My hands-on expertise in research includes project and portfolio management, hit triage, hit-to-lead and lead optimization from hits found using enabling technologies such as high-throughput screening, fragment based drug discovery, and virtual screening. In these efforts I have employed ligand and structure based SAR methods, analytical chemistry, multi-step synthesis, parallel synthesis, data management. I obtain and use ADME and PK data in the design of our analogs.

I have become involved with Prof. Harty on evaluation of egress inhibitors (namely mammalian Nedd4 / viral L domain inhibitors) of RNA viruses as an approach toward new antiviral drugs to combat Ebola, Marburg, Lassa and AIDS. In this capacity I have provided Prof. Harty with medicinal chemistry leadership on a Phase I STTR grant application and along with my colleagues at FCCDC have discovered new egress inhibitor leads that are more potent, stable and suitable for composition of matter patent status than what we (FCCDC and Harty lab) discovered prior to the STTR-funded work (see *Journal of Virology*, **2014**, *88*(13), 7294-7306). We have since published an SAR account of our further work (*Bioorg. Med. Chem. Lett*. **2016** *26*(15), 3429-35). We have obtained an issued U.S. patent on the initial series and have make great advances during the Phase I STTR period of study resulting in in vivo proof of concept efficacy in a mouse model of Marburg infection.

I also have additional experience with infectious diseases including antiviral small molecules therapeutics (Nef inhibitors-HIV) and antibacterial agents against drug resistant pathogens.

My role in this project would be to implement and oversee all aspects of the medicinal chemistry program including design of analogs (with participation of the bench chemists), day to day and long term management of medicinal chemists, directing all ADME, PK and drug safety studies with advice from our CRO partners. We will use a project flow scheme I devised to find the best compounds and I will also be involved in any major project decisions with PI Ron Harty.

Loughran HM, Han Z, **Wrobel JE**, Decker SE, Ruthel G, Freedman BD, Harty RN, Reitz AB. Quinoxaline-based inhibitors of Ebola and Marburg VP40 egress. Bioorg Med Chem Lett. 2016 Aug 1;26(15):3429-35. doi: 10.1016/j.bmcl.2016.06.053. Epub 2016 Jun 23. PubMed PMID: 27377328; PubMed Central PMCID: PMC4955528

Han Z, Lu J, Liu Y, Davis B, Lee MS, Olson MA, Ruthel G, Freedman BD, Schnell MJ, **Wrobel JE**, Reitz AB, Harty RN. Small-molecule probes targeting the viral PPxY-host Nedd4 interface block egress of a broad range

of RNA viruses. J Virol. 2014 Jul; 88(13): 7294-306. doi: 10.1128/JVI.00591-14. Epub 2014 Apr 16. PubMed PMID: 24741084; PubMed Central PMCID: PMC4054416.

Emert-Sedlak LA, Loughran HM, Shi H, Kulp JL 3rd, Shu ST, Zhao J, Day BW, **Wrobel JE**, Reitz AB, Smithgall TE. Synthesis and evaluation of orally active small molecule HIV-1 Nef antagonists. Bioorg Med Chem Lett. 2016 Mar 1;26(5):1480-4. doi: 10.1016/j.bmcl.2016.01.043. Epub 2016 Jan 21. PubMed PMID: 26852364; PubMed Central PMCID: PMC4756635.

Rubin H, Selwood T, Yano T, Weaver DG, Loughran HM, Costanzo MJ, Scott RW, **Wrobel JE**, Freeman KB, Reitz AB. Acinetobacter baumannii OxPhos inhibitors as selective anti-infective agents. Bioorg Med Chem Lett. 2015 Jan 15; 25(2): 378-83. doi: 10.1016/j.bmcl.2014.11.020. Epub 2014 Nov 22. PubMed PMID: 25496770.

B. Positions and Honors

1983-1987 Senior Scientist, Medicinal Chemistry, Ayerst, Inc., Princeton, NJ
1987-1988 Research Associate, Medicinal Chemistry, Wyeth-Ayerst Research, Inc., Princeton, NJ
1988-1996 Principal Scientist, Chemical Sciences, Wyeth-Ayerst Research, Inc., Princeton, NJ
1996-2001 Associate Director, Chemical Sciences, Wyeth-Ayerst Research, Inc., Radnor, Pa
2001-2007 Director, Chemical Sciences, Wyeth Research, Inc., Collegeville, Pa
2007-2010 Senior Director, Chemical Sciences, Wyeth/Pfizer, Inc., Collegeville, Pa
2011-2012- Senior Research Fellow, Fox Chase Chemical Diversity Center, Inc., Doylestown, Pa
2012-present Vice President, Academic Relations, Fox Chase Chemical Diversity Center, Inc., Doylestown, Pa

Member of the American Chemical Society 1978-present
Alternate Councilor, American Chemical Society, Princeton, NJ Local Section, 2012-2014
Member, DOD Study Section for review of applications, 2012, 2013.

C. Contribution to Science

In terms of discovering other novel drug candidates, I have worked extensively in cardiovascular areas on LXR modulators for atherosclerosis. As a LXR Modulator co-team leader and Medicinal Chemistry coordinator for the Wyeth/Karo-Bio LXR alliance, I delivered the first in-human LXR modulator (LXR-623) to phase I clinical studies for atherosclerosis indication in 2006. I also brought forth second and third generation LXR modulators devoid of mechanism and physiochemical-based side effects.

Wrobel J, Steffan R, Bowen SM, Magolda R, Matelan E, Unwalla R, Basso M, Clerin V, Gardell SJ, Nambi P, Quinet E, Reminick JI, Vlasuk GP, Wang S, Feingold I, Huselton C, Bonn T, Farnegardh M, Hansson T, Nilsson AG, Wilhelmsson A, Zamaratski E, Evans MJ. Indazole-based liver X receptor (LXR) modulators with maintained atherosclerotic lesion reduction activity but diminished stimulation of hepatic triglyceride synthesis. J Med Chem. 2008 Nov 27; 51(22): 7161-8. doi: 10.1021/jm800799q. PubMed PMID: 18973288.

Quinet EM, Basso MD, Halpern AR, Yates DW, Steffan RJ, Clerin V, Resmini C, Keith JC, Berrodin TJ, Feingold I, Zhong W, Hartman HB, Evans MJ, Gardell SJ, DiBlasio-Smith E, Mounts WM, LaVallie ER, Wrobel J, Nambi P, Vlasuk GP. LXR ligand lowers LDL cholesterol in primates, is lipid neutral in hamster, and reduces atherosclerosis in mouse. J Lipid Res. 2009 Dec; 50(12): 2358-70. doi: 10.1194/jlr.M900037-JLR200. Epub 2009 Mar 24. PubMed PMID: 19318684; PubMed Central PMCID: PMC2781308.

Hu B, Unwalla RJ, Goljer I, Jetter JW, Quinet EM, Berrodin TJ, Basso MD, Feingold IB, Nilsson AG, Wilhelmsson A, Evans MJ, Wrobel JE. Identification of phenylsulfone-substituted quinoxaline (WYE-672) as a tissue selective liver X-receptor (LXR) agonist. J Med Chem. 2010 Apr 22; 53(8): 3296-304. doi: 10.1021/jm100034x. PubMed PMID: 20350005

I was on a team that evaluated and brought in a FXR candidate from external alliance partner Exelixis. This candidate later became the first non-steroid based FXR agonist (FXR-450) taken to Phase I clinical studies for dyslipidemia in 2006. I later helped discover a FXR follow-on candidate with improved physiochemical properties (WYE-108635) that was taken to advanced preclinical animal toxicity evaluation in 2007.

Flatt B, Martin R, Wang TL, Mahaney P, Murphy B, Gu XH, Foster P, Li J, Pircher P, Petrowski M, Schulman I, Westin S, Wrobel J, Yan G, Bischoff E, Daige C, Mohan R. Discovery of XL335 (WAY-362450), a highly potent, selective, and orally active agonist of the farnesoid X receptor (FXR). J Med Chem. 2009 Feb 26; 52(4): 904-7. doi: 10.1021/jm8014124. PubMed PMID: 19159286.

Lundquist JT, Harnish DC, Kim CY, Mehlmann JF, Unwalla RJ, Phipps KM, Crawley ML, Commons T, Green DM, Xu W, Hum WT, Eta JE, Feingold I, Patel V, Evans MJ, Lai K, Borges-Marcucci L, Mahaney PE, Wrobel JE. Improvement of physiochemical properties of the tetrahydroazepinoindole series of farnesoid X receptor (FXR) agonists: beneficial modulation of lipids in primates. J Med Chem. 2010 Feb 25; 53(4): 1774-87. doi: 10.1021/jm901650u. PubMed PMID: 20095622.

I co-invented the first non-steroidal PR antagonists that were delivered to development track (Phase 0 and beyond) for contraception or fibroid indications in 2003-2007. And I brought forth the first non-steroid based PR agonist (tanaproget) to human clinical trials for contraception indication in 2001. This compound successfully completed Phase II clinical trials and was licensed to Teva Pharmaceuticals.

Fensome A, Adams WR, Adams AL, Berrodin TJ, Cohen J, Huselton C, Illenberger A, Kern JC, Hudak VA, Marella MA, Melenski EG, McComas CC, Mugford CA, Slayden OD, Yudt M, Zhang Z, Zhang P, Zhu Y, Winneker RC, Wrobel JE. Design, synthesis, and SAR of new pyrrole-oxindole progesterone receptor modulators leading to 5-(7-fluoro-3,3-dimethyl-2-oxo-2,3-dihydro-1H-indol-5-yl)-1-methyl-1H-pyrrole-2-carbonitrile (WAY-255348). J Med Chem. 2008 Mar 27;51(6): 1861-73. doi: 10.1021/jm701080t. Epub 2008 Mar 5. PubMed PMID: 18318463.

Zhang Z, Lundeen SG, Slayden O, Zhu Y, Cohen J, Berrodin TJ, Bretz J, Chippari S, Wrobel J, Zhang P, Fensome A, Winneker RC, Yudt MR. In vitro and in vivo characterization of a novel nonsteroidal, species-specific progesterone receptor modulator, PRA-910. Ernst Schering Found Symp Proc. 2007; (1):171-97. PubMed PMID: 18540573.

Fensome A, Bender R, Chopra R, Cohen J, Collins MA, Hudak V, Malakian K, Lockhead S, Olland A, Svenson K, Terefenko EA, Unwalla RJ, Wilhelm JM, Wolfrom S, Zhu Y, Zhang Z, Zhang P, Winneker RC, Wrobel J. Synthesis and structure-activity relationship of novel 6-aryl-1,4-dihydrobenzo[d][1,3]oxazine-2-thiones as progesterone receptor modulators leading to the potent and selective nonsteroidal progesterone receptor agonist tanaproget. J Med Chem. 2005 Aug 11;48(16): 5092-5. PubMed PMID: 16078826.

Zhang Z, Olland AM, Zhu Y, Cohen J, Berrodin T, Chippari S, Appavu C, Li S, Wilhem J, Chopra R, Fensome A, Zhang P, Wrobel J, Unwalla RJ, Lyttle CR, Winneker RC. Molecular and pharmacological properties of a potent and selective novel nonsteroidal progesterone receptor agonist tanaproget. J Biol Chem. 2005 Aug 5; 280(31): 28468-75. Epub 2005 Jun 3. PubMed PMID: 15937332.

I invented a mixed PPARα,γ,δ agonist that completed Phase II clinical trials for type II diabetes but was not selected to go further for business reasons.

I have an excellent external scientific and technical record based on 75 papers and 81 presentations. I am an inventor on 79 issued U.S. Patents. My publications can be found online at http://www.ncbi.nlm.nih.gov/myncbi/collections/bibliography/47703212/?reload=deleteSuccess&dnum=1

D. Research Support

Current

Development of Small Molecule Therapeutics Targeting Hemorrhagic Fever Viruses
R41AI138630-01A1 (Role: Key Personnel) 06/01/18 – 05/31/21 (NCE)
This is the Phase I STTR that provided most of the preliminary data that forms the basis of this Phase II STTR application. It was a two year Phase I which is currently in a third year no cost extension.

Soft LXR Agonists for Idiopathic Pulmonary Fibrosis
R43 HL154852-01 (Role: PI) 07/20/20 – 06/30/21

This Phase I SBIR involves the discovery of soft drug LXR agonists for the treatment of Idiopathic Pulmonary Fibrosis (PDF).
Overlap – none.

Molecule Antagonists of PF4 for the Treatment and Prevention of HIT
R42 HL12312602 (Role: PI) 09/15/2019 – 06/30/2022
This Phase IIB SBIR is directed toward the optimization of novel small molecule PF4 tetramer antagonists for the treatment of Heparin-Induced Thrombocytopenia (HIT).
Overlap – none.

Completed

Inhibitors of the PHD2 Zinc Finger to Treat Anemia
R32HL137458 (Role: PI) 04/01/2017 – 3/31/2018
This Phase I SBIR involves identification of SAR with intent to go to full-fledge drug discovery program in the Phase II portion for novel small molecule inhibitors the PHD2 Zinc Finger as a treatment for anemia

Molecule Antagonists of PF4 for the Treatment and Prevention of HIT
R42 HL12312602 (Role: Key Personnel) 10/01/2016 – 10/31/2018
This Phase II STTR is a full-fledge drug discovery program toward the optimization of novel small molecule PF4 tetramer antagonists for the treatment of heparin induced thrombocytopenia.

Small Molecule Inhibitors of HIV1 Nef Virulence Factor for Treatment of HIV-Aids
2 R42 GM112516-02A1 (Role: Key Personnel) 10/01/2016 – 10/31/2018
This Phase II STTR is a full-fledge drug discovery program toward the optimization for novel small molecule inhibitors of the HIV-1 virulence factor, Nef. This viral protein is critical to HIV-1 replication in vivo, immune escape of HIV-infected cells, and AIDS progression

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Shtanko, Olena

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Staff Scientist I

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Harvard University, Cambridge, MA	A.L.B.	06/2002	Natural Sciences
University of Wisconsin, Madison, WI	Ph.D.	09/2010	Microbiology
Texas Biomedical Research Institute, San Antonio, TX	Post-Doc	12/2016	Virology & Antivirals

A. Personal Statement

I am interested in understanding how filoviruses and other emerging viruses establish productive infection and trigger disease in affected individuals. My thesis research focused on the mechanisms of budding and incorporation of virus nucleocapsids into nascent virions of Mopeia virus, a close relative of Lassa Fever virus. As a Postdoctoral Scientist, I studied mechanisms of entry and replication of highly pathogenic viruses as well as developed and tested novel therapeutics to combat these pathogens. My work has identified cell factors critical for Ebola virus and Crimean-Congo hemorrhagic fever virus entry into cells. Since advancing to the Staff Scientist position, I have established my own laboratory to study mechanisms of pathogenesis and dissemination of filoviruses and other high-containment pathogens. I have also extensively collaborated with a number of investigators, including Dr. Harty, to coordinate and perform virus studies to identify host factors critical for replication and spread of filoviruses as well as test novel antiviral therapeutics using a mouse model of Ebola and Marburg virus disease. I provide sophisticated molecular biology and cell biology techniques for studies of highly pathogenic viruses. I am well-trained in BSL4 procedures and have been working in Texas Biomed's ABSL4 laboratory since 2011. I will be responsible for all BSL4 studies as well as virus data analysis.

B. Positions and Honors

Positions and Employment

2004-2010 Graduate Research Assistant, Department of Pathobiological Sciences, Advisor: Yoshihiro Kawaoka, University of Wisconsin, Madison, WI
2011-2017 Postdoctoral Scientist, Department of Virology and Immunology, Advisor: Robert A. Davey, Texas Biomedical Research Institute, San Antonio, TX
2017- Staff Scientist I, Department of Virology and Immunology, Texas Biomedical Research Institute, San Antonio, TX

Professional Memberships

2008- Member, American Society for Microbiology
2009- Member, American Society for Virology
2016- Member, American Society for Cell Biology

Honors

2012 Douglass Foundation Post-doctoral Award
2014 Travel Award recipient, American Society for Virology Annual Meeting
2017 Texas Biomed Forum Research Support Grant
2017 Texas Biomed Founder's Council Grant

C. Contributions to Science

Characterization of mechanisms of assembly, egress, and spread of emerging viruses

Understanding how viruses invade and establish productive infection in host cells is essential to identify novel therapeutics and to reveal how the cell itself functions. My work on assembly of Mopeia virus, a close relative of Lassa Fever virus, has revealed that ALIX/AIP1, which is required for the biogenesis of multivesicular bodies (MVBs) of the cell, binds to and drives incorporation of virus nucleocapsids into nascent virions. Recently, in collaboration with Dr. Ronald Harty, I have helped to identify angiomotin (Amot), a key regulator of YAP nuclear/cytoplasmic localization and function, as essential for egress and spread of authentic Marburg and Ebola viruses.

1. **Shtanko O**, Imai M, Goto H, Lukashevich IS, Neumann, G, Watanabe T and Kawaoka Y. *A role for the C terminus of Mopeia virus nucleoprotein in its incorporation into Z protein-induced virus-like particles. J Virol.* 2010; 84(10):5415-5422. PMID: 20200234. PMCID: PMC2863806.
2. **Shtanko O**, Watanabe S, Jasenosky LD, Watanabe T and Kawaoka Y. *ALIX/AIP1 is required for NP incorporation into Mopeia Z-induced virus-like particles. J Virol.* 2011; 85(7):3631-3641. PMID: 21248028. PMCID: PMC3067881.
3. Han Z, Dash S, Sagum CA, Ruthel G, Berry CT, Freedman BD, Bedford M, Fan H, Sidhu S, Sudol M, **Shtanko O** and Harty RN. *Modular mimicry and engagement of the Hippo pathway by Marburg virus VP40: implications for filovirus biology and budding*. *PLoS Path*. 2020; 16(1):e1008231. PMID: 31905227. PMCID: PMC6977764.
4. Han Z, Ruthel G, Dash S, Berry CT, Freedman BD, Harty R and **Shtanko O**. *Angiomotin regulates budding and spread of Ebola virus*. *J Biol Chem*. 2020; 295(25):8596-8601. PMID: 32381509. PMCID: PMC7307192.

Identification of host factors critical for entry and replication of high-containment viruses

My work on Crimean-Congo Hemorrhagic Fever virus spearheaded the identification of the MVB as the site of release of viral genome into the cell cytoplasm. Since disruption of this compartment completely abolishes infection, I have been working on identifying compounds that interfere with MVB function. My research on EBOV has led to the finding that proteins that are known to initiate and coordinate autophagy, a catabolic process where cellular membranes serve as a source of vesicles whose role is to engulf cell contents for degradation, were also essential for macropinocytosis, a primary route of virus uptake. Interestingly, these cellular proteins appear to coordinate macropinosome formation close to the cell surface, an unexpected finding since these proteins were thought to function exclusively internally. I have been involved in several collaborative projects dedicated to mapping cellular networks critical for virus replication. For one such study, in collaboration with Dr. Schlesinger, I have developed assays to manipulate clinically relevant primary human cells to demonstrate the novel finding that E3 ubiquitin-protein ligase RBBP6 binds components of EBOV polymerase complex to modulate virus transcription and replication.

1. **Shtanko O**, Nikitina RA, Altuntas CZ, Chepurnov AA and Davey RA. *Crimean-Congo hemorrhagic fever virus entry into host cells occurs through the multivesicular body and requires ESCRT regulators. PLoS Pathog*. 2014; 10(9):e1004390. PMID: 25233119. PMCID: PMC4169490.
2. **Shtanko O**, Reyes AN, Jackson, WT and Davey RA. *Autophagy-associated proteins control Ebola virus internalization into host cells. J Infect Dis*. 2018; Jun 27. doi: 10.1093/infdis/jiy294. PMID: 29947774. PMCID: PMC6249560.
3. Batra J, Hultquist JF, Liu D, **Shtanko O,** Von Dollen J, Satkamp L, Jang GM, Luthra P, Schwarz TM, Small GI, Arnett E, Anantpadma M, Reyes AN, Leung DW, Kaake R, Haas P, Schmidt CB, Schlesinger LS, LaCount DJ, Davey RA, Amarasinghe GK, Basler CF and Krogan NJ. *An Ebola virus-human protein-protein interaction map identifies the host ubiquitin ligase RBBP6 as a negative regulator of infection. Cell*. 2018; 175(7):1917-1930. PMID: 30550789. PMCID: PMC6366944.

Antivirals discovery to combat filoviruses

As part of a multi-collaborative effort, I was involved in establishing methods to perform high-throughput screens to identify novel compounds with antiviral activity. I also initiated development of multiple assays to study the mechanism of virus entry into cells. These efforts have successfully identified novel potent inhibitors of macropinocytosis and consequently virus infection.

1. Anantpadma M, Kouznetsova J, Wang H, Huang R, Kolokoltsov A, Guha R, Lindstrom AR, **Shtanko O**, Simeonov A, Maloney DJ, Maury W, LaCount DJ, Jadhav A and Davey RA. *Large-scale screening and identification of novel Ebola virus and Marburg virus entry inhibitors. Antimicrob Agents Chemother*. 2016; 60(8):4471-4481. PMID: 27161622. PMCID: PMC4958205.
2. **Shtanko O**, Sakurai Y, Noël R, Cintrat JC, Gillet D, Barbier J and Davey RA. *Retro-2 and its dihydroquinazolinone derivatives inhibit filovirus infection. Antiviral Res*. 2018; 149:154-163. PMID: 29175127.
3. Wu Y, Pons V, Noël R, Kali S, **Shtanko O**, Davey RA, Popoff MR, Tordo N, Gillet D, Cintrat JC and Barbier J. *DABMA: A derivative of ABMA with improved broad-spectrum inhibitory activity of toxins and viruses. ACS Med Chem Lett*. 2019; 10(8):1140-1147. PMID: 31413797. PMCID: PMC6691562.

Macrophage signaling pathways to control acute infection

In collaboration with Dr. Wendy Maury, I investigated how acute infection with *Plasmodium* parasite, a causative agent of malaria, affected EBOV challenge in a BALB/c mouse model of virus disease. The work demonstrated that acute *Plasmodium* infection protected the animals from lethal viral challenge and that such protection was dependent on parasite-elicited IFN-γ and associated macrophage signaling events. In addition to elucidating a factor critical for the outcome of EBOV disease in malaria-virus coinfected populations, the study carefully analyzed what virus challenge dose was appropriate for research on EBOV pathogenesis. Inoculation of mice with either 1, 10, or 100 PFU resulted in uniform lethality and similar virus loads in blood and major tissues. Interestingly, the protective effect of acute parasitemia was apparent only in 1 PFU-inoculated animals, demonstrating the critical need for appropriate experimental set up. In a separate study, I co-investigated the mechanisms of tissue macrophage-mediated control of acute virus infections. The study identified a previously unappreciated role for macrophage-intrinsic CD40 signaling in controlling EBOV infection.

1. Rogers K, **Shtanko O**, Vijay R, Mallinger L, Joyner C, Galinski M, Butler N and Maury W. *Acute Plasmodium infection promotes interferon-gamma dependent resistance to Ebola virus infection. Cell Reports.* 2020; 30(12):4041-4051. PMID: 32209467. PMCID: PMC7172281.
2. Rogers K, **Shtanko O**, Stunz L, Mallinger L, White J, Schmidt M, Varga S, Butler N, Bishop G and Maury W. *Frontline Science: CD40 signaling restricts RNA virus replication in MΦs, leading to rapid innate immune control of acute virus infection. J Leukoc Biol.* 2020; May 22. PMID: 32441445.

For full publication record, see: https://www.ncbi.nlm.nih.gov/myncbi/1jeRfa5Kb3tko/bibliography/public/

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

R41 AI138630 Harty (PI) 06/01/2018-05/31/2021 (2nd NCE)
Development of small molecule therapeutics targeting hemorrhagic fever viruses
NIH/NIAID
The purpose of this study is to develop novel small molecule, broad-spectrum therapeutics against viral infections caused by filoviruses that depend on the PPxY L-domain motif for virus egress and spread of infection.
Role: Subaward PI

R21 AI144215 Maury (PI) 03/11/2020-03/10/2022
CD40 regulation of acute virus infection
NIH/NIAID
The purpose of this study is to clarify the role of host macrophages and the CD40 signaling pathway in regulating early immune responses to filovirus infection.
Role: Subaward PI

Completed Research Support

R21 AI139902 Maury (PI) 06/01/2018-05/31/2020
Mechanisms and consequences of plasmodium/Ebola virus co-infections
NIH/NIAID
This project determined the impact of malaria on EBOV infection and defined the roles of specific pro- and anti-inflammatory cytokines in regulating EBOV disease severity in a mouse co-infection model.
Role: Subaward PI

HDTRA118C0031 Westfall (PI) 02/01/2019-12/31/2019
Grow, Expand, and Extract Total RNA
Defense Threat Reduction Agency
The purpose of this study was to develop a method to effectively extract and inactivate nucleic acids from select agents.
Role: Subaward co-PI

HHSN272201500015C McDonough (PI) 06/23/2015-5/31/2019
Targeting therapeutics development to relieve bottlenecks: optimizing lead therapeutic compounds against infectious pathogens
NIH/NIAID
The purpose of this study was to characterize the efficacy of tetrandrine derivatives as inhibitors of filovirus disease using a mouse intraperitoneal Ebola virus exposure model.
Role: Subaward PI

[redacted] Shtanko (PI) 12/01/2017-03/31/2019
This grant explored how VPS34 complex regulates macropinocytosis and EBOV infection in primary human macrophages and iPSC-derived hepatocytes.
Role: PI

BIOGRAPHICAL SKETCH

NAME: John L. Kulp III

POSITION TITLE: Director of Computational Chemistry, FCCDC

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Drew University, Madison, NJ	BA	05/2001	Chemistry, Mathematics
New York University, NY, NY	MS, PhD	05/2004, 06/2006	Chemistry
Naval Research Laboratory, Washington, DC	Postdoctoral	12/2009	Chemistry

A. PERSONAL STATEMENT

I have 11 years of computational chemistry experience with 17 peer reviewed publications. My background includes extensive experience in computer-aided drug design with a specialty focus on computational fragment-based methods. In 2010, I joined BioLeap, a small business that specializes in protein-protein interactions and that has a proprietary fragment-based computational chemistry software platform. I participated in the development of novel inhibitors for DHFR, PCSK9, 11β-HSD, and recA. During that time, I integrated molecular dynamics and docking software into BioLeap's proprietary fragment-based discovery platform. From 2013 to 2014, I was the project leader for BioLeap's PCSK9 program, which consisted of modeling (BioLeap), assays (Broad Institute of MIT and Harvard), and chemical synthesis (WuXi and BioDuro). As Director of Computational Chemistry at FCCDC, I apply structural biology to SAR diversification efforts in areas of innovative pharmacology. For example, I work closely with Dr. Wrobel and his team on multiple projects currently. I will apply my knowledge and expertise in the area of pharmacophore development to achieves the potency, efficacy and ADME gains of this grant application targeting the host Nedd4:virus PPxY interaction for the treatment of Marburg, Ebola and related diseases.

B. POSITIONS AND HONORS

Positions and Employment

2009-11 Federal Staff Scientist, Naval Research Laboratory, Washington, DC
2011-14 Distinguished Senior Scientist, BioLeap, Inc., Pennington, NJ
2013- Assistant Professor, Baruch S. Blumberg Institute, Doylestown, PA
2014- CSO, Small Molecule PPI Mimics LLC, College Station, TX
2014- CEO, Conifer Point Pharmaceuticals LLC, Doylestown, PA
2017- Director of Computational Chemistry, FCCDC

Other Experience and Professional Memberships

2014 Consultant on NIH Phase I STTR grant entitled "Small Molecule Inhibitors of HIV1 Nef Virulence Factor for Treatment of HIV/AIDS" Fox Chase Chemical Diversity Center, R41 GM112516-01.
2015 Consultant on NIH Direct to Phase II SBIR entitled "Development of Small Molecule Therapeutics Against Smallpox and Other Poxviruses" Fox Chase Chemical Diversity Center, R44AI115759-01.

Honors

2002-6 MacCracken Fellowship, New York University
2004 Kramer Fellowship, New York University
2006-9 American Society for Engineering Educations Postdoctoral Fellowship
2009 Young Investigator Award, American Peptide Society

C. CONTRIBUTIONS TO SCIENCE

1. My vision is to create better drugs by rigorous molecular design. Fragment-based lead discovery (FBLD) involves constructing drugs from small chemical building blocks (fragments) that bind to a protein target. Numerous companies, such as Abbott, Astex, and others, are pursuing this strategy by screening fragment libraries, followed by X-ray crystallography or NMR. In contrast, my research starts with a detailed hypothesis of what protein binding interactions are required of a drug for inhibition or activation. Commercial and proprietary design tools, some of which I created, are then used to search ligand-protein or fragment-protein simulation data for poses, ranked by predicted binding affinity, which can be assembled or optimized into custom compounds that satisfy the requirements. The designed compounds are synthesized and tested in assays. With success, this establishes a predictive model to guide subsequent lead optimization. This is a protein-centric strategy, complementary to ligand-centric SAR approaches. This methodology provides access to broad chemical diversity, which is crucial for solving difficult problems in lead identification and optimization.

- "A Fragment-based Approach to the SAMPL3 Challenge." J.L. Kulp III, S.N. Blumenthal, Q. Wang, R. Bryan, F. Guarnieri, *Journal of Computer-Aided Molecular Design* **2012**, *26(5)*, 583-594.
- “Diverse Fragment Clustering and Water Exclusion Identify Protein Hot Spots,” J.L. Kulp III, J.L. Kulp Jr., D.L. Pompliano, F. Guarnieri, *Journal of the American Chemical Society* **2011**, *133(28)*, 10740-1074.
- “Simulations of Nanocylinders Self-Assembled from Cyclic β-Tripeptides,” N. Bernstein, J.L. Kulp III, M.A. Cato Jr., T.D. Clark, *Journal of Physical Chemistry A*, **2010**, *114(44)*, 11948-11952.

2. The field of foldamer design promises new routes to important compounds for use in sensors, smart materials, therapeutics and catalysts. The term “foldamer” refers to a molecule that folds into a structurally stable state in solution. Proteins and peptides are an important class of natural foldamers that carry out a host of essential functions in biology, including molecular recognition, information storage, catalysis, and controlled crystallization of inorganic materials. The desire to mimic such functions with synthetic molecules inspires the field of foldamer design. I developed cyclic β-peptides as stochastic sensing elements and as a model chiral system for studying surface interactions. Other work included a universal method for restraining short peptides in helical conformations by a main-chain hydrogen bond surrogate (HBS) approach. These HBS peptides inhibited a variety of pharmaceutically relevant protein-protein interactions and the resulting technology was licensed by Aileron Therapeutics for clinical development.

- “Synthesis and characterization of cyclic peptides that are β-helical in trifluoroethanol.” K.P. Fears, S.J. Photiadis, J.L. Kulp III and T.D. Clark, *Journal of Peptide Science* **2014,** *20(5)*, 366-374.
- “Engineering a β helical D,L-peptide for folding in polar media.” J.L. Kulp III, T.D. Clark. *Chemistry: A European Journal* **2009,** *15(44)*, 11867-11877.
- “Trapping a Folding Intermediate of the α-Helix: Stabilization of the π-Helix.” R.N. Chapman, J.L. Kulp III, A. Patgiri, N.R. Kallenbach, C. Bracken, P.S. Arora, *Biochemistry* **2008,** *47(14)*, 4189-4195.
- “Evaluation of Biologically Relevant Short α-Helices Stabilized by a Main-Chain Hydrogen-Bond Surrogate.” D. Wang, K. Chen, J.L. Kulp III, P.S. Arora, *Journal of the American Chemical Society* **2006**, *128(28)*, 9248-9256.

3. My postdoctoral fellowship and subsequent two year federal employment was in The Molecular Interfaces and Tribology Section of the Department of Chemistry, Naval Research Laboratory. This section studies interdisciplinary questions in basic and applied interface and surface science. Our group’s research stretched from advancing broad analytical methods, such as vibrational circular dichroism, to evaluating both prototypical and realistic interfaces to crafting and improving molecular surfaces and peptide architectures for applications such as controlling biological activity, anti-fouling, or lowering contact friction. One focus area was in the design and employment of bioinspired materials that conserve and utilize the exceptional characteristics of biomaterials while combining added functionality, such as fluorescence or conductivity, not originating from natural materials.

- “Vibrational Circular-Dichroism Spectroscopy of Homologous Cyclic Peptides Designed to Fold into β Helices of Opposite Chirality,” J.L. Kulp III, K.P. Fears, R. Lombardi, D.Y. Petrovykh, J.C. Owrutsky, L.A.

Nafie, T.D. Clark, *Biointerphases*, **2011,** *6(1),* 1-7.

- "Characterization of the adhesive plaque of the barnacle Balanus Amphitrite: Anyloid-like nanofibrils are a major component," D.E. Barlow, G.H. Dickinson, B. Orihuela, J.L. Kulp III, D. Rittschof, K.J. Wahl, *Langmuir* **2010,** *26(9)*, 6549-6556. Highlighted in *Chemical and Engineering News* **2010,** *88(9)*, 34.
- "Molecular Recognition and Supramolecular Self-Assembly of a Genetically Engineered Gold Binding Peptide on Au {111}." C. So, J.L. Kulp III, E.E. Oren, H. Zareie, H. Zareie, C. Tamerler, J.S. Evans, M. Sarikaya, *ACS Nano* **2009,** *3(6)*, 1525-1531.
- "Laser Ablation of Micropores for Formation of Artificial Planar Lipid Bilayers." T.J. O'Shaughnessy, J.E. Hu, J.L. Kulp III, S.M. Daly, F.S. Ligler, *Biomedical Microdevices* **2007**, *9(6)*, 863-868.

4. The beginning of my computational chemistry career began when I was using nuclear magnetic resonance (NMR) spectroscopy to determine the structures of biological macromolecules like peptides, polypeptides, and proteins. To do this, I would rely on 1D, homo- and hetero-nuclear 2D, and triple resonance, 3D, spectra. Some other examples of this work are cited in scientific contribution 3, above. After extracting all available information about the atomic distances and torsion angles from the NMR spectra, computer-aided structure calculations would convert distance and torsion angle data (constraints) into the 3D atomic model. For these calculations, a molecular dynamics simulation was used with simulated annealing; increasing temperature to generate an ensemble of conformations then decreasing temperature with increasing constraints based on the NMR data. The result is a family of structures that span a relatively narrow conformational space (low RMSD), unless intrinsically flexible, and obeys the constraint criteria.

- "Structural Properties of an Artificial Protein that Regulates the Nucleation of Inorganic and Organic Crystals." J.L. Kulp III, T. Minamisawa, K. Shiba, M. Tejani, J.S. Evans, *Langmuir* **2007,** *23(7)*, 3857-3863.
- "Probing the Conformational Features of a Phage Display Polypeptide Sequence Directed Against Single Walled Carbon Nanohorn Surfaces." J.L. Kulp III, K. Shiba, J.S. Evans. *Langmuir* **2005**, *21(25)*, 11907-11914.
- "Affinity Selection of Peptide Phage Libraries Against Single-Wall Carbon Nanohorns Identifies a Peptide Aptamer with Conformational Variability" D. Kase, J.L. Kulp III, M. Yudasaka, J.S. Evans, S. Iijima, K. Shiba, *Langmuir* **2004**, *20(20)*, 8939-8941.
- "Molecular Characterization of a Prokaryotic Polypeptide Sequence that Catalyzes Au Crystal Formation." J.L. Kulp III, M. Sarikaya, J.S. Evans, *J. Mat. Chem.***2004**, *14(14)*, 2325-2332.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/john.kulp.2/bibliography/48391280/public/?sort=date&direction=ascending

D. RESEARCH SUPPORT

Current:

Molecular Mechanism of the Dual Effects of Capsid Assembly Effectors on HBV Capsid Assembly and Disassembly (DoD/PRMRP). Role: Investigator 9/1/2017 – 8/31/2020
The goal of this project is to obtain molecular insights on the mechanism of two distinct chemotypes of hepatitis B virus (HBV) core protein allosteric modulators (CpAMs) on the assembly and disassembly (or uncoating) of HBV nucleocapsids.

Previous:

[redacted] Role: Investigator 7/1/2018 – 6/30/2019
The goal of this project is to progress disubstituted aminothiazoles that demonstrate remarkable selective toxicity for HCC-derived cell lines versus non-HCC liver lines and most other cancer lines.

Small Molecule Inhibitors of HIV1 Nef Virulence Factor for Treatment of HIV-Aids
R42GM112516-01 (PI: Smithgall; Role: Key Personnel) 9/01/2016–8/31/2018
This Phase II STTR involves the discovery of novel small molecule inhibitors of the HIV-1 virulence factor Nef, and has a substantial structural biology and computational chemistry component.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES**.

NAME: Reitz, Allen Bernard

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Chief Executive Officer

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of California, Santa Barbara	B.A.	08/1977	Biochemistry and Molecular Biology
University of California, San Diego	M.S.	06/1979	Chemistry
	Ph.D.	01/1982	Chemistry
University of Pennsylvania (Wharton)	M.S.	06/2003	Management of Technology

A. Personal Statement

Dr. Reitz has had >38 years of experience in the pharmaceutical industry including ~26 years with Johnson & Johnson in various drug discovery research and management roles. He is an inventor of eight compounds that have entered clinical trials, including two from FCCDC which he founded in 2008 (www.fc-cdci.com). For example, he is a coinventor of troriluzole which emerged from an Phase I/II SBIR-funded project at FCCDC on which he was the Principal Investigator, and which is currently in Phase II/III clinical trial having recently passed an interim futility analysis for the treatment of Alzheimer's disease. He is co-author of >160 scientific publications, co-inventor on 71 issued U.S. patents, Editor-in-Chief of the journal *Current Topics in Medicinal Chemistry*, on the Editorial Advisory Board of *ACS Med. Chem. Lett.* Dr. Reitz has extensive experience in project and portfolio management, target validation, hit triage, eADME profiling, and preclinical candidate selection. He is currently serving as an ad hoc reviewer on two of the rapid review COVID-19 study sections in NIAID. Dr. Reitz has worked with Drs. Wrobel and Prof. Harty since the inception of the FCCDC – U. Penn. collaboration of this novel host directed approach to treat Ebola and Marburg infection. He will continue to provide overall guidance as much as possible, working with Dr. Wrobel, and will seek to ensure a successful transition at the end of Phase II to further development and eventual commercialization including through taking advantage of various initiatives of the FDA such as the Neglected Disease Priority Review Voucher program.

Loughran HM, Han Z, Wrobel JE, Decker SE, Ruthel G, Freedman BD, Harty RN, **Reitz AB**. Quinoxaline-based inhibitors of Ebola and Marburg VP40 egress. Bioorg Med Chem Lett. 2016 Aug 1;26(15):3429-35. doi: 10.1016/j.bmcl.2016.06.053. PubMed PMID: 27377328; PubMed Central PMCID: PMC4955528

Han Z, Lu J, Liu Y, Davis B, Lee MS, Olson MA, Ruthel G, Freedman BD, Schnell MJ, Wrobel JE, **Reitz AB**, Harty RN. Small-molecule probes targeting the viral PPxY-host Nedd4 interface block egress of a broad range of RNA viruses. J Virol. 2014 Jul; 88(13): 7294-306. doi: 10.1128/JVI.00591-14. PubMed PMID: 24741084; PubMed Central PMCID: PMC4054416.

B. Positions and Honors

Positions and Employment

1982-1983 Postdoctoral Research Fellow, McNeil Pharmaceutical
1983-1984 Research Scientist, McNeil Pharmaceutical
1984-1987 Senior Scientist, Janssen Research Foundation

1987-1992 Principal Scientist, R W Johnson Pharm. Res. Inst.
1992-1998 Research Fellow, R W Johnson Pharm. Res. Inst.
1999-2007 Senior Research Fellow, JNJPRD
2008 Vice President, Research and Development, AlsGen, Inc.
2008-present Founder and CEO, Fox Chase Chemical Diversity Center, Inc.
2009-present Co-Founder and CEO, ALS Biopharma, LLC
2010-present Founder and President, Pennsylvania Drug Discovery Institute (non-profit)

Other Experience and Professional Memberships

2015 National Co-Chair of the BIO Boot Camp, associated with the national BIO meeting
2014-present Editorial Advisory Board member, *ACS Med. Chem. Lett.*
2014-2016 Co-Editor, *Technology Transfer and Entrepreneurship*
2008-present Adjunct Professor, Drexel University College of Medicine
2008-2009 Consultant to the World Health Organization for the Tropical Diseases (TDR)
2005-present Have served on >42 NIH Study Sections including Drug Discovery for the Nervous System (2012-2014), and am currently an ad hoc member of COVID-19 rapid review study section study sections ZAI1 RG-W (S2) and ZAI1 RG-W (J2), July 14 and Oct. 15 respectively.
2000-present Editor-in-Chief, *Current Topics in Medicinal Chemistry*
1995-1996 Philadelphia Organic Chemists' Club, Assistant Chairman, and then Chairman
1985-1986 Philadelphia Organic Chemists' Club, Assistant Secretary, and then Secretary

Honors

2016 Cornerstone Award CEO Honoree, Winner for Bucks County, PA
2011 Gold Award, Best Early-Stage Company, Philadelphia Business Journal
2006 Ronald J. Borne Distinguished Lectureship Award, in Recognition for Outstanding Contributions to Medicinal Chemistry, Department of Medicinal Chemistry, School of Pharmacy, University of Mississippi (April 18, 2006). Highlighted on pg. 6 of *AACP News***2006**, *37(9)*.
2003 Industrial Chemistry Award of the Philadelphia Organic Chemists' Club.Poster Session, Reception, and Lecture: May 29, 2003
1999 Johnson & Johnson Corporate Office of Science and Technology Excellence in Science Award
1997 Johnson & Johnson Significant Achievement Award for Creating the POP Process to Rapidly Move New Compounds from the Laboratory into Clinical Trials
1994 Philip B. Hofmann Research Scientist Award for Outstanding Achievement
1990 Johnson & Johnson Achievement Award for Outstanding Contributions

C. Contribution to Science

1. Target Validation. Research in this area has been to understand the relation of structure to function among related compound libraries, using modern methods of synthetic chemistry and SAR development. Along those lines, FCCDC has submitted >12,000 new chemical entities in the context of individual collaborations with academic and biotech research partners.

    a. Punchi Hewage, A. N. D.; Yao, H.; Nammalwar, B. Gnanasekaran, K. K.; Lovell, S.; Bunce, R. A.; Eshelman, K.; Phaniraj, S. M.; Lee, M. M.; Peterson, B. R.; Battaile, K. P.; **Reitz, A. B**.; Rivera, M. Small Molecule Inhibitors of the BfrB-Bfd Interaction Decrease Pseudomonas aeruginona Fitness and Potentiate Fluoroquinoline Activity. *J. Am. Chem. Soc.* **2019**, *141*, 8171-8184.
    b. Karpova, Y.; Wu, C.; Divan, A.; McDonnell, M. E.; Hewlett, E.; Makhov, P.; Gordon, J.; Ye, M.; **Reitz, A. B**.; Childers, W. E.; Skorski, T.; Kolenko, V.; Tulin, A. V. Non-NAD-like PARP-1 inhibitors in prostate cancer treatment. *Biochem. Pharmacol.* **2019**, *167*, 149-162.
    c. Robinson, R. M.; Reyes, L.; Duncan, R. M.; Bian, H.; **Reitz, A. B**.; Manevich, Y.; McClure, J. J.; Champion, M. M.; Chou, C. J.; Sharik, M. E.; Chesi, M.; Bergsagel, P. L.; Dolloff, N. G. Inhibitors of the protein disulfide isomerase family for the treatment of multiple myeloma. *Leukemia* **2019**, *33*, 1011-1022.
    d. Martin, M. D.; Calcul, L.; Smith, C.; Jinwal, U. K.; Fontaine, S. N.; Darling, A.; Seeley, K.; Woitas, L.; Narayan, M.; Gestwicki, J. E.; Smith, G. R.; **Reitz, A. B**.; Baker, B. J.; Dickey, C. A. Synthesis, Stereochemical Analysis, and Derivatization of Myricanol Provide New Probes That Promote Autophagic Tau Clearance. *ACS Chem. Biol.* **2015**, *10*, 1099-1109.

2. Hit to Lead Medicinal Chemistry. Much effort has focused on this area, in which libraries of related compounds are prepared in order to obtain maximum diversity based upon common themes dictated by the structure of the target, pharmacophore analysis, and the creation of new intellectual property. Multiple projects (e.g. BACE, EBNA1) have involved real-time X-ray co-crystallography collaboration and analysis.

    a. Baxter, E. W.; Conway, K. A.; Kennis, L.; Bischoff, F.; Mercken, M. H.; De Winter, H. L.; Reynolds, C. H.; Tounge, B. A.; Luo, C.; Scott, M. K.; Huang, Y.; Braeken, M.; Pieters, S. M. A.; Berthelot, D. J. C.; Masure, S.; Bruinzeel, W. D.; Jordan, A. D.; Parker, M. H.; Boyd, R. E.; Qu, J.; Alexander, R. S.; Brenneman, D. E.; **Reitz, A. B.** 2-Amino-3,4-dihydroquinazolines as Inhibitors of BACE-1 (β-Site APP Cleaving Enzyme): Use of Structure Based Design to Convert a Micromolar Hit into a Nanomolar Lead. *J. Med. Chem.* **2007**, *50*, 4261-4264.
    b. Huang, Y.; Strobel, E. D.; Ho, C. Y.; Reynolds, C. H.; Conway, K. A.; Piesvaux, J. A.; Brenneman, D. E.; Yohrling, G. J.; Arnold, H. M.; Rosenthal, D.; Alexander, R. S.; Tounge, B. A.; Mercken, M.; Vandermeeren, M.; Parker, M. H.; **Reitz, A. B**.; Baxter, E. W. Macrocyclic BACE Inhibitors: Optimization of a Micromolar Hit to Nanomolar Leads. *Bioorg. Med. Chem. Lett.* **2010**, *20*¸3158-3160.
    c. McDonnell, M. E.; Vera, M. D.; Blass, B. E.; Pelletier, J. C.; King, R. C.; Fernandez-Metzler, C.; Smith, G. R.; Wrobel, J.; Chen, S.; **Reitz, A. B.** Riluzole prodrugs for melanoma and ALS: design, synthesis, and in vitro metabolic profiling. **2012**, *Bioorg. Med. Chem.*, *20*, 5642-5648.
    d. Rubin, H.; Selwood, T.; Yano, T.; Weaver, D. G.; Loughran, H. M.; Costanzo, M. J.; Scott, R. W.; Wrobel, J. E.; Freeman, K. B.; **Reitz, A. B**. *Acinetobacter baumannii* OxPhos inhibitors as selective anti-infective agents. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 378-383.

3. Lead Optimization (LO) Medicinal Chemistry Advancing to Preclinical and Clinical Development. Considerable emphasis has been placed on this phase of the drug discovery process, for which eight NMEs have entered human clinical trials. The 71 issued U.S. patents are largely devoted to LO SAR programs. There are three basic considerations for a medicinal chemist which apply to all indications: (1) route of administration (e.g. p.o, i.v., etc.), and (2) dosing regimen (acute, subchronic, chronic), and (3) standard of care (crowded marketplace or keen unmet need).

    a. **Reitz, A. B**.; Baxter, E. W.; Bennett, D. J.; Codd, E. E.; Jordan, A. D.; Maryanoff, B. E.; McDonnell, M. E. ; Ortegon, M. E.; Renzi, M. J.; Scott, M. K.; Shank, R. P.; Sherrill, R. G.; Vaught, J. L.; Wustrow, D. J. *N*-Aryl-*N*'-Benzylpiperazines as Potential Antipsychotic Agents. *J. Med. Chem.* **1995**, *38*, 4211-4222. **[Description of mazapertine, which advanced to Phase II clinical trials.]**
    b. Parker, M. H.; Smith-Swintosky, V. L.; McComsey, D. F.; Huang, Y.; Brenneman, D.; Klein, B.; Malatynska, E.; White, H. S.; Milewski, M. E.; Herb, M.; Finley, M. F. A.; Liu, Y.; Lubin, M. L.; Qin, N.; Iannucci, R.; Leclercq, L.; Cuyckens, F.; **Reitz, A. B**.; Maryanoff, B. E. Novel, Broad-Spectrum Anticonvulsants Containing a Sulfamide Group: Advancement of N-[(Benzo[*b*]thien-3-yl)methyl]sulfamide (JNJ-26990990) into Human Clinical Studies. *J. Med. Chem*., **2009**, *52*, 7528-7536. **[Anticonvulsant JNJ-26990990 which advanced into Phase I clinical trials.]**
    c. Ross, T. M.; Battista, K.; Bignan, G. C.; Brenneman, D. E.; Connolly, P.. J.; Liu, J.; Middleton, S. A.; Orsini, M.; **Reitz, A. B**.; Rosenthal, D. I.; Scott, M. K.; Vaidya, A. H. A selective small molecule NOP (ORL-1 receptor) partial agonist for the treatment of anxiety. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 602-606. **[Anxiolytic ORL-1 receptor agonist that advanced into Phase I clinical trials.]**
    d. Messick, T. E.; Smith, G. R.; Soldan, S. S.; McDonnell, M. E.; Deakyne, J. S.; Malecka, K. A.; Tolvinski, L.; van den Heuvel, A. P. J.; Gu, B. W.; Cassel, J. A.; Tran, D. H.; Wasserman, B. R.; Zhang, Y.; Velvadapu, V.; Zartler, E. R.; Busson, P.; **Reitz, A. B**.; Lieberman, P. M. Structure-based design of small-molecule inhibitors of EBNA1 DNA binding blocks Epstein-Barr virus latent infection and tumor growth. *Sci. Tranl. Med.* **2019**, *11*, pii: eaau5612. doi: 10.1126/scitranslmed.aau5612. **[EBNA1 inhibitor currently in Phase II clinical trials, discovered at FCCDC]**

4. Synthetic Organic Chemistry. Dr. Reitz was inspired by organic faculty as an undergraduate, which was reinforced by a positive experience as a graduate student with Murray Goodman at UCSD. Highly cited publications include a *Chem. Rev.* article on the Wittig reaction and Vol. 59 of *Organic Reactions* on reductive aminations using boron-containing reagents consisting of >700 pages and an entire volume. Research has been conducted in the areas of conformational analysis and new synthetic methods.

a. Larsen, B. J.; Rosano, R. J.; Ford-Hutchinson, T. A.; **Reitz, A. B**.; Wrobel, J. A. A method for C2 arylation of 1,3-indandiones. *Tetrahedron* **2018**, *74*, 2762-2768.
b. Pelletier, J. C.; Velvadapu, V.; McDonnell, M. E.; Wrobel, J. E.; **Reitz, A. B.** Intramolecular rearrangement of α-amino acid amide derivatives of 2-aminobenzothiazoles.*Tetrahedron Lett.* **2014**, *55*, 4193-4195.
c. Maryanoff, B. E.; **Reitz, A. B**. The Wittig Olefination Reaction and Modifications Involving Phosphoryl-Stabilized Carbanions. Stereochemistry, Mechanism, and Selected Synthetic Aspects. *Chem. Rev.***1989**, *89*, 863-927.
d. Baxter, E. W; **Reitz, A. B.** "Reductive Aminations of Carbonyl Compounds with Borohydride and Borane Reducing Agents" In *Organic Reactions*: Wiley, New York, p. 1-714, Vol. 59, **2002**.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/1nyMq8FpqE0A5/bibliography/47313087/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

Riluzole Prodrugs for Melanoma and ALS
R44 CA156781-04A1 (PI: Reitz) 9/1/18-8/31/21
This Phase II SBIR Bridge application involves the further pre-clinical development of troriluzole, an actively-transported (PepT1) prodrug of riluzole, . A Phase II clinical trial for the treatment of solid tumors is included in the grant, to be conducted at the Dana Farber Cancer Institute. Overlap – none.

Troriluzole for the Treatment of Cocaine Addiction
R41 DA047169-01 (PI: Rawls, Temple University; Role, Key Personnel) 07/01/18-7/30/21 (NCE)
In this project, Prof. Rawls is testing whether troriluzole can counter the effects of cocaine in rats. Currently in the no cost extension phase. Overlap – none.

Psychoactive bath salts and the glutamate system
R01 DA039139-02 (PI: Rawls, Temple University; Role, Key Personnel) 8/1/16-7/31/21
This R01 from Prof. Rawls at Temple University involves understanding the pharmacology of bath salts in CNS receptor and behavioral assays. Overlap – none.

Targeting Telomerase for Pancreatic Cancer Therapeutics
R01 CA208439-01 (PI: Skordalakes; Role, Key Personnel) 8/1/18-7/31/21
This work is to discover new telomerase inhibitors for the treatment of pancreatic cancers. Overlap – none.

DART2.0: comprehensive cell type-specific behavioral neuropharmacology
RF1 MH117055-01 (PI: Tadross, Duke University; Role, Key Personnel) 9/14/18-9/13/21
Prof. Tadross is the PI of a Brain R01 looking to identify new DART bifunctional probes to understand the localization and function of important receptor systems in the CNS. Our role is to perform synthetic chemistry to obtain suitable probes which are then studied in his laboratory. Overlap – none

FCCDC Budget Justification

Personnel

Jay E. Wrobel, Ph.D. Vice President, Academic Affairs and Senior Research Fellow, Key Personnel, FCCDC Project Leader (Yrs 1-3: 3 cal. months). Dr. Wrobel has >35 years of experience in the pharmaceutical industry focused on drug design and discovery on a variety of drug discovery programs. He was formerly Senior Director of Medicinal Chemistry at Wyeth/Pfizer in Collegeville, PA where he mentored and guided the efforts of up to 31 medicinal chemists and worked successfully with outside alliance partners in the design and synthesis of novel agents for multiple drug discovery programs (hit to Preclinical Development). Dr. Wrobel was directly involved in bringing forward nine development track candidates (phase 0 and beyond). He has 78 peer reviewed articles and is an inventor on 83 U.S. Patents. Dr. Wrobel has expertise in project and portfolio management, hit triage, hit-to-lead and lead optimization from hits found using enabling technologies such as high-throughput screening, fragment based drug discovery, and virtual screening. In these efforts he has employed ligand and structure based SAR methods, analytical chemistry, multi-step synthesis, parallel synthesis, data management and ADME analysis.

In his current position as VP Academic Relations at Fox Chase Chemical Diversity Center, Dr. Wrobel has collaborated and co-published with Professor Harty on *in vitro* and *in vivo* characterization of first-in-class novel inhibitors of filovirus PPxY interaction with host protein Nedd4 as an approach toward broad-spectrum antiviral therapy. Dr. Wrobel will be responsible for FCCDC project design, direction of the project and data interpretation. He will also organize regular project meetings among team members and consultants to maximize efficiency.

Allen B. Reitz, Ph.D., CEO, Key Personnel (Yrs 1-3: 0.6 cal. months). Dr. Reitz has had >38 years of demonstrated accomplishment as a medicinal chemist in the pharmaceutical industry, including 26 years with Johnson & Johnson. He is a co-inventor as well as the project leader in most cases of nine compounds that have entered human clinical trials, two of which are currently in the clinic. He has >150 scientific publications and 71 issued U.S. patents, and is the Editor of the journal *Current Topics in Medicinal Chemistry*. He has extensive experience in project and portfolio management, target validation, hit triage, hit to lead and lead optimization medicinal chemistry, eADME profiling, and preclinical candidate selection. He is also Adj. Prof. at Drexel University, College of Medicine. Dr. Reitz founded FCCDC in 2008 to provide translational biomedical research in the area of early drug discovery research including medicinal chemistry hit to lead and lead optimization research. Dr. Reitz will assist Dr. Wrobel on project design and data interpretation. He will also pay particular attention to developing the required transition beyond Phase II, including a suitable project strategy, implementation of the Commercialization Plan, and interface with potential development partners and other interested parties.

John Kulp III, Ph.D., Director of Computational Chemistry, Key Personnel (Yrs 1-3: 0.6 cal. months). Dr. Kulp has 9 years of computational chemistry experience with 17 peer reviewed publications. After his PhD and postdoctoral training he spent 4 years at BioLeap, a small business that specializes in protein-protein interactions and that has a proprietary fragment-based computational chemistry software platform. There he was the project leader for BioLeap's PCSK9 program. He will perform computer-assisted drug design studies, and help to provide insight by the creation of computational pharmacophores during the course of the program.

Katie B. Freeman, Ph.D., Director of Biology, FCCDC (Yrs 1-3: 0.6 cal. months/yr). Dr. Freeman has extensive experience with numerous *in vitro* assay formats, in microbiological methodologies, mammalian cell culture, bioanalytical methodologies. She will conduct cytotoxicity assays at FCCDC in liver HepG2 and endothelial HUVEC to compare the relative in vitro therapeutic index between series and individual compounds. In addition, she will perform formulations development as required to support the in vivo evaluation at TBRI.

Hong Ye, Scientist (Yrs 1-3: 12 cal. months), Ms. Ye is an exceptionally talented bench medicinal chemist, who consistently ranks among the top staff at FCCDC in terms of productivity and creativity. She previously had 20 years of experience at Johnson & Johnson in Spring House PA and other companies before joining FCCDC in June 2015. She has been the primary laboratory medicinal chemistry on the Harty Marburg/Ebola project and will continue to do so working under the direction of Dr. Wrobel with proper attention being paid to laboratory safety, confidentiality, productivity, notebook keeping and spectral data records retention.

Leo J. Adalbert, M.B.A. Technical and Business Assistance (TABA). ($50,000 over three years requested) Mr. Adalbert is a Harvard MBA who will be our business development consultant for this project. He has more than 25 years of deep cross-functional commercial and clinical development experience across both small molecules and biologics. Prior to founding StraNexa LLC, Leo held various leadership roles across marketing, business development, and alliance leadership at leading firms such as AstraZeneca, Merck, Astra Merck, and Regeneron. Mr. Adalbert earned his MBA from Harvard Business School while on a fellowship, and holds executive leadership certifications from Northwestern (Kellogg) and the University of Pennsylvania (Wharton). He will interface with government agencies (FDA, BARDA) and investors as appropriate.

Equipment

No new equipment is requested.

Supplies

Materials will be purchased as needed for routine medicinal chemistry, including the following:

	Year 1	Year 2	Year 3	Total
Solvent and reagents:	[redacted]	[redacted]	[redacted]	[redacted]
Consumables lab supplies:				
Glassware:				
Total per year:				

Other Expenses

Contracted Research Organization Expenses: [redacted] over three years

Contract Research Organization (CRO) Expenses – ADMET and PK testing will be performed as in the following schedule. Contractor names are in parentheses, and have all worked effectively with FCCDC in the past.

		year 1		year 2		year 3	
In Vitro ADMET, Alliance Pharm	**Cost**	**# compds or assays**	**total**	**# compds or assays**	**total**	**# compds or assays**	**total**
Metabolic Stability in Mouse Liver Microsomes	[redacted]	15	[redacted]	15	[redacted]	6	[redacted]
Metabolic Stability in Human Liver Microsomes		6		6		10	
Metabolic Stability in rat Liver Microsomes		2		2		2	
Metabolic Stability in Dog Liver Microsomes		2		2		2	
CYP IC50 Using LC-MS-MS in Human Liver Microsomes (3A4, Testostrone)		6		6		4	
CYP IC50 Using LC-MS-MS in Human Liver Microsomes (1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4-Midazolam)		2		2		2	
Mouse Plasma Protein Binding Determined by Equilibrium Dialysis		1		1		1	
Human Plasma Protein Binding Determined by Equilibrium Dialysis		2		2		2	
Stability in Mouse Plasma		2		2		2	
Stability in Human Plasma		2		2		1	
Aqueous Solubilty		6		6		4	
Permeability in MDCK		2		2		1	
Total ADME							
In Vivo PK (Alliance Pharma)		Y		Y2			
PO exposure in Mice		1		1		1	
IV and PO exposure in Mice		6		6		6	
Total Mouse PK							
In Vivo PK (Absorption Sytsems)							
IV/ PO exposure in Rat		1		1		1	
IV /PO exposure in Dog		1		1		1	
Total rat and dog In Vivo PK							
Safety Pharm		Y		Y1			
hErg (Cyprotex)		1		1		1	
Ames test (Cyprotex)		1		1		1	
EuroFins SafteyScreen44 panel		1		1			
EuroFins SafteyScreen87 panel						1	
Total (Saftey Pharm)							
Total ADME/PK and Saftey Pharm							

Indirect Cost and Fee

Indirect costs of 40% are requested. A fee of 7% is requested.

Justification for budget above statutory budget limitations

According to National Institutes of Health SBA-Approved SBIR/STTR Topics for Awards over Statutory Budget Limitations as listed in Appendix A of the omnibus 2020 solicitation (https://sbir.nih.gov/sites/default/files/NIH_Topics_for_Budget_Waivers.pdf), the topic "discovery and development of therapeutics for infectious diseases" (the prime concern of this proposal) is listed in NIAID, Division of Microbiology and Infectious Disease (DMD) as eligible for budgets over the statutory maximum amounts.

Small molecule drug discovery is very expensive and labor intensive, involving the synthesis and evaluation of a large number of test compounds in an iterative fashion. In this STTR Phase II grant application, we embark on a full lead optimization program with the goal of obtaining preclinical drug candidates for the treatment of Marburg, Ebola and related viral infections. Our current lead series are novel and suitable for composition of matter patent protection, and a US patent has issued on the original series which will be supplemented by additional provisional patent applications over time. We expect the optimized compounds at the end of Phase II to have increased potency, and be safe and suitable for oral administration.

LEO J. ADALBERT

GREATER PHILADELPHIA AREA, PA 18901

[redacted] www.stranexa.com [redacted]

BIO-PHARMA EXECUTIVE – MARKETING | STRATEGY | GLOBAL COMMERCIALIZATION | BD

- Dynamic, creative, and strategic business leader with proven expertise in strategic commercialization, go-to-market strategy, product launches, business development, and consulting across all phases of development, inclusive of biologics and small molecules.
- Strong neuroscience, immunology, oncology, and rare disease/Orphan Drug commercialization and scientific expertise across multiple disease areas.
- Outstanding track record of success in identifying and evaluating opportunities, developing a vision for growth, and building differentiated franchises.
- Lead teams to exceed expectations utilizing high initiative, strong team-building skills, creative problem-solving, cross-functional leadership, and collaboration. Harvard MBA (*Fellowship*).

CORE COMPETENCIES

• Partnering & Collaboration • Leadership/Vision • New Product Launches & Promotion
• Opportunity Identification • Customer, Competitor & Market Analysis • Product Development
• Negotiations • Transactions • Capability Creation • Innovation/Medical Science
• Strategic & Tactical Agility • Critical Thinking/Problem Solving • Talent Development
• KOL and Advocacy Group Partnering • Metrics & Governance • Life-Cycle Management

PROFESSIONAL EXPERIENCE AND KEY ACCOMPLISHMENTS

STRANEXA, LLC (www.stranexa.com) (2014-Present) **DOYLESTOWN, PA**
Founder and Principal
Lead bio-pharmaceutical strategic commercialization consulting practice with focus on specialty and rare disease/Orphan Drug markets. Passion for building differentiated brands and capabilities transformative for patients and communities across for-profit and non-profit enterprises through a science-driven, market-led, patient-focused approach.

- Led significant projects across multiple domains that consistently exceeded client expectations (portfolio and commercialization strategy, go-to-market strategy, product launch planning, BD/alliance leadership, competitor and market analyses, communications strategy and publications, non-profit market development).
- Strong scientific foundation across diverse diseases and conditions.
- Expert instructor with Biotech Primer www.biotechprimer.com (*Strategic Commercialization Within BioPharma* and *Drug Development Immersion*).

CSL BEHRING, INC. (2012-2013) **KING OF PRUSSIA, PA**
Global Marketing Director, Immunoglobulin (IgG) Marketing & Strategic Commercialization ('12-13)
Consultant ('12)

Led global marketing strategy, commercialization, launch planning and core tactics for global IgG portfolio with focus on HIZENTRA® and PRIVIGEN®. Led global commercial strategy for CSL362 (hematological tumors).

- Chaired global business team and led major cross-functional initiatives guiding market leadership for key brands (globally PRIVIGEN® revenues up 17.9% to $1B+; HIZENTRA® revenues up 45.2% to $402MM).
- Developed innovative strategies and major initiatives to build market leadership and differentiation.

AXCAN PHARMA, INC. (2008-2011) **BRIDGEWATER, NJ**
Director Business Development and Alliance Management *(company restructured in 2011)*

- Successfully divested global PHOTOFRIN®/PHOTOBARR® oncology drug/device business, exceeding internal objectives (strong cash return, elimination of significant ongoing costs, and placement of commercial and R&D employees in acquiring firm). Led entire transaction process, including prospectus development, buyer identification, business case preparation, due diligence, negotiations and transition planning.
- Created new Alliance Management capability (vision, strategies, metrics, tools and roll-out plan). Effectively managed ongoing and new alliance relationships.
- Selected to lead LACTEOL® Global Product Team, and helped advance critical lifecycle initiatives and global presence in new markets.

LEO J. ADALBERT
[redacted]

PROFESSIONAL EXPERIENCE AND KEY ACCOMPLISHMENTS (CONTINUED)

ASTRAZENECA PHARMACEUTICALS (1999–2008) **WILMINGTON, DE**
Advanced rapidly through a series of increasingly responsible roles, providing effective leadership in driving major commercialization and business development initiatives (multiple recipient of *AstraZeneca Excellence Award*).

Alliance and Program Leader, Strategic Planning & Business Development
- Spearheaded and managed collaboration to commercialize CRESTOR®/fenofibrate fixed-dose combination. *Collaboration progressed six months ahead of schedule and $3.5M under budget.*
- Instrumental in design and implementation of new Alliance Management capability within the firm.

Senior Director, Licensing Commercial Evaluations
Managed commercialization strategy in collaboration with Global Product Teams. Led evaluations of Phase II/III licensing/acquisition opportunities across multiple therapeutic areas.
- Led product and company alliance and acquisition evaluations covering oncology and neuroscience, driving successful progression of target opportunities through formal diligence to deal completion.
- Defined and implemented novel commercialization approaches for various CRESTOR® fixed dose combination opportunities.

Senior Director, US Marketing & Commercial Operations – Oncology & Infection
Directed commercialization strategy, market development, and licensing support for oncology/infection emerging brands portfolio (seven drug candidates from Discovery through Phase III clinical development covering lung, prostate, breast, and colorectal cancers).
- Directed overall strategic direction, including creation of integrated target product profiles and patient-centered claims across entire brand portfolio encompassing multiple pathways (SRC kinase, VEGF/EGF, endothelin, vascular targeting agents, and prenylation inhibitors.)
- Proactively identified new business opportunities and resolved strategic gaps with global product teams to consistently maintain momentum through key decision-points.

Transaction Director, Licensing – North America
Led transaction process through all stages involving analyzing, negotiating, closing, and managing transitions of select partnership opportunities (product divestments, co-promotions, and in-licenses.)
- Directed all facets of buyer identification, business case prep, due diligence, negotiations, and transition planning. Successfully closed multiple divestment transactions under tight timelines, delivering significant operating income, tax benefits, and business efficiencies based on significant premiums to retention values.
- Consistently earned Distinguished/Excellent performance ratings (top 2%) and achieved "*Deal of the Year*" recognition.

EARLIER RELEVANT EXPERIENCE AND KEY ACCOMPLISHMENTS

ELAN PHARMACEUTICALS, INC. **SAN FRANCISCO, CA**
Director, Neuromuscular Marketing *(business restructured via SEC obligations)*
Developed and led execution of strategic and tactical marketing plans to effectively support all stages of lifecycle/market entry. Developed comprehensive product pre-launch and launch deployment plans that expanded Élan's neuromuscular franchise into attractive new market segments.

ASTRA-MERCK PHARMACEUTICALS, INC. *(JV dissolved)* **WAYNE, PA**
Director, Neuroscience Marketing & Business Strategy
Marketing Manager, Neuroscience ('96-'97)
Directed franchise development of Astra-Merck's neuroscience business as commercial lead in formulating strategic direction and integration activities with Astra Sweden corporate portfolio.
- Established strategic alliances and launched market development activities with patient advocacy groups, medical associations, and payers. Recognized with Leadership Award by Huntington's Disease Society.
- Recipient of ***Astra-Merck Leadership Award for Excellence*** for development and implementation of an innovative, comprehensive business and licensing strategy encompassing multiple neurological and psychiatric diseases, indications and product opportunities globally.

LEO J. ADALBERT
[redacted]

EARLIER RELEVANT EXPERIENCE AND KEY ACCOMPLISHMENTS (CONTINUED)

RHONE-POULENC RORER PHARMACEUTICALS, INC. **COLLEGEVILLE, PA**
Senior Product Manager – U.S.
Led market development and successful introduction of RILUTEK®, the *first* and *only* medical therapy approved for the treatment of amyotrophic lateral sclerosis (Lou Gehrig's Disease) prolonging survival. *Achieved 95% formulary acceptance within six months and sales 121% to plan*.

REGENERON PHARMACEUTICALS, INC. **TARRYTOWN, NY**
Manager, Market Development
Established commercial direction for the company's first biologic product CNTF (ciliary neurotrophic factor) for the treatment of multiple neurodegenerative diseases. (*Product failed in late-Phase III clinical trials.)*

MERCK HUMAN HEALTH U.S. – *a Division of Merck & Company* **WEST POINT, PA**

Senior Promotion Manager – *Promoted from Promotions Manager*
Directed $20M product promotion and market development pre-launch and launch program. Drove successful introduction of PROSCAR® in the U.S., the first medical therapy treatment of benign prostatic enlargement.
- *Physician and DTC Program acknowledged with several industry awards; achieved 119% to plan.*

Marketing Analyst – *Promoted from Associate Marketing Analyst*
Designed, implemented, and analyzed strategic and product-specific marketing research.
- Developed and presented recommendations to senior management based on research findings, *resulting in >$20M in media efficiencies.*
- Recognized with ***Merck Leadership Award of Excellence*** for developing methodology to analyze the impact of changes in promotion mixes and budgets that *decreased planning time and increased efficiencies by 33%.*

Professional Sales Representative, Western Pennsylvania Territory
Managed $4 million office and hospital sales territory. Conducted educational forums, and presented product and clinical information to nurses, pharmacists and physicians in primary care, cardiology, neurology, orthopedics and ophthalmology. (*Achieved 124% of goal)*

EDUCATION & PROFESSIONAL DEVELOPMENT

HARVARD UNIVERSITY GRADUATE SCHOOL OF BUSINESS ADMINISTRATION **BOSTON, MA**
Master of Business Administration (MBA) - Concentration: Marketing & Finance
Awarded Harvard Business School Fellowship

WEST VIRGINIA UNIVERSITY **MORGANTOWN, WV**
Bachelor of Science in Business Administration – *Highest Honors (Summa Cum Laude) / Rhodes Scholarship Finalist*
Beta Gamma Sigma National Business Honor Society; *Phi Kappa Phi* & *Mortar Board* National Honor Societies; *Phi Sigma Kappa* Fraternity (*Repeat Merit Scholar*); Varsity Track Team (*400 meters/relay*).

CONTINUING EDUCATION:

- Harvard Medical School HMX Program (completed with *Achievement Honors* Pharmacology and Immunology; Genetics and Physiology in progress to complete 2019)
- Villanova University/Bucks County Community College – Completion of full pre-med/health sciences program with labs (anatomy/chemistry/biology) - *recognized with highest honors and selected for membership in Phi Theta Kappa*
- Harvard School of Public Health (TH Chan School)
  - *Measurement, Design, and Analysis Methods for Health Economics Research*
  - *Navigating the US Bio-Pharmaceutical Sector – Policy, Politics, Pricing, and Payment*
  - *Leading in Health Systems: Integrating Effort, Improving Outcomes*

LEO J. ADALBERT
[redacted]

EDUCATION & PROFESSIONAL DEVELOPMENT (CONTINUED)

- Northwestern University Graduate School of Management: *Graduate of Kellogg Executive Scholar Program*
- Wharton School (University of Pennsylvania): *Graduate of Professional Development Program*
- Center for Creative Leadership: *Leadership Development Program*
- Dartmouth: *Global Leaders Program*
- GAP International: *Leadership for Breakthrough Outcomes*
- Harvard Law School: *Negotiations Strategy Program*
- Merck & Company: *Strategic Marketing Plan Development*
- MIT: *Systems Thinking Program*

CERTIFICATIONS: Certified Licensing Professional & Certified Strategic Alliance Professional

COMMUNITY / PERSONAL

- Special Projects Leader – Castleman Disease Collaborative Network (CDCN) www.cdcn.org
- Co-Creator & Executive Board member of Camden, NJ "*Dream Center*" (youth community outreach center) www.camdendreamcenter.org
- Graduate of Chester County non-profit Leadership Development Program (*partnership with United Way*)
- Competitive masters track and field athlete (400 and 800 meters) and road cyclist

Quotation Date: 10 August 2020 **Quotation Number: APQ-19-0548**

Sponsor	**Alliance Pharma, Inc.**
Jay Wrobel, PhD Vice President, Academic Relations Fox Chase Chemical Diversity Center [redacted] Doylestown, PA 18902 Phone: [redacted] Mobile: [redacted] E-mail: [redacted]	Ryan Klein, PhD Director, Business Development [redacted] Malvern, PA 19355 Mobile: [redacted] Phone: [redacted] Fax: [redacted] Email: [redacted] Web: [redacted]

Non-GLP ADME Studies of Compounds for Fox Chase Chemical Diversity Center (Three Year Plan)

1 Plasma Protein Binding Study By Rapid Equilibrium Dialysis

1.1 Scope of Service

To assess the protein binding of compounds in mouse and human plasma using a rapid equilibrium dialysis (RED) device (Thermo Fisher Scientific).

1.2 General Procedures

The test compounds will be spiked into plasma samples to achieve a final concentration of 5 µM. Triplicate aliquots of the spiked plasma will be added to the red chambers of the RED device inserts, and phosphate buffered saline will be added into the white chambers of the inserts. The RED device plate will be sealed and incubated for 4 hours at 37°C with shaking. Warfarin, a known highly bound drug in plasma, will be tested as the positive control for this assay.

After incubation, samples from both the red and white chambers will be analyzed by LC-MS/MS. The percentages of free and bound fractions will be determined using the peak area ratios of each analyte to internal standard.

1.3 General Information and Sample Requirement

- Molecular weights, including formula weights and exact mass units, of the compounds.
- Sufficient amount of the compounds.
- Stability information for the compounds such as light, temperature, and solvents, if available.
- Safety information for handling the compounds.
- Solubility of the compounds, if available.

1.4 Deliverable

A Microsoft Excel summary report including raw data, methodology, calculated percent bound, and percent recovery values will be provided.

1.5 Turnaround Time

Report will be delivered within 7 business days from receipt of the compounds and any additional items specific to the assay that need to be sourced externally.

1.6 Documentation/Records

Data from this non-GLP study will be preserved for 1 year after submission of the results to the Sponsor.

1.7 Fee Schedule

The cost of plasma protein binding analysis is [redacted] per compound.

Item		Year 1	Year 2	Year 3	Cost
Human Plasma Binding	Number of compounds	2	2	2	[redacted]
	Cost	[redacted]	[redacted]	[redacted]	
Mouse Plasma Binding	Number of compounds	1	1	1	[redacted]
	Cost	[redacted]	[redacted]	[redacted]	

Total: [redacted]

2 Metabolic Stability Studies (Intrinsic Clearance) in Liver Microsomes

2.1 Scope of Service

To assess the relative metabolic stability (intrinsic clearance) of compounds using mouse, rat, canine and human liver microsomes.

2.2 General Procedures

The test compounds at a concentration of 0.5 µM will be incubated with 0.5 mg/mL of liver microsomes and an NADPH-regenerating system (cofactor solution) in potassium phosphate buffer (pH 7.4). At 0, 5, 15, 30, and 45 minutes, an aliquot will be taken, and reactions will be quenched with an acetonitrile solution containing an internal standard. Additionally, controls will be measured that do not contain the cofactor solution. Following completion of the experiment, samples will be analyzed by LC-MS/MS. Results will be reported as peak area ratios of each analyte to internal standard. The intrinsic clearance (CL_{int}) will be determined from the first-order elimination constant by nonlinear regression.

2.3 General Information and Sample Requirement

- Molecular weights, including formula weights and exact mass units, of the compounds.
- Sufficient amounts of the compounds.
- Stability information for the compounds such as light, temperature, and solvents, if available.
- Safety information for handling the compounds.
- Solubility of the compounds, if available.

2.4 Deliverables

A Microsoft Excel summary report of intrinsic clearance (CL_{int}), half-life ($t_{1/2}$), rate constant, peak area ratios of each analyte to internal standard, and graphic profiles will be provided.

2.5 Turnaround Time

Report will be delivered within 7 business days from Sponsor's approval of the study and receipt of all necessary information and assay-specific materials/reagents by Alliance Pharma.

2.6 Documentation/Records

Data from this non-GLP study will be preserved for 1 year after submission of the results to the Sponsor.

2.7 Fee Schedule

The cost of metabolic stability analysis using liver microsomes is [redacted] per compound.

Item		Year 1	Year 2	Year 3	Cost
Microsomal Clearance (Mouse)	Number of compounds	15	15	6	[redacted]
	Cost	[redacted]	[redacted]	[redacted]	
Microsomal Clearance (Rat)	Number of compounds	2	2	2	[redacted]
	Cost	[redacted]	[redacted]	[redacted]	
Microsomal Clearance (Dog)	Number of compounds	2	2	2	[redacted]
	Cost	[redacted]	[redacted]	[redacted]	
Microsomal Clearance (Human)	Number of compounds	6	6	10	[redacted]
	Cost	[redacted]	[redacted]	[redacted]	
				Total:	[redacted]

3 Direct Cytochrome P450 (CYP 3A4) Inhibition Study

3.1 Scope of Service

To evaluate the direct inhibition potential of compounds on the major human hepatic CYP3A4 isoform using pooled human liver microsomes and the isoform-specific probe substrate testosterone.

3.2 General Procedures

Pooled human liver microsomes will be used for this study. Incubation mixtures will contain *a)* potassium phosphate buffer, *b)* 0.1 mg/mL microsomal protein; *c)* CYP3A4 probe substrate testosterone (12.5 µM); *d)* specific concentrations of test compounds, blank solvent or positive controls; and (*e*) an NADPH-regenerating cofactor solution. The mixtures will be incubated at 37°C and 5% CO_2 with orbital shaking at 200 rpm for 5 minutes. Upon completion of the incubation, the reactions will be quenched with an acetonitrile solution containing an internal standard. The samples will be prepared and analyzed by LC-MS/MS to monitor for substrate metabolite formation. The enzyme activity in the presence of the compounds will be normalized with the enzyme activity in the absence of the compounds (controls) and expressed as a percentage of activity. The inhibitory potential (IC50) of the compounds will be determined using nonlinear regression.

3.3 General Information and Sample Requirement

- Molecular weights, including formula weights and exact mass units, of the compounds.
- Sufficient amounts of the compounds.
- Stability information for the compounds such as light, temperature, and solvents, if available.
- Safety information for handling the compounds.
- Solubility of the compounds, if available.

3.4 Deliverables

A Microsoft Excel summary report including IC_{50} summary tables and plots of test compound concentration versus peak area ratios will be provided.

3.5 Turnaround Time

Report will be delivered within 8 business days from Sponsor's approval of the study and receipt of all necessary information and assay-specific materials/reagents by Alliance Pharma.

3.6 Documentation/Records

Data from this non-GLP study will be preserved for 1 year after submission of the results to the Sponsor.

3.7 Fee Schedule

The cost of CYP inhibition analysis is [redacted] per compound, per probe substrate (one isoform-specific probe substrate used).

Item		Year 1	Year 2	Year 3	Cost
Direct CYP IC_{50} (1 substrate)	Number of compounds	6	6	4	[redacted]
	Cost	[redacted]	[redacted]	[redacted]	

Total: [redacted]

4 Direct Cytochrome P450 (CYP) Inhibition Study – Full Panel

4.1 Scope of Service

To evaluate the direct inhibition potential of compounds on 7 major human hepatic cytochrome P450 (CYP) isoforms (1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4) using pooled human liver microsomes and the isoform-specific probe substrates.

4.2 General Procedures

Pooled human liver microsomes will be used for this study. The incubation mixtures will contain the following: (*a*) potassium phosphate buffer; (*b*) 0.1 mg/mL microsomal protein; (*c*) isoform-specific probe substrates; (*d*) specific concentrations of the test compounds, blank solvent, or positive controls (isoform-specific inhibitors); and (*e*) an NADPH-regenerating cofactor solution. **Table 1** below lists the inhibitors, substrates, and associated concentrations that will be used for each incubation.

Table 1: Isoform-Specific Probe Substrates and Inhibitors

CYP Isoform	Inhibitor	Final Concentrations (µM)	Substrate	Final Concentration (µM)
1A2	Fluvoxamine	0.0033, 0.001, 0.033, 0.1, 0.33, 1, 3.3	Phenacetin	35
2B6	Clopidogrel	0.033, 0.1, 0.33, 1, 3.3, 10, 33	Bupropion	50
2C8	Quercetin	0.033, 0.1, 0.33, 1, 3.3, 10, 33	Amodiaquine	1.5
2C9	Sulfaphenazole	0.0033, 0.01, 0.033, 0.1, 0.33, 1, 3.3	Diclofenac	5
2C19	Ticlopidine	0.033, 0.1, 0.33, 1, 3.3, 10, 33	*S*-mephenytoin	25
2D6	Quinidine	0.0033, 0.01, 0.033, 0.1, 0.33, 1, 3.3	Dextromethorphan	5
3A4	Ketoconazole	0.00033, 0.001, 0.0033, 0.01, 0.033, 0.1, 0.33	Midazolam	1.25

The mixtures will be incubated at 37°C and 5% CO_2 with orbital shaking at 200 rpm for 5 minutes with reactions containing midazolam, for 10 minutes with reactions containing phenacetin, bupropion, diclofenac, or dextromethorphan, and for 20 minutes with reactions containing amodiaquine or *S*-mephenytoin. Upon completion of the incubation, the reactions will be quenched with an acetonitrile solution containing an internal standard. The samples will be prepared and analyzed by LC-MS/MS to monitor for substrate metabolite formation. The enzyme activity in the presence of the compounds will be normalized with the enzyme activity in the absence of the compounds (controls) and expressed as a percentage of activity. The inhibitory potential (IC_{50}) of the compounds will be determined using nonlinear regression.

4.3 General Information and Sample Requirement

- Molecular weights, including formula weights and exact mass units, of the compounds.
- Sufficient amounts of the compounds.
- Stability information for the compounds such as light, temperature, and solvents, if available.
- Safety information for handling the compounds.
- Solubility of the compounds, if available.

4.4 Deliverables

A Microsoft Excel summary report including IC_{50} summary tables and plots of test compound concentration versus peak area ratios will be provided.

4.5 Turnaround Time

Report will be delivered within 8 business days from Sponsor's approval of the study and receipt of all necessary information and assay-specific materials/reagents by Alliance Pharma.

4.6 Documentation/Records

Data from this non-GLP study will be preserved for 1 year after submission of the results to the Sponsor.

4.7 Fee Schedule

The cost of the full-panel CYP inhibition assay is [redacted] per compound ([redacted] per compound, per probe substrate).

Item		Year 1	Year 2	Year 3	Cost
Direct CYP IC_{50} (7 substrates)	Number of compounds	2	2	2	[redacted]
	Cost	[redacted]	[redacted]	[redacted]	
				Total:	[redacted]

5 Plasma Stability Studies

5.1 Scope of Service

To assess the relative stability of compounds in mouse and human plasma.

5.2 General Procedures

Test compounds will be incubated at a concentration of 1 μM in plasma samples. At 0, 15, 30, 60 and 120 minutes, an aliquot will be removed and reactions will be quenched with an acetonitrile solution containing an internal standard. Following completion of the experiment, samples will be analyzed by LC-MS/MS. Results will be reported as peak area ratios of analyte to internal standard.

5.3 General Information and Sample Requirement

- Molecular weights, including formula weights and exact mass units, of the compounds.
- Sufficient amounts of the compounds.
- Stability information for the compounds such as light, temperature, and solvents, if available.
- Safety information for handling the compounds.
- Solubility of the compounds, if available.

5.4 Deliverables

A Microsoft Excel summary report including half-life ($t_{1/2}$), elimination rate constant, peak area ratios of each analyte to internal standard, and graphic profiles will be provided.

5.5 Turnaround Time

Report will be delivered within 6 business days from Sponsor's approval of the study and receipt of all necessary information and assay-specific materials/reagents by Alliance Pharma.

5.6 Documentation/Records

Data from this non-GLP study will be preserved for 1 year after submission of the results to the Sponsor.

5.7 Fee Schedule

The cost of plasma stability analysis is [redacted] per compound.

Item		Year 1	Year 2	Year 3	Cost
Mouse Plasma Stability	Number of compounds	2	2	2	[redacted]
	Cost	[redacted]	[redacted]	[redacted]	
Human Plasma Stability	Number of compounds	2	2	1	[redacted]
	Cost	[redacted]	[redacted]	[redacted]	
				Total:	[redacted]

6 Aqueous Solubility Study in Phosphate-Buffered Saline

6.1 Scope of Service

To assess the solubility of compounds in phosphate-buffered saline (PBS).

6.2 General Procedures

Approximately 1 mL of PBS buffer (pH 7.4) will be added to approximately 10 mg of each test compound. The solutions will be sonicated for 30 minutes and vortexed at a low speed for at least 30 minutes. The solutions will then sit at room temperature for 16-28 hours. After such time, the solutions will be filtered through a 0.22-micron filter followed by diluting 1:10, 1:100, and 1:1000, and 1:10,000 in triplicate in 50% acetonitrile in water (1:1, v/v). The final sample solutions will be mixed with an internal standard solution (1:1) followed by LC MS/MS analysis. Solubility will be determined according to the calibration curve generated from 5 concentration standards.

6.3 General Information and Sample Requirement

- Molecular weights, including formula weights and exact mass units, of the compounds.
- Approximately 10 mg of each compound (minimum requirement).
- Stability information for the compounds such as light, temperature, and solvents, if available.
- Safety information for handling the compounds.
- Solubility of the compounds, if available.

6.4 Deliverables

A Microsoft Excel summary report including standard curve information and sample solubility data will be provided.

6.5 Turnaround Time

Report will be delivered within 7 business days from receipt of compounds by Alliance Pharma.

6.6 Documentation/Records

Data from this non-GLP study will be preserved for 1 year after submission of the report to the Sponsor.

6.7 Fee Schedule

The cost of aqueous solubility analysis is [redacted] per compound.

Item		Year 1	Year 2	Year 3	Cost
Aqueous Solubility	Number of compounds	6	6	4	[redacted]
	Cost	[redacted]	[redacted]	[redacted]	
				Total:	[redacted]

7 Permeability in MDCKII-MDR1 cells

7.1 Scope of Service

To assess the permeability of compounds in MDCKII-MDR1 cells.

7.2 General Procedures

The permeability of the test compound in the MDCKII-MDR1 cell monolayer system will be assessed in duplicate in both apical to basolateral (A→B) and basolateral to apical (B→A) directions.

MDCK cells will be seeded onto multiwell permeable supports in culture medium. The permeability study will be conducted approximately 4 days post-seeding. The Transepithelial Electrical Resistance (TEER) of the cell monolayer will serve as a quality control for monolayer integrity. On the day of the transport assay, TEER will be measured in all wells.

Transport medium will be added to the basolateral and apical sides of the system for a 30-minute incubation to wash the monolayer. After removing transport medium, receiving solution and compound-specific dosing solution (containing Lucifer yellow) will be added to the appropriate wells of the system for A→B and B→A analysis. The multiwell system will be incubated at 37°C with 5% CO_2. After incubation, samples will be collected from both the apical and basolateral sides of the system for LC-MS/MS analysis. Lucifer yellow will be fluorometrically determined in all receiving samples and will serve as an in-well quality control for monolayer integrity.

7.3 General Information and Sample Requirements

Sponsor shall provide the following:

- Molecular weights, including formula weights and exact mass units, of the compounds.
- Sufficient amount of each compound.
- Stability information (e.g., light, temperature, and solvents) for the compounds, if available.
- Safety information for handling the compounds.
- Solubility of the compounds, if available.

7.4 Deliverables

A spreadsheet summary report (Microsoft Excel file) will be provided that includes the permeability coefficient of the compound from basolateral side to apical side ($P_{app,\ B\ to\ A}$) and from apical side to basolateral side ($P_{app,\ A\ to\ B}$).

7.5 Turnaround Time

The spreadsheet summary report will be delivered to the Sponsor within approximately 11 business days from Sponsor's approval of the study and receipt of all necessary information and assay-specific materials/reagents by Alliance Pharma.

7.6 Documentation/Records

Data from this non-GLP study will be preserved for 1 year after submission of the results to the Sponsor.

7.7 Fee Schedule

The cost of MDCKII-MDR1 permeability analysis is [redacted] per compound.

Item		Year 1	Year 2	Year 3	Cost
Permeability	Number of compounds	2	2	1	[redacted]
	Cost	[redacted]	[redacted]	[redacted]	
				Total:	[redacted]

8 Non-GLP *in vivo* Pharmacokinetic Studies in Balb/C Mice by PO Administration

8.1 Scope of Service

To provide the services of in-life animal dosing and sample bioanalysis for pharmacokinetic studies conducted in mice.

8.2 General Procedures

One group of 21 mice (n=3 mice per time point) will be administered an oral (PO) dose of the compound of interest. Dosing formulation will be prepared according to Sponsor-provided protocol. Terminal blood plasma samples will be collected from each animal at 7 established time points within a 24 hour period. After all study samples are collected, the samples will be analyzed by LC-MS/MS. Calculated concentrations will be reported to the Sponsor.

8.3 General Information and Sample Requirement

- Predicted concentration range and/or dosing, C_{max} information, if available
- Compound molecular weight and its purity information
- Sufficient amount of standard compound
- Sample stability information, such as light sensitivity, temperature and solvent, if available
- Safety information for handling compounds/samples
- Solubility of the compound, if available

8.4 Deliverables

A report of abnormal in-life observations will be provided to the Sponsor, if applicable. A Microsoft Excel spreadsheet summary of sample analysis results will be delivered to the Sponsor.

8.5 Turnaround Time

The pharmacokinetic study will be completed and results will be delivered within approximately 10 business days from receipt of the test compound and all other necessary study materials.

8.6 Documentation and Records

Data from this non-GLP study will be preserved for 1 year after submission of the report to the Sponsor.

8.7 Fee Schedule

The cost of the in vivo pharmacokinetic study (dosing and bioanalysis) is [redacted] per compound.

Item		Year 1	Year 2	Year 3	Cost
In Vivo PO PK in Mice	Number of compounds	1	1	1	[redacted]
	Cost	[redacted]	[redacted]	[redacted]	
				Total:	[redacted]

9 Non-GLP *in vivo* Pharmacokinetic Studies in Balb/C Mice by IV/PO Administration

9.1 Scope of Service

To provide the services of in-life animal dosing and sample bioanalysis for pharmacokinetic studies conducted in mice.

9.2 General Procedures

Two groups of 21 mice (n=3 mice per time point) will be administered an intravenous (IV) or oral (PO) dose of the compound of interest. Dosing formulation will be prepared according to Sponsor-provided protocol. Terminal blood plasma samples will be collected from each animal at 7 established time points within a 24 hour period. After all study samples are collected, the samples will be analyzed by LC-MS/MS. Calculated concentrations will be reported to the Sponsor.

9.3 General Information and Sample Requirement

- Predicted concentration range and/or dosing, C_{max} information, if available
- Compound molecular weight and its purity information
- Sufficient amount of standard compound
- Sample stability information, such as light sensitivity, temperature and solvent, if available
- Safety information for handling compounds/samples
- Solubility of the compound, if available

9.4 Deliverables

A report of abnormal in-life observations will be provided to the Sponsor, if applicable. A Microsoft Excel spreadsheet summary of sample analysis results will be delivered to the Sponsor.

9.5 Turnaround Time

The pharmacokinetic study will be completed and results will be delivered within approximately 10 business days from receipt of the test compound and all other necessary study materials.

9.6 Documentation and Records

Data from this non-GLP study will be preserved for 1 year after submission of the report to the Sponsor.

9.7 Fee Schedule

The cost of the in vivo pharmacokinetic study (dosing and bioanalysis) is [redacted] per compound.

Item		Year 1	Year 2	Year 3	Cost
In Vivo IV/PO PK in Mice	Number of compounds	6	6	6	[redacted]
	Cost	[redacted]	[redacted]	[redacted]	
			Total:	**Total:**	[redacted]

10 Total Fee Schedule

Assay	Year 1	Year 2	Year 3
1. Plasma Protein Binding	[redacted]	[redacted]	[redacted]
2. Microsomal Clearance	[redacted]	[redacted]	[redacted]
3. Direct CYP IC50 (3A4 - Testosterone)	[redacted]	[redacted]	[redacted]
4. Direct CYP IC50 (1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4-Midazolam)	[redacted]	[redacted]	[redacted]
5. Plasma Stability	[redacted]	[redacted]	[redacted]
6. Aqueous Solubility	[redacted]	[redacted]	[redacted]
7. Permeability	[redacted]	[redacted]	[redacted]
8. In Vivo PK in Mice (PO)	[redacted]	[redacted]	[redacted]
9. In Vivo PK in Mice (IV/PO)	[redacted]	[redacted]	[redacted]
Annual Total	[redacted]	[redacted]	[redacted]
Grand Total	[redacted]		

TERMS AND CONDITIONS

1. SERVICES

1.1 Compliance — Alliance Pharma shall perform itself or, as the case may be, through its affiliate(s) for the benefit of Sponsor, the Services described herein in accordance with, as applicable: (i) this Proposal; (ii) applicable standard operating procedures; (iii) current standards of Good Laboratory Practice/Good Clinical Practice (GLP/GCP) as applicable in the country where the Services are performed; and (iv) any other applicable professional standards, codes, guidelines, laws, rules, and regulations, including, without limitation, those of any regulatory authority or agency having jurisdiction over the Services (collectively, the "***Applicable Laws***"). **Performance of such Services shall only be initiated after the full execution of this Proposal**.

1.2 Mutual non-solicitation — During the term of this Proposal and for a two (2) year period thereafter, neither party nor its respective affiliates shall, directly or indirectly: (i) recruit, solicit or induce, or attempt to recruit, solicit or induce, any employee of the other party to terminate his or her employment or engagement with the other party, or (ii) hire any employee of the other party (including, without limitation, employees of Alliance Pharma that are providing services to Sponsor on behalf of Alliance Pharma at the time of hiring).

NOTE.—This Proposal is valid **for sixty (60) calendar days** from quotation date.

2. CHEMICAL AND BIOLOGICAL SAMPLES

2.1 Delivery — Sponsor shall deliver to Alliance Pharma, at Sponsor's risk and expense and at the location of Alliance Pharma identified in this Proposal or to such other location of Alliance Pharma as identified to Sponsor in writing, the "***Chemical Samples***" and "***Biological Samples***" in sufficient and necessary quantities for Alliance Pharma to perform the Services in compliance with the provisions of Section 1.1 above and as required under the Applicable Laws, along with instructions for the handling, storage, and safety requirements and conditions of such Chemical or Biological Samples. Alliance Pharma will receive shipment(s) of Chemical or Biological Samples only if the equipment required to safely process the samples exists at Alliance Pharma.

2.2 Restricted Use — Alliance Pharma shall neither use nor analyze any Chemical or Biological Samples for any purposes other than to perform the Services without Sponsor's prior written consent.

2.3 Storage and Disposal of Chemical Samples— Alliance Pharma will store the Chemical Samples at the appropriate storage conditions as directed by the Sponsor for 3 months after submission of the final deliverable hereunder. Alliance Pharma will dispose of the Chemical Samples following the 3-month expiration date, unless requested otherwise by the Sponsor in writing prior to said date.

2.4 Storage, Return or Destruction of Biological Samples— For regulatory (e.g., GLP/GCP) studies, Alliance Pharma will store the Biological Samples at the appropriate storage conditions as directed by the Sponsor for 6 months after submission of the final service deliverable herein. **Alliance Pharma will continue to store the Biological Samples following the 6-month expiration date at the rate of US $0.50 per Biological Sample per month,** unless otherwise directed by the Sponsor in writing prior to said date. Sponsor will retain the option of having the Biological Samples destroyed, stored under Alliance Pharma's responsibility, or returned to Sponsor at Sponsor's expense. An authorization request form will be sent to Sponsor at sponsor's request. For non-regulated studies, Alliance Pharma will destroy Biological Samples **two (2) weeks after the Sponsor acknowledges receipt of final Service deliverables** unless otherwise requested by the Sponsor in writing prior to such date.

If Biological Samples are received by Alliance Pharma that are not intended for bioanalysis under the scope of this Proposal, such as back up samples, the Sponsor will be contacted and offered the option of having the Biological Samples destroyed, stored under Alliance Pharma's responsibility, or returned to the Sponsor at Sponsor's expense. If stored under Alliance Pharma's responsibility, the samples, either regulatory or non-regulatory, will be stored and invoiced at the rate of **US $0.50 per Biological Sample per month.**

All sample storage fees are based on standard 1.2 – 2 mL cryogenic storage vials with a maximum height of 49 mm and diameter of 13.5 mm. Larger sample containers may incur additional fees to be agreed upon between the parties to this Proposal.

3. BUDGET AND PAYMENT TERMS

3.1 Service Fees — In accordance with the following, Sponsor shall provide Alliance Pharma with a payment amount corresponding to the Service fees identified herein. The per-sample pricing provided in this Proposal is based on the number of samples requested by the Sponsor. Pricing may be renegotiated if the sample count decreases from the quoted count by more than 20%. Sample re-assays/repeats requested by Sponsor, dilutional rea-assays, and incurred sample reanalysis will be invoiced at the sample unit rate. Re-assays/repeats due to failure to meet the pre-defined criteria will be at no charge to Sponsor. However, any special requirements from the Sponsor not included in such budget will be invoiced to the Sponsor at additional cost.

3.2 Pass-Through Costs — Pricing includes all standard laboratory consumables; however, the cost of non-standard items (e.g. chromatographic columns, reference standards, internal standards, SPE plates, commercial ELISA kits, enzymes, specific reagents etc.) purchased by Alliance Pharma will be passed-through to the study sponsor. The fees identified herein may include estimated costs that are expected at the start of the study; however, additional materials may be required. All pass-through costs are subject to a mark-up fee for purchasing, handling and accounting services.

3.3 Installments - Alliance Pharma will invoice **upon delivery of the first draft report for each study**. (Any additional authorized dollars from contract modifications, if any, will be added to the next billing cycle.)

3.4 Payment Terms — Sponsor shall pay each invoice **within thirty (30) days** of receipt thereof. Any amount owed by Sponsor under this Proposal that is not received by Alliance Pharma on or before the date due shall bear interest at a per annum rate of two percent (2%) above the prime rate in effect at Bank of America on the date due. Sponsor shall also pay all reasonable collection costs at any time incurred by Alliance Pharma in obtaining payment of amounts past due, including reasonable attorneys' fees. If any portion of an invoice is disputed, the Sponsor shall pay the undisputed amounts as set forth therein, and the parties shall use good faith efforts to reconcile the disputed portion of the invoice within thirty (30) days of receipt by Sponsor of the applicable invoice.

IN WITNESS WHEREOF, this Agreement has been accepted and executed by duly authorized representatives of both parties as follows:

Alliance Pharma Inc.		**SPONSOR: Fox Chase Chemical Diversity Center**	
By:	_____	**By:**	_____
Print Name:	Ryan Klein, PhD	**Print Name:**	_____
Title	Director, Business Development	**Title:**	_____
Date:	_____	**Date:**	_____

Contact PD/PI: Harty, Ronald N.

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Quotation

Customer Delivery Information

Fox Chase Chemical Diversity Center Inc
[redacted]
2, Doylestown, PA 18902, United States
Name: Jay Wrobel
Phone: [redacted]
Email: [redacted]

Customer Invoice Information

Fox Chase Chemical Diversity Center Inc
[redacted] Doylestown, PA 18902, United States
Name: Accounts Payable
Phone: [redacted]
Email: [redacted]

Quotation Number	Study Code	Customer Account No	Eurofins Contact
[redacted]	[redacted]	[redacted]	Andrew Lord
Date Issued	**Customer reference**	**Payment Terms**	**Eurofins Contact Phone**
04-Aug-2020		30 Days	[redacted]
Quote Valid Until	**Promo Code**	**Loyalty Category**	**Eurofins Contact Email**
03-Sep-2020		Standard	[redacted]

Summary

Price	Discount	Charges	Net Price	Sales tax	Grand Total Price	Currency
[redacted]	0.00	0.00	[redacted]	0.00	[redacted]	USD

Details

Item #	Description	Assay Mode	Cmpd	Conc	Rep	Qty	Option	TAT*	Unit price	Disc unit price	Net Price
PP241	SafetyScreen44 Panel, Panlabs	Flexible	1	1	2	1.00	Standard	10	[redacted]	[redacted]	[redacted]
Special Requirement: Default test concentrations 10 uM											

Cmpd = compounds; Conc = Concentrations; Rep = Replicates; Qty = Quantity; TAT = Turnaround Time.
*please see “Delivery Dates, Turnaround Time” section of Terms and Conditions (web link) for more details.

Additional Charges	0.00

Special Instructions: N/A

Orders can be placed by purchase order or credit card.

Purchase orders should detail the following company address:

Eurofins Panlabs Discovery Services Taiwan Ltd
[redacted]
New Taipei City, Taiwan 24891

Please note that the address for sending check payment is as following:
[redacted]
Birmingham, AL 35246-5588

Please note that if arranging payment from a bank outside of Taiwan, any intermediary or sender’s charges should be borne by the sender.

AUTHORIZATION AND ACCEPTANCE

Submitting a purchase order with reference to this quotation number or sending a scanned copy of the signed quotation to the address stated below, is considered an acceptance of prices and all other special conditions listed on the quotation.

Client Services
Email: [redacted]
Fax: [redacted]

By authorization and acceptance of this quotation as indicated above, authorizes Eurofins to perform the work as described in this quotation, subject to the terms and conditions of the MSA by and between the Client and Eurofins or its affiliate, if any, or if none, or in the event that the MSA is not active or found to be invalid as of the date hereof, then the work described in this quotation shall be subject to the then current version of the Eurofins General Terms & Conditions of Sale of Products and Services, as amended from time to time, which can be viewed at the following link: https://www.eurofinsdiscoveryservices.com/cms/cms-content/misc/tc/order-terms-condition-products-and-services-dec19/. This quotation constitutes a separate and distinct contract between Client and Eurofins. In no event shall an affiliate of Eurofins be liable for its obligations under this quotation, and Client shall look exclusively to Eurofins in relation to any rights or remedies it may have with respect to this quotation.

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Quotation

AGREED TO AND ACCCEPTED

For: Fox Chase Chemical Diversity Center Inc

By: _____

Name: _____

Title: _____

Date: _____

Eurofins Panlabs Discovery Services Taiwan, Ltd.

[redacted] Wugu District, New Taipei City, 24891, Taiwan

Phone +[redacted] Fax +[redacted]

www.eurofinspdiscoveryservices.com

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Quotation

Customer Delivery Information

Fox Chase Chemical Diversity Center Inc
3805 Old Easton Road
[redacted] PA 18902, United States
Name: Jay Wrobel
Phone: [redacted]
Email: [redacted]

Customer Invoice Information

Fox Chase Chemical Diversity Center Inc
[redacted] Doylestown, PA 18902, United States
Name: Accounts Payable
Phone: [redacted]
Email: [redacted]

Quotation Number	Study Code	Customer Account No	Eurofins Contact
[redacted]	[redacted]	[redacted]	Andrew Lord
Date Issued	**Customer reference**	**Payment Terms**	**Eurofins Contact Phone**
04-Aug-2020		30 Days	[redacted]
Quote Valid Until	**Promo Code**	**Loyalty Category**	**Eurofins Contact Email**
03-Sep-2020		Standard	[redacted]

Summary

Price	Discount	Charges	Net Price	Sales tax	Grand Total Price	Currency
[redacted]	0.00	0.00	[redacted]	0.00	[redacted]	USD

Details

Item #	Description	Assay Mode	Cmpd	Conc	Rep	Qty	Option	TAT*	Unit price	Disc unit price	Net Price
PP223	SafetyScreen87 Panel, Panlabs	Flexible	1	1	2	1.00	Standard	10	[redacted]	[redacted]	[redacted]
Special Requirement: Default test concentrations 10 uM											

Cmpd = compounds; Conc = Concentrations; Rep = Replicates; Qty = Quantity; TAT = Turnaround Time.
*please see “Delivery Dates, Turnaround Time” section of Terms and Conditions (web link) for more details.

Additional Charges	0.00

Special Instructions: N/A

Orders can be placed by purchase order or credit card.

Purchase orders should detail the following company address:

Eurofins Panlabs Discovery Services Taiwan Ltd
[redacted]
New Taipei City, Taiwan 24891

Please note that the address for sending check payment is as following:
[redacted]
Birmingham, AL 35246-5588

Please note that if arranging payment from a bank outside of Taiwan, any intermediary or sender’s charges should be borne by the sender.

AUTHORIZATION AND ACCEPTANCE

Submitting a purchase order with reference to this quotation number or sending a scanned copy of the signed quotation to the address stated below, is considered an acceptance of prices and all other special conditions listed on the quotation.

Client Services
Email: [redacted]
Fax: [redacted]

By authorization and acceptance of this quotation as indicated above, authorizes Eurofins to perform the work as described in this quotation, subject to the terms and conditions of the MSA by and between the Client and Eurofins or its affiliate, if any, or if none, or in the event that the MSA is not active or found to be invalid as of the date hereof, then the work described in this quotation shall be subject to the then current version of the Eurofins General Terms & Conditions of Sale of Products and Services, as amended from time to time, which can be viewed at the following link: https://www.eurofinsdiscoveryservices.com/cms/cms-content/misc/tc/order-terms-condition-products-and-services-dec19/. This quotation constitutes a separate and distinct contract between Client and Eurofins. In no event shall an affiliate of Eurofins be liable for its obligations under this quotation, and Client shall look exclusively to Eurofins in relation to any rights or remedies it may have with respect to this quotation.

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Quotation

AGREED TO AND ACCCEPTED

For: Fox Chase Chemical Diversity Center Inc

By: _____

Name: _____

Title: _____

Date: _____

Eurofins Panlabs Discovery Services Taiwan, Ltd.

[redacted] New Taipei City, 24891, Taiwan

Phone [redacted] Fax [redacted]

www.eurofinspdiscoveryservices.com

SUBAWARD BUDGET JUSTIFICATION

Senior/Key Personnel

Ronald Harty, PhD, PI (25% effort – 3.0 ca mo). Dr. Harty is a Professor in the Department of Pathobiology at the University of Pennsylvania School of Veterinary Medicine and a member of the Cell and Molecular Biology Graduate Group. His laboratory is focused on the molecular events that lead to virus assembly and budding and has developed models of virus budding that recapitulate biological events occurring during infection with a number of Category A pathogens. Dr. Harty's laboratory utilizes a wide-array of techniques for these studies such as, virus-like particle (VLP) budding assays, reverse-genetics to generate VSV recombinants, bimolecular complementation, ubiquitination and ISGylation assays, and fluorescence lifetime imaging microscopy (FLIM). Dr. Harty will direct studies to test lead compounds for their ability to block VLP egress from mammalian cells, block the specific virus-host interaction targeted by the lead compound using BiMC, and block budding of live virus using a surrogate model of filovirus and arenavirus infection. Dr. Harty will be responsible for the overall conduct of this study in the role of PI, dedicating 25% effort to the project and being responsible for communication/joint lab meetings with the Fox Chase Chemical Diversity Center and Texas Biomedical Research Institute to track the progress of the project.

Other Personnel

Ziying Han, MD, Ph.D. (100% effort – 12.0 ca mo). Budget is requested to support Research Specialist Dr. Han, who has provided all of the BSL2 preliminary virology and VLP budding data. Dr. Han will be responsible for maintaining the tissue culture models needed for the experiments and for all molecular biological techniques necessary for testing lead compounds for their ability to block VLP and live virus egress and specific virus-host interactions in live mammalian cells using the BiMC assay.

Other direct costs: [redacted] (Year 1), [redacted] /year (Year 2-3)

Materials and supplies: [redacted] is requested in Year 1 to cover the cost of consumables and reagents necessary for the tissue culture and molecular virology studies associated with this project. These funds will also cover the costs of equipment or facility rental/user fees associated with using the University of Pennsylvania Microscopy Core facility. The imaging core operates on a fee for use cost recovery system that is heavily subsidized by departments and centers in the School of Veterinary Medicine. All users, regardless of their affiliation are charged the same fee for using the Leica SP5 confocal systems, which is [redacted] /hr. This fee helps to defray maintenance and operation costs of the core.

Fringe Benefits:
Fringe benefits have been requested for the salaries in this budget at the DHHS negotiated rate, which is currently 29.5%

Indirect Costs: [redacted] (Year 1), [redacted] /year (Year 2-3)
A total modified direct cost rate has been requested in this budget and is the DHHS negotiated rate. It is currently 62% through June 30, 2021 and then it changes to 62.5% starting July 1, 2020.

BUDGET JUSTIFICATION

Texas Biomedical Research Institute

PERSONNEL

Olena Shtanko, Ph.D., Subaward Principal Investigator (2 calendar months/year): Dr. Olena Shtanko has extensive experience in researching and handling BSL4 agents, including Ebola, Marburg, and Lassa viruses, both *in vitro* and a mouse model of filovirus disease, as evidenced by her publications in the field. Dr. Shtanko is fully authorized to work with select agents and has the appropriate Homeland Security and CDC approvals for this work. Dr. Shtanko will be responsible for the overall experimental design and execution of the project at Texas Biomed. She will supervise the conduct of all studies at Texas Biomed, participate in review and interpretation of the data, and prepare all reports and communications with Dr. Harty. In addition, she will perform certain infection and all imaging assays.

Gloria Rodriguez, B.S., Research Assistant (4 calendar months/year): Gloria Rodriguez, B.S., has training and experience in the cell culture and molecular biology experiments to be executed in the BSL2 setting. She is also fully authorized and experienced to work with replication-competent wild-type viruses in the Texas Biomed BSL4 facility and has been doing so for >5 years. Gloria Rodriguez will help with the extensive cell culture, qPCR, and compound treatments in the BSL2 facility, and will maintain supplies and reagents. She will also perform virus expansion, plaque assays, infection assays, virus inactivation and any other virus-associated work.

SUPPLIES

A total amount of [redacted] is budgeted to cover costs of cell culture media, media supplements, culture vessels, and reagents for cell maintenance, donor recruitment to obtain peripheral blood cells, generation and characterization of virus stocks, testing compounds for antiviral activity and cytotoxicity, and plaque assays; molecular biology reagents; antibodies and materials associated with immunofluorescence assays.

OTHER DIRECT COSTS

***In vivo* toxicity studies**

We request a total of [redacted] for studies aimed to access toxicity of compound treatments in a mouse model. These costs include purchase and shipment of 540 4-5-week old BALB/c mice of either sex from the Jackson Laboratories, housing fees at Texas Biomed ABSL2 Vivarium, therapeutic dosing, necropsy, gross pathology, histopathology, required supplies and reagents, and veterinary personnel effort.

Item	Qty	Freq	Total	Subtotal
Animal purchase costs				[redacted]
BALB/c, either gender, 5 weeks, JAX	45	12	[redacted]	
Shipping	5	12		
Shipping container	5	12		

Item	Qty	Freq	Total Direct	Subtotal Direct
Per diem				[redacted]
ABSL2 ventilated racks (cage rate; 5 animals/cage)	108	18	[redacted]	
Procedures				
ABSL2 PO gavage x 2 WEEKDAY	24	8		
ABSL2 PO gavage AM WEEKEND	12	2		
ABSL2 PO gavage PM WEEKEND AFTER HOURS	24	2		
ABSL2 Organ Collection with Euthanasia	9	12		
SNPRC HISTOLOGY: Mouse, Necropsy	45	12		
Total				
Total Costs				

***In vivo* compound accumulation studies**

We request a total of [redacted] for studies to determine the concentration and accumulation of compounds over time in blood and organs of treated mice. These costs include purchase and shipment of 360 BALB/c mice from the Jackson Laboratories, housing fees at Texas Biomed Vivarium, therapeutic dosing, blood and organ harvesting, isolation of plasma from blood, required supplies and reagents, and veterinary personnel effort.

Item	Qty	Freq	Total	Subtotal
Animal purchase costs				[redacted]
BALB/c, either gender, 5 weeks, JAX	60	6	[redacted]	
Shipping	6	6		
Shipping container	6	6		

Item	Qty	Freq	Total Direct	Subtotal Direct
Per diem				[redacted]
ABSL2 ventilated racks (cage rate; 5 animals/cage)	72	9	[redacted]	
ABSL2 ventilated racks (cage rate; 5 animals/cage)	48	4		
ABSL2 ventilated racks (cage rate; 5 animals/cage)	24	5		
Procedures				
ABSL2 PO gavage x 2 WEEKDAY	12	8		
ABSL2 PO gavage AM WEEKEND	6	2		
ABSL2 PO gavage PM WEEKEND AFTER HOURS	12	2		
ABSL2 Organ Collection with Euthanasia	12	6		
SNPRC PROCEDURES: Mouse (per box of 5), Necropsy with blood collection	36	1		
Total				
Total Costs				

A total amount of [redacted] is requested to ship animal samples to an external source for analysis of compound concentration.

ABSL4 efficacy studies using a mouse model of Marburg virus disease

Animal procurement (a total of [redacted]) is budgeted to purchase and ship 180 4-5-week old BALB/c mice of either sex from the Jackson Laboratories.

Efficacy studies (a total of [redacted]) cover animal housing fees, virus challenge, therapeutic dosing, necropsy, required supplies and reagents, and veterinary personnel effort.

	Unit Price	Quantity	Frequency	Total #	Direct
BSL-4 Mice study					
ABSL-4 Mice Dosing of therapeutic cage (10 mice)	[redacted]	18	20	360	[redacted]
ABSL-4 Mice weight cage (10 mice)		18	10	180	
ABSL-4 Mice Virus challenge cage (10 mice)		18	1	18	
ABSL-4 Mice Per diem cage (10 mice)		18	28	504	
ABSL-4 Mice Necropsy cage (10 mice)		18	1	18	
[redacted] ABSL-4 enhanced observations/hr		[redacted]	72	5	
ABSL-4 Weekend back-up radio/hr			24	4	
[redacted] **BSL-4 Mice study Subtotal**					
Subtotal Cost					

The indirect cost rate is the Texas Biomed Federal MTDC Rate of 98%. Fringe is calculated at 29.51% according to our federally negotiated rate.

Total Direct Costs less Consortium F&A

NIH policy (NOT-OD-05-004) allows applicants to exclude consortium/contractual F&A costs when determining if an application falls at or beneath any applicable direct cost limit. When a direct cost limit is specified in an FOA, the following table can be used to determine if your application falls within that limit.

Total Direct Costs less Consortium F&A	[redacted]	[redacted]	[redacted]	0	0	[redacted]

OMB Number: 4040-0010
Expiration date: 12/31/2022

SBIR/STTR Information

Agency to which you are applying (select only one)*

❍ DOE ● HHS ❍ USDA ❍ Other:

SBC Control ID:* [redacted]

Program Type (select only one)*

❍ SBIR ● STTR

❍ Both (See agency-specific instructions to determine whether a particular agency allows a single submission for both SBIR and STTR)

Application Type (select only one)*

❍ Phase I ● Phase II ❍ Fast-Track ❍ Direct Phase II ❍ Phase IIA ❍ Phase IIB ❍ Phase IIC

❍ Commercialization Readiness Program (See agency-specific instructions to determine application type participation.)

Phase I Letter of Intent Number:

* Agency Topic/Subtopic:

Questions 1-7 must be completed by all SBIR and STTR Applicants:

1a. Do you certify that at the time of award your organization will meet the eligibility criteria for a small business as defined in the funding opportunity announcement?* ● Yes ❍ No

1b. Anticipated Number of personnel to be employed at your organization at the time of award.* 28

1c. Is your small business majority owned by venture capital operating companies, hedge funds, or private equity firms?* ❍ Yes ● No

1d. Is your small business a Faculty or Student-Owned entity?* ❍ Yes ● No

2. Does this application include subcontracts with Federal laboratories or any other Federal Government agencies?* ❍ Yes ● No

If yes, insert the names of the Federal laboratories/agencies:*

3. Are you located in a HUBZone? To find out if your business is in a HUBZone, use the mapping utility provided by the Small Business Administration at its web site: http://www.sba.gov * ❍ Yes ● No

4. Will all research and development on the project be performed in its entirety in the United States?* ● Yes ❍ No

If no, provide an explanation in an attached file. Explanation:*

5. Has the applicant and/or Program Director/Principal Investigator submitted proposals for essentially equivalent work under other Federal program solicitations or received other Federal awards for essentially equivalent work?* ❍ Yes ● No

If yes, insert the names of the other Federal agencies:*

6. Disclosure Permission Statement: If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and email address of the official signing for the applicant organization to state-level economic development organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?* ❍ Yes ● No

7.Commercialization Plan: The following applications require a Commercialization Plan: Phase I (DOE only), Phase II (all agencies), Phase I/II Fast-Track (all agencies). Include a Commercialization Plan in accordance with the agency announcement and/or agency-specific instructions.*

Attach File:* Commercialization_Plan_Harty_Final.pdf

Funding Opportunity Number: PA-20-265. Received Date: 2020-09-08T12:43:45.000-04:00

OMB Number: 4040-0001
Expiration date: 10/31/2019

SBIR/STTR Information

SBIR-Specific Questions:

Questions 8 and 9 apply only to SBIR applications. If you are submitting ONLY an STTR application, leave questions 8 and 9 blank and proceed to question 10.

8. Have you received SBIR Phase II awards from the Federal Government? If yes, provide a company commercialization history in accordance with agency-specific instructions using this attachment.* ❍ Yes ❍ No

Attach File:*

9. Will the Project Director/Principal Investigator have his/her primary employment with the small business at the time of award?* ❍ Yes ❍ No

STTR-Specific Questions:

Questions 10 - 12 apply only to STTR applications. If you are submitting ONLY an SBIR application, leave questions 10 - 12 blank.

10. Please indicate whether the answer to BOTH of the following questions is TRUE:* ● Yes ❍ No

(1) Does the Project Director/Principal Investigator have a formal appointment or commitment either with the small business directly (as an employee or a contractor) OR as an employee of the Research Institution, which in turn has made a commitment to the small business through the STTR application process; AND

(2) Will the Project Director/Principal Investigator devote at least 10% effort to the proposed project?

11. In the joint research and development proposed in this project, does the small business perform at least 40% of the work and the research institution named in the application perform at least 30% of the work?* ● Yes ❍ No

12. Provide DUNS Number of non-profit research partner for STTR.*

[redacted]

Funding Opportunity Number: PA-20-265. Received Date: 2020-09-08T12:43:45.000-04:00

Development of Small Molecule Therapeutics against RNA Viruses

Fox Chase Chemical Diversity Center, Inc.

Commercialization Plan

We have developed small molecule leads that impede the progression and transmission of RNA viruses including Marburg and Ebola viruses, both potential bioterrorism agents and an area of focus for National Institute of Allergy and Infectious Diseases (NIAID) small business funding. In addition, other viruses that may be therapeutic targets of our agents include Lassa and Human T Cell Leukemia virus. A therapeutic agent arising from our efforts will be taken orally alone or in combination with other agents by persons infected with these RNA viruses, or taken as prophylactic agents for individuals deemed to be in high risk situations such as military or healthcare workers. Our current lead candidate ***FC-10696*** *was shown to be a potent inhibitor of virus budding (egress) and transmission in vitro and is active in vivo (mouse). Its mechanism is novel (inhibition of the viral matrix protein interaction with mammalian target cell proteins). In this Phase II STTR application we plan to fully characterize preclinical candidates suitable for IND enabling toxicology studies. We present here our Commercialization Plan from our joint program of study with researchers at University of Pennsylvania and the Texas Biomedical Research Institute, covering the topics listed in the guidelines for STTR submissions.*

A significant portion of this report was taken from a proprietary Technology Niche Analysis supported by the NIH and prepared for FCCDC by Bruce Inwood of Foresight Science and Technology.

1. Value of the STTR Project, Expected Outcomes and Impact.

We review here our key technology objectives for this STTR, commercial need for our technology, our competitive advantage relative to standard of care, and the impact of this technology upon drug discovery and society as a whole.

1.1 Key technology objectives, therapeutic benefit, commercial need and competitive advantage relative to current standard of care of the STTR project.

Marburg and Ebola viruses cause hemorrhagic diseases that afflict humans and nonhuman primates. Our proposed drug candidate will be a novel small molecule oral agent used alone or in conjunction with other antiviral agents for individuals infected with filoviruses Marburg and Ebola or other RNA viruses, including arenaviruses (Lassa), which can cause severe hemorrhagic fever syndromes in humans with high mortality rates, or rhabdoviruses (Rabies). “Viral hemorrhagic fevers (Ebola, Lassa fever, etc.)” are a focus for NIAID SBIR/STTR research and funding (Omnibus Solicitation for SBIRs and STTRs, PHS 2020-, p. 34). These viruses depend on an interaction between their matrix protein PPxY motif and select host proteins for efficient virus egress and spread of infection. Thus, drugs that block or disrupt viral-host interactions required for virus budding are expected to effectively block virus transmission and disease progression.

The 2014-2015 outbreak of Ebola in western Africa resulted in over 28,000 infected individuals and over 11,000 deaths (WHO: Ebola Situation Report 2015). More recently the Democratic Republic of the Congo has second largest Ebola epidemic on record, which is ongoing, and as of June, 2020, with more than 2,200 lives lost, 3,400 confirmed infections and 1,160 survivors since the outbreak was declared on 1 August 2018. These unprecedented epidemics have spurred a call to action to discover new, cost effect therapies that combat this deadly pathogen. Among these efforts are several vaccines and antiviral drug candidates. However, the current vaccines are not a complete defense since they must be given pre-exposure. Oral antiviral agents used alone or in combination may be of value for individuals who respond adversely to the vaccine, and could be of value as prophylactic agents for individuals deemed to be in high risk situations such as military or healthcare workers. Therefore, effective therapeutics are needed to safeguard the largely immunologically naive human population by providing immediate protection.

We have discovered two novel series of small molecule early leads that have nanomolar potency at inhibiting RNA virus budding and are

effective at reducing spread of live viruses including Marburg and Ebola virus. We are currently pursuing both series in order to increase our chance of success. Our most advanced lead is compound, FC-10696 and close structural analogs have the following attributes, tested to the extent afforded by the Phase I level of funding:

- Potent activity (IC_{50} < 100 nM) in the Marburg and Ebola VLP cellular budding (egress) assays,
- Evidence that analogs of FC-10696 directly target the host cell and virus molecular machinery that effect viral cellular egress,
- Live virus activity against RNA viruses (Marburg, Ebola),
- Metabolic stability in mouse and human liver microsomes,
- No cytochrome P450 3A4 activity which translates to a lower risk of drug-drug interactions,
- Good drug levels upon IP administration in mice, and
- Proof of concept in vivo reductions of Marburg virus (IP administration) in mouse models.

However, it is important to note that improved analogs and additional novel composition of matter are expected to be discovered during the course of the Phase II STTR period of study, with fully characterized ADME properties suitable as antiviral oral drug candidates for preclinical development.

1.2 Expected outcomes: path to commercialization including timelines.

The goal of this STTR Phase II application is to produce one or more preclinical drug candidates by medicinal chemistry synthesis optimization strategies that would be ready for GMP scale up and IND enabling studies. At the end of this award period we would have one or more drug candidates that are:

- Potent, orally available inhibitors of RNA virus egress, due to inhibition of the viral matrix protein PPxY interaction with cellular Nedd4,
- Broad spectrum activity against RNA viruses (Ebola, Marburg, Ebola, Lassa, RSV, etc).
- Are not cytotoxic at concentrations 20-fold or higher than the live virus assay IC_{50},
- Have suitable ADME and PK properties in mice for once a day oral administration,
- Have oral activity in a mouse antiviral assay,
- Have little or no cytochrome P450 inhibitory (Cyp) activity against the seven Cyp isoforms most likely to cause exposure variability and drug-drug interactions (DDIs),
- Have little or no activity in a manual patch clamp assay to assess hERG (human Ether-à-go-go-Related Gene product), suggesting a greatly lower risk of the compound causing cardiac arrhythmias,
- Have little or no activity in an Ames testing with and without metabolic activation, suggesting a greatly lower risk of the compound being mutagenic,
- Have chemical syntheses that are efficient and scalable, and
- Provide the basis for one or more composition of matter U.S. patent applications.

We anticipate being in an ideal position to pursue IND enabling studies with our candidate compound and will require additional funding to support IND filing and early clinical development. Many of the future IND-enabling activities would be funded from sources listed later in this application. Such post-Phase II SBIR activities include:

- Preparation of 300 g of non-GMP active pharmaceutical agent,
- Selection of a suitable salt form (if necessary), vehicle and formulation, and evaluation of crystallinity and the propensity for polymorph formation,
- Analytical methods development,
- Drug substance stability testing, including standard pharmaceutics development work-up involving shelf-life and stress testing,
- File additional provisional and PCT patent applications as necessary, and
- Prepare 5 kg of GMP quality active pharmaceutical ingredient.

With the completion of GMP and non-GMP batches we plan to initiate the necessary studies to support an IND filing, specifically investigation of metabolites, *in vitro* toxicity and acute *in vivo* safety pharmacology and toxicity assessment. Activities include:

- Metabolite ID (human, rat and monkey), characterization for toxicity and biological testing,
- Full PK parameters in three relevant safety pharmacology species, mice, rabbit, and non-human primate (NHP), chosen since they are used in relevant *in vivo* pharmacology studies,
- Investigation of Cyp450 inhibition and induction and transporter modulation in detail, for the potential for unexpected drug-drug interactions,
- Enhanced survival in two or more GLP double blind placebo-controlled animal models of Ebola according to the Animal Rule of Efficacy (some of these studies might be done during the IND phase). Viral load and survival will also be assessed,
- Completion of *in vitro* GLP toxicology (hERG, Ames, genotoxicity),
- Conducting dose ranging and 28-day safety profiles in mice and NHPs,
- Safety Pharmacology: Cardiovascular safety study in dogs, CNS safety in rats, respiratory safety in rats (if needed),
- Reproductive toxicology in mice and rabbits (possibly do after/during Phase I studies).
- Conducting a pre-IND meeting with the FDA to establish and understand expectations, and
- Initiating preparation of an IND to facilitate the transition into clinical evaluation.

We have established a world-class **PPxY Project Leadership Team (PPLT)** to interpret data and provide strategic direction on a real time basis. The PPLT will regularly provide advice and guidance (at least monthly) in person, by Zoom, or by email. The PPLT will interpret data most appropriately and coordinate experimental design in close coordination with the Harty group and FCCDC management. The PPLT will conduct more formal quarterly meetings that Prof. Harty will chair, and any member of the PPLT may add items to the agenda prepared in advance of the meeting. The PPLT shall consist of the following members:

Ronald N. Harty, Ph.D., Professor, University of Pennsylvania. Dr. Harty is Professor of Pathobiology and Microbiology, at the School of Veterinary Medicine. His research interests are focused on understanding the molecular mechanisms of filovirus (Ebola and Marburg viruses) and arenavirus (Lassa fever and Junin) assembly/budding, in order to identify broad-spectrum inhibitors of virus-host interactions. His research has directly led to the discovery of the antiviral agents described in this application. He is also interested in understanding the innate immune responses to virus infection that provide a critical first line of defense for the host against the invading pathogen. Prof. Harty has co-authored >80 scientific publications.

Jay E. Wrobel, Ph.D., Vice President, Business Development, FCCDC. Dr. Wrobel has spent 37 years as a medicinal chemist in the pharmaceutical industry, 26 years at Wyeth (later Pfizer), with his last position as Senior Director of Medicinal Chemistry at the Collegeville, PA facility. At Wyeth, he mentored and guided the efforts of 31 Ph.D./MS medicinal chemists and worked successfully with outside alliance partners. He was directly involved in bringing forward nine development track candidates (Phase 0 and beyond) in a variety of therapeutic areas**.** He has co-authored 78 scientific publications and is an inventor on 83 issued US patents.

Bruce Freedman, VMD, Ph.D., Associate Professor, University of Pennsylvania. Dr. Freedman is an Associate Professor of Pathobiology and Microbiology, at the School of Veterinary Medicine. Dr. Freedman is Director of the Microscopy Core facility at PennVet which houses state-of-the-art confocal and live cell imaging instruments that will be critical for assessing the efficacy of our budding inhibitors. Dr. Freedman is a long-standing collaborator with Dr. Ronald Harty, the PI on this proposal.

Olena Shtanko, Ph.D., Staff Scientist, Texas Biomedical Research Institute. Dr. Shtanko researches fundamental molecular processes used by Ebola and Marburg viruses to disseminate within the host to understand the extraordinary capacity of these emerging viruses to trigger disease in affected individuals, with the aim to design and test antiviral therapies. Dr. Shtanko has over 16 years of conducting research on viruses,

with 9 years spent at the maximum containment laboratory (BSL-4) at Texas Biomedical Research Institute. Dr. Shtanko's work has identified factors critical for Ebola virus and Crimean-Congo Hemorrhagic Fever virus entry into cells. Dr. Shtanko also extensively collaborates with a number of investigators to coordinate and perform virus studies to identify host factors critical for replication and spread of high-containment viruses as well as test novel antiviral therapeutics using a small animal model of Ebola and Marburg virus disease.

Allen B. Reitz, Ph.D. CEO, FCCDC. Dr. Reitz has spent 38 years as a medicinal chemist in the pharmaceutical industry, 26 years with Johnson and Johnson (Spring House, PA). At J&J he mentored and guided a staff of 16 Ph.D./MS medicinal chemists and was co-inventor on 7 compounds that have entered human clinical trials. He has co-authored >150 scientific publications and is an inventor on 71 issued US patents. He is Editor-in-Chief and founding editor of *Current Topics in Medicinal Chemistry*. He has established two biotechnology companies: FCCDC and ALS Biopharma. FCCDC has already successfully partnered a separate technology platform for the treatment of Alzheimer's disease, which has passed an interim futility analysis in Phase II/III.

Leo J. Adalbert, M.B.A. Mr. Adalbert will be a business development consultant for this project. An insightful, collaborative, and creative leader, Leo carries more than 25 years of deep cross-functional commercial and clinical development experience across both small molecules and biologics. Core therapeutic areas of expertise include cardiovascular, neuroscience, oncology, immunology, and GI, as well as biomarkers. Prior to founding StraNexa LLC, Leo held various leadership roles across marketing, business development, and alliance leadership at leading firms such as AstraZeneca, Merck, Astra Merck, and Regeneron. Leo earned an MBA from Harvard Business School (fellowship), and executive leadership certifications from Northwestern University (Kellogg) and University of Pennsylvania (Wharton). He also completed extensive coursework in biological sciences (Villanova University/Bucks CC), earning high honors (*Phi Theta Kappa* distinction), and is a graduate of the HMX Program at Harvard Medical School (pharmacology and immunology).

FCCDC has established a world-class Scientific Advisory Board, as posted on the company website, who are on call for questions regarding drug development strategy, flowchart design, chemistry and pharmacology.

Nicholas A. Meanwell, Ph.D., Bristol-Myers Squibb Research & Development, Princeton, NJ. Nicholas A. Meanwell is currently Vice President, Discovery Chemistry Platforms, Small Molecule Drug Discovery where he has led drug discovery programs in the cardiovascular, neurosciences and virology therapeutic areas, work that has resulted in the advancement of over 30 clinical candidates for the prevention of thrombosis, the treatment of stroke and therapy for viral infections, including human immunodeficiency virus-1 (HIV-1), hepatitis C virus (HCV) and respiratory syncytial virus (RSV). Significant compounds in the HCV arena include daclatasvir (Daklinza™), asunaprevir (Sunvepra™), Daklinza™ and Sunvepra™. Dr. Meanwell has authored/co-authored more than 250 publications, review articles, book chapters and editorials and is named as an inventor/co-inventor on 138 issued U.S. Patents. Dr. Meanwell is the co-recipient of a PhRMA Research and Hope Award for Biopharmaceutical Industry Research, 2014 and the recipient of the 2015 Philip S. Portoghese Medicinal Chemistry Lectureship Award. He was inducted into the ACS Division of Medicinal Chemistry Hall of Fame on August 18th, 2015 and he is a member of the team that was recognized by a 2017 "Heroes of Chemistry" Award sponsored by the American Chemical Society.

Eileen Jaffe, Ph.D., Fox Chase Cancer Center, Temple University School of Medicine & Drexel University School of Medicine. Eileen Jaffe received her PhD in Biochemistry from the University of Pennsylvania, working with Mildred Cohn. She was then a Postdoctoral Fellow at Harvard University with Jeremy Knowles. Since 2003 she has been at the Fox Chase Cancer Center in Philadelphia, PA, and is currently also an Adjunct Professor of Biochemistry at Temple University School of Medicine and Drexel University College of Medicine. Her research interests lie in understanding protein structure-function relationships using both biochemical and biophysical approaches. Prof. Jaffe is currently focused on the roles of protein quaternary structure and quaternary structure rearrangements in the control of protein function as this relates to phenylalanine hydroxylase and phenylketonuria. She has been associated with FCCDC since 2008.

Patrick Lam, Ph.D., Baruch S. Blumberg Institute, Drexel University College of Medicine & Lam Drug Discovery Consulting. Patrick Lam is currently a Distinguished Professor of Chemistry at Baruch S. Blumberg Institute and Adjunct Professor at Drexel University College of Medicine. He is responsible for the discovery of a total of eight

clinical candidates. At Bristol Myers Squibb, Dr. Lam was the group leader/co-inventor responsible for the discovery of Eliquis®/Apixaban, a novel Factor Xa anticoagulant with annual sales of >$2 B. Eliquis® was chosen as the “Best New Medicine of 2012” by Med Ad News. He and his team were awarded 2015 American Chemical Society Heroes of Chemistry Award for the discovery of Eliquis®. Patrick is also internationally known as the co-discoverer of the powerful Chan-Lam Coupling Reaction, which is complementary to the Nobel-prize winning Suzuki-Miyaura Coupling Reaction. Patrick has authored 98 papers/reviews/book chapters and is an inventor on 36 patents/patent applications. He has presented 110 invited seminars worldwide.

Assuming that our candidate has progressed to IND status, our next objective will be clinical evaluation. Collectively, the management of FCCDC has been responsible for more than a dozen compounds that have entered human clinical trials, with one currently in Phase III and two in Phase II. Based on this experience, we propose the following path to commercialization with the associated timelines.

- A pre-IND meeting will be held with the FDA prior to initiation of IND enabling studies, to obtain guidance as to proper preclinical and clinical study design and requirements. We expect to file an Investigational New Drug (IND) in ~2Q/2024, with First in Human (FIH) dosing to begin ~1Q/2025. If we have partnered with an appropriate mid-level or major pharmaceutical company, then our development partner will monitor and lead the drug development process.

- Since our primary indication is as a Marburg antiviral medication, effectiveness of our candidate may not easily be determined in human clinical trials for ethical reasons, particularly if there is not an outbreak of the virus. Thus, we will resort to the “Animal Rule" (21CFR Part 314 Subpart 1) as the regulatory pathway for approval of this drug.

- Initial Phase I clinical evaluation will take place in normal individuals looking for safety and tolerability in a single ascending and multiple ascending dose format and drug-drug interactions. Clinical read-outs will include standard hemodynamic parameters involved in cardiovascular safety testing. Adverse events would be monitored. The study would most likely take 12 months, enroll 40 participants and cost $3 million.

- Phase II trial beginning ~1Q/2027 will be an intervention trial for healthy patients with human T cell leukemia virus, (a virus with a similar mode of egress to Marburg). A dose of candidate compound would be administered orally with a placebo. The endpoints would be change in viral load. The study would most likely take 10 months, enroll 140 participants and cost $10 million.

- A New Drug Application (NDA) will be filed with the U.S. FDA containing all of the CMC and other ICH regulatory and safety pharmacology documentation required for commercialization. This would be schedule to occur at ~2Q/2029.

- Initial FDA approval would be for use as an antiviral therapeutic agent for Marburg infection. This would occur on or about 1Q/2030. The Target Product Profile for our candidate is shown in Table 1.

Table 1. Desired Product Profile

	Preferred	**Minimally acceptable**
Efficacy (% survive)	>95%	>85%
Efficacy (virus)	Undetectable levels of virus	Undetectable levels of virus
Adverse events	Comparable to placebo	Minor
Administration	Oral*	Oral
Dosing schedule	Once daily	Twice a day
Drug resistance	None	None
Stability	No special packaging required	Modest precautions acceptable

* An IV formulation may be preferable for the extremely ill, and a liquid formulation useful for children

1.3. Impact on drug discovery and society.

In the outbreak of a terrorist exposure to Marburg, Ebola or related virus, thousands if not greater numbers of people could die before vaccines become available to treat the affected population. Our technology, an oral antiviral medicine for Ebola, Marburg, Lassa and other deadly viruses, could be used alone or with other antiviral medications to protect unvaccinated individuals for the time required to mount an immune response to the vaccine. These agents may also be of value for individuals who respond adversely to the vaccine, and could be of value as prophylactic agents for individuals deemed to be in high risk situations such as military or healthcare workers. In addition, our agent may be effective against the related human T cell leukemia virus that can lead to a rare but highly aggressive cancer. This agent also has the potential to be used as an HIV medication used in conjunction with other HIV antiviral agents. Since current HIV medications can have reduced efficacy over time due to resistance mechanisms, other agents used in combination can reduce resistance and side effects, new drugs are of great need. Finally, our agents that block Nedd4 activity, such as FC-10696, have the potential to show clinical effectiveness against other areas of medical need including various cancers, and obesity (Huang, 2019, Li, 2015; Wan, 2019, Ye, 2014; Zou, 2015).

Continued successful prosecution of this project will allow Fox Chase Chemical Diversity Center to benefit financially and be able to expand and hire additional staff, thereby creating jobs. In addition, the development, clinical validation and commercial exploitation of this platform technology will serve as a great testament to the value of the SBIR/STTR program. In summary, patients will be treated with an effective and safe therapy that had not been available before, Fox Chase Chemical Diversity Center and partners will realize potentially substantial financial return, and the STTR program will have served its mission to society.

2. Company

Fox Chase Chemical Diversity Center, Inc., (FCCDC, www.fc-cdci.com) founded in 2008, is an emerging biotechnology company located in **Doylestown, PA** whose mission is to advance basic scientific discoveries by providing value-added early drug discovery and medicinal chemistry research support for the translation of viable preclinical drug and diagnostic candidates prior to eventual entry into human clinical trials. Our goal is to partner with suitable biomedical research collaborators on selected projects or act as their company's proprietary medicinal chemistry research service. We bring a unique and highly experienced team of internal scientists and collaborative external partners in order to leverage cutting edge scientific advances. The staff at FCCDC has vast drug discovery experience, drawing from a diverse background of pharmaceutical and biotech companies including Johnson & Johnson, Wyeth and Merck. FCCDC has two fully functional, multisite medicinal chemistry laboratories with two 300-MHz NMRs, multiple LC/MS machines, microwave reactor, and multiple Gilson semi-prep HPLCs for compound and library purification. We have prepared >12,000 new chemical entities (NCEs) so far, with two compounds advancing into human clinical trials, both funded initially by SBIR grant support. FCCDC has a >30,000 member onsite chemical inventory of reagents and starting materials for ready use, which are bar-coded, computer searchable, and segregated according to chemical class.

As the basis for our internal technology platform, we have the ability to empower research programs at multiple stages of their development: provide insight into probe molecule identification and synthesis, *in silico* identification of custom and commercial high throughput screening libraries, perform hit triage, hit to lead medicinal chemistry, and lead optimization. Importantly, we also prepare or assist in preparation of intellectual property documents. We seek to improve efficiencies in early drug discovery by quickly derisking the overall process using modern methods and wisdom regarding drug suitability and compound library evaluation.

3. Market, Customers and Competition

3.1 Market. In response to credible scenarios of bioterrorist attacks using a filovirus or other infectious agents, the US Congress enacted Project BioShield legislation that was signed in 2004 which provided $5.6 billion over 10 years to encourage the private sector to develop medical countermeasures against chemical, biological and other terrorism agents (Grotton, 2011). Following this legislation, the Biomedical Advanced Research and Development Authority (BARDA) was created two years later to oversee BioShield's advanced development and procurement efforts. In 2013 Congress renewed laws to extend funding by passing the Pandemic All Hazards

Preparedness Reauthorization Act that provided an additional $2.8 billion and extended BARDA until 2018 (Genome Web Daily News, 2013). As an example, the US has granted Fast Track designation to the Arbutus Ebola drug and has funded $140 million for R&D on EVD (PR Newswire 2014). BioCryst was awarded $12 million for development of BCX4430 (Barda 2015). In addition to the Barda program, the commercial market for future natural outbreaks is growing at a CAGR of 95.2% and the market is expected to reach $56 million by 2020 (Allied Market Research 2015).

Lassa fever is another viral disease treatable with our egress inhibitors. This malady is caused by the Lassa virus and results in 300,000 to 500,000 cases annually, mainly in western Africa and causes about 5,000 deaths each year (Ogbua 2007). Transmission of Lassa virus to humans occurs most commonly through ingestion or inhalation due to rodent infestation.

A drug in this category could also be effective against human T cell leukemia virus (HTLV), which can lead to adult T-cell leukemia/lymphoma (ATLL). ATLL is a rare and fast-growing T-cell lymphoma that can be found in the blood (leukemia), lymph nodes (lymphoma), skin, or multiple areas of the body (Lymphoma Research Foundation, 2015). Around 20 million people worldwide are infected with HTLV. It is common in Japan, South and Central America, the Caribbean and Western Africa, and a large percentage (>30%) of African Americans are infected in certain areas of the US (e.g. New Jersey and New Orleans). It is transmitted through sexual contact or exposure to infected blood and blood products. Only a small percentage of carriers (<5%) actually come down with ATLL however, but there is no way to predict those that do. Survival depends on the ATLL subtype and varies from 4-6 months to more than five years (Gonçalves, 2010). Treatment options include standard anti-cancer protocols but at present no therapy targets the virus. Other diseases and disorders from HTLV include the incapacitating neurological disease (HTLV-associated myelopathy/tropical spastic paraparesis [HAM/TSP]), uveitis, rheumatic syndromes, and predisposition to helminth and bacterial infections (Gonçalves, 2010).

As mentioned earlier, the compounds in this application may block other biological functions of Nedd4. For example, Nedd4 is overexpressed in a variety of cancers including bladder, breast, prostate, gastric carcinomas, colorectal, breast and non-small cell lung carcinomas and inhibits several tumor suppressor systems (e.g. PTEN, Beclin). Suppression of Nedd4 has been shown to reduce tumor cell growth (Boase, 2015; Wan, 2019, Ye, 2014; Zou 2015). Thus, these compounds could find applications in the treatment of such cancers.

Nedd4 also negatively regulates interferon (IFN)-induced transmembrane protein 3 (IFITM3) that restricts cellular infection by influenza virus. Therefore, blocking Nedd4 could lead to therapeutic treatment of influenza (Chesarino, 2015)

3.2 Customers. As mentioned previously, BARDA is the most obvious customer for our RNA virus antiviral agent; however several potential governmental partners could provide development funds and/or resources toward development including the National Institute of Allergy and Infectious Diseases (NIAID), Office of Biodefense Research (OBRA), Department of Defense (DOD), The Defense Threat Reduction Agency (DTRA), The United Sates Army Medical Research Institute for Infectious Diseases (USAMRIID), Defense Science and Technology Laboratories (DSTL), and the Centers for Disease Control and Prevention (CDC). In addition to pursuing the government funding options outlined above, we may seek to partner with small biotechnology companies such as Siga, Tekmira, Chimerix, MappBio Pharm, Athene, Biocryst and Tetraphase, and large companies such as Merck, Johnson & Johnson, and GSK who currently are developing one or more therapeutics targeting a bioterrorism threat as most of these companies have a stated company goal of identifying and developing additional therapeutics in this area.

3.3 Competition. Since there are currently no other agents that inhibit virus egress that are being developed as broad spectrum RNA antiviral agents, an oral drug product based on FC-10696 is potentially a first-in-class treatment either as a mono-therapeutic prophylactic agent, or in combination with other agents currently in development for treatment of RNA viral infections. With respect to Ebola efforts, presently there are several therapeutics agents being evaluated that are summarized here.

- Recombinant vesicular stomatitis virus–Zaire Ebola virus (rVSV-ZEBOV), sold under the brand name Ervebo (Merck) is a vaccine that prevents Zaire-strain Ebola. When used in ring vaccination, Ervebo has shown a high level of protection. Around half the people given the vaccine have mild to moderate adverse effects that include headache, fatigue, and muscle pain. Ervebo does not protect against other species of Ebolavirus or Marburg virus. Also, rural distribution has been a challenge and there is no data on longevity of the vaccine's protection and how often an additional dose boost is required to extend immunity.

- Johnson and Johnson is developing a vaccine regiment that consists of two doses leveraging different vaccines. One is the Ad26.ZEBOV monovalent vaccine based on adenovirus type 26 (Ad26) vector expressing the glycoprotein (GP) of the Ebola virus Mayinga variant (Ad26.ZEBOV). The other is multivalent Modified Vaccinia Virus Ankara (MVA) strain containing ZEBOV, SEBOV, Marburg Virus GP and Tai Forest nucleoprotein inserts (MVA-BN®-Filo). More than 6,500 individuals have now participated in clinical studies for the Ebola vaccine across the U.S., Europe, and Africa. So far, the data from these studies suggest that the vaccine stimulates a robust immune response and has a favorable safety profile. Janssen has also assembled a stockpile of 1.5 million vaccine regimens for potential use in public health emergencies.

- ZMapp (MappBio USA) is a cocktail of three monoclonal antibodies with neutralizing activity against Ebola virus in animal models. Phase II trial is underway. A similar produce MIL-77 (MabWorks, China) is undergoing Phase I study. A trial conducted in 71 patients, 36 of whom received ZMapp in addition to the standard of care, did not show a significant effect on mortality. At 28 days, death had occurred in 84 of 169 (49.7%) of patients in the ZMapp group.

- REGN-EB3 (Regeneron) is an experimental biopharmaceutical antiviral drug that is a cocktail of three monoclonal antibodies (REGN3470, 3471 and 3479) for treatment of Ebola virus disease. It is being developed, tested and manufactured as part of an agreement established in 2015 with BARDA, part of the Office of the Assistant Secretary for Preparedness and Response at the U.S. Department of Health and Human Services. REGN-EB3 is currently under clinical development and its safety and efficacy have not been fully evaluated by any regulatory authority. It was developed against the Zaire species of Ebola virus, but the Sudan and Bundibugyo strains have also caused outbreaks and it is unlikely that EB3 would be effective against these strains. It was tested in the PALM trial that had enrolled nearly 700 participants across four Ebola Treatment Centers in the DRC. At 28 days, death occurred in 52 of 155 (33.5%) patients in the REGN-EB3 group.

- mAb114 is a monoclonal antibody (Ridgeback Biotherapeutics LP) that is being evaluated as a treatment for Ebola virus disease. A license for mAb114 was obtained by in 2018 from the National Institutes of Health-National Institute of Allergy and Infectious Diseases. In the PALM trial that had enrolled nearly 700 participants across four Ebola Treatment Centers in the DRC. At 28 days, death occurred in 61 of 174 (35.1%) patients in the MAb114 group.

- Galidesivir (BCX4430, BioCryst) is a broad-spectrum small molecule antiviral in advanced development under the Animal Rule for the treatment of viruses including Ebola, Marburg, Yellow Fever and Zika viruses. A Phase 1 clinical safety and pharmacokinetics study in healthy subjects has been completed, and in animal studies, galidesivir has demonstrated survival benefits. Galidesivir is a viral RNA-dependent RNA polymerase inhibitor that has also demonstrated broad-spectrum activity in vitro against more than 20 ribonucleic acid (RNA) viruses in nine different families, including filoviruses, togaviruses, bunyaviruses, arenaviruses, paramyxoviruses, coronaviruses and flaviviruses. Galidesivir may be suitable for administration by intravenous, intramuscular, and oral routes.

There remains a critical need for additional agents, particularly with alternative modes of action. First many of the therapeutic agents above, particularly among the vaccine, antibody, siRNA and/or antisense oligonucleotides are specific for individual viruses and sometimes only one or more strains within a virus. Biological agents require iv or infusion administration, and also need to be kept in the cold during storage, which are not ideal conditions in many remote areas of virus prevalence. Our product would be broad spectrum for RNA viruses that have PPxY motifs. Our product differentiates further from vaccines in that it would be an oral agent with a great probability of more economic manufacturing and ease of distribution. Our agent could be

used in conjunction with other oral agents, like the cocktail approach to HIV drug administration, as synergistic therapeutic effects and a decrease in the risk of drug resistance are possible.

4. Intellectual Property Protection

FCCDC and the Trustees of the University of Pennsylvania obtained U.S. Patent 10,160,756, December 25, 2018. This application contains composition of matter and use claims specific to earlier analogs of FC-10696. FC-10696 and its present suite of analogs are outside the scope of this patent and will be subject to new composition of matter applications. We will seek to create additional value in this project by the continued prosecution of the other U.S. provisional patent applications and those that would be expected to emerge in the future. We will file PCT applications and pursue patent protection in major foreign markets, including in Europe, Asia and the BRIC countries (Brazil, Russia, India, China). No special restrictions or licenses have been or are imposed on the rights of the key personnel, FCCDC or the University of Pennsylvania that fall outside of the project description in this grant application.

Additional new compositions of matter will be produced during our execution of this proposal. Therefore, new patent applications will be filed during the course of this grant award time period. The preparation of the applications will commence shortly after wrap-up of medicinal chemistry new analog synthesis. Our Project Leadership Team includes those with extensive pharmaceutical industry experience, who are well versed in the mechanics and opportunities afforded by patent filing and prosecution, with >100 issued U.S. patents between them.

5. Finance Plan

5.1. Initial funding strategy and current status. Our initial funding has been provided by1R41AI113952-01A1 (Ron Harty, PI) 03/01/2015 – 02/29/2016 for a total of [redacted]. We obtained another Phase I STTR R41 AI138630-01A1 (Ron Harty, PI) 06/01/2018 – 05/31/2020 for a total of [redacted].

5.2. Models of commercialization. A likely outcome for these studies is licensure of the Ebola/Marburg therapeutic to the US government and Department of Defense. While there are challenges to this type of business model (Bolken 2008), several companies have demonstrated success, including SIGA, PharmAthene and more recently Regeneron. Furthermore, there appears to be substantial funds from the US government, primarily the Biomedical Advanced Research and Development Authority (BARDA), that would help support late stage development costs for IND- and NDA-enabling studies which would be needed once funding under this grant has stopped. Of direct relevance to this direct application, BARDA awarded SIGA a $433M 5-year base contract for development and licensure of the smallpox antiviral agent, tecovirimat, which could rise to $2.3B with additional treatment courses. Other potential sources for support are the Defense Threat Reduction Agency (DTRA), the Centers for Disease Control (CDC), the NIH and the Office of Biodefense Research Resources and Translational Research (OBRRTR).The types of support available from these agencies include direct research and development funding and/or work-in-kind where development studies are conducted within or under the direction of the respective agencies. We will also seek, when appropriate, to establish partnerships with the private sector to help complete the required development studies in a timely and cost-effective manner. There are a number of companies that perform highly specialized tasks important for regulatory approval, such as those addressing preclinical toxicity, safety pharmacology and genotoxicity, while other companies have substantial CMC expertise for drug substance and drug product manufacturing. As described previously, there is substantial pharmaceutical development expertise at FCCDC so we have hand-on experiences with many of these companies and can make informed decisions on the most appropriate partner for each task. During the course of the development phase, there are three primary models of commercialization.

- Commercialization model #1. This involves licensing for all indications to a mid-level or large pharmaceutical company, for development within the partner company. This is routinely conducted, and these types of agreements typically involve an upfront cash payment followed by a combination of royalties and milestone payments as the asset progresses further. We believe that we need additional validation data such as provided in this Phase II SBIR program of study to sufficiently interest in such a partner. This model is a probable outcome of this Phase II SBIR grant research effort.

- Commercialization model #2. In this model, FCCDC continues the development program through the Animal Rule efficacy and Phase 1 safety studies. This type of derisking adds tremendous value to the asset and often multiple groups will show interest at this time. The value of a transaction at this point intime can be a substantial upfront payment with a combination of royalties and milestones later. The funding to get to this decision point could come from a corporate partner who has first rights of refusal, angel or venture capital support, government (e.g. NIH or DOD) or foundation (e.g. Wellcome Trust) support. NIAID offers a Phase IIB grant opportunity to assist small businesses such as FCCDC to continue their project further if they are not sufficiently advanced to be able to attract an investment partner.
- Commercialization model #3. This is a hybrid of plans 1 and 2, in which transition or licensing to a development partner occurs prior to the conclusion of the Phase 1 safety studies, perhaps during the course of the Animal Rule efficacy studies or at IND filing. Model #3 is a model of opportunity, in that as we aggressively pursue alternate funding to empower our research program to advance a candidate into clinical evaluation, an unforeseen opportunity for joint development, acquisition or licensing may prove too good to pass up.

Several general concepts permeate throughout all of our activities, irrespective of which Commercialization Model we adopt. These are:

- Most important: Ebola and Marburg are disease targets that are eligible for the U.S. Neglected Disease Voucher program. In this program, if we can obtain an approved NDA for Ebola from the U.S. FDA for the treatment of Ebola-virus infection, we will obtain a saleable voucher for priority review of a subsequent drug candidate in any therapeutic category, even if in reality the treatment for Ebola is never used therapeutically for any reason. Such vouchers have recently been receiving a high premium on the secondary market, often in excess of $100 million. NDA approval from the U.S. FDA for Marburg or Ebola would involve the "animal rule" in which efficacy would be inferred from in vivo efficacy studies, and of course no human clinical trials would be conducted in human patients with the disease.
- We will file for Orphan Drug designation for Marburg or Ebola infection since infection affects less than 200,000 people/year in the US. This designation results in seven-year market exclusivity for companies that develop the orphan drug, tax credits equal to half of the development costs, grants for drug development, and fast-track approvals of drugs indicated for rare diseases. SIGA was successful in getting this designation for tecovirimat (Love, 2011)
- FCCDC will actively solicit support from academic investigators and clinicians who manage anti-infective clinical trials.
- FCCDC will seek to obtain financial support, consultation and coordination from various non-profits and charitable organizations working diligently to find new medicines for infectious disease.
- The FDA frequently waives application fees for small businesses (<500 employees), especially when this would be a significant barrier to innovation. We will apply for these application waivers and expect no difficulty in obtaining them given the compelling need for new treatments for RNA virus infections as a result of bioterrorism.
- We will seek to expand the therapeutic indications that can be treated by our RNA virus antiviral drug lead in order to address the unmet medical need of a greater patient population and to attract further investment and/or interest from suitable development partners.
- We will actively cultivate relationships with the external licensing and evaluation offices of major pharmaceutical companies with established product pipelines and portfolios in the area of bioterrorism infectious diseases, such as Siga, Tekmira, Chimerix, MappBio Pharm, Athene Biocryst, and Tetraphase, Merck, Johnson & Johnson.

6. Production and Marketing Plan.

Our current laboratory capabilities enable us to prepare 100 g batches of any candidate, to a high level of purity (>99%). As we require GMP-grade drug substance, we will engage an appropriate contract research organization who is properly certified for this type of chemistry project. Once a facility is selected, we will perform a site visit to ensure that our projects are performed under standard good laboratory practices. We will increase awareness of our candidate and related platform compounds in the scientific community by publications in appropriate peer-reviewed journals and presentations at national scientific meetings, such as the Infectious Disease Society of

America annual meeting. We will engage key opinion leaders in the area as we move forward, as part of the FCCDC Scientific Advisory Board, in order to select the appropriate clinical safety study design.
We have established an internal business development function within FCCDC, to be able to effectively market and seek out partners or funding for transition into full development and commercialization. Our CFO (Kathleen Czupich, MBA) has also participated in multiple discussions with potential partners and investors, marketing our technology. If we are awarded the Phase II SBIR funding and successfully carry out the research described, we will have more derisking validation data with which to market the technology and attract substantial investment.

7. Revenue Stream

FCCDC and the University of Pennsylvania have negotiated and signed an Intellectual Property Management Agreement (IPMA) to codify the terms of distributions of milestones and royalties from this project, and to establish the mechanisms for project governance and patent protection.

A primary source of revenue for this program will most likely be from licensure to the US government and the Department of Defense. As described in Section 5, there is relevant precedence for the potential of significant income with the smallpox antiviral agent, tecovirimat where SIGA was awarded from BARDA a $433M 5-year base contract for development and licensure that could rise to $2.3B with additional treatment courses. Additional examples of successful Biodefense licensures include $1.2B for the SparVax® anthrax vaccine, >$500M for the nerve agent bioscavenger, rBChE and >$500M for the anthrax anti-toxin, Valortim®. FCCDC intends to generate a revenue stream for our product platform, depending on our ultimate production and marketing strategy. The revenue stream we envision will be as follows:

In the scenario where we license rights to our product to a development partner, we expect that additional trained and qualified business development staff will be hired in order to manage these and other relationships. This dedicated business development function will manage the commercial exploitation of our intellectual property platform. A licensing deal would generate income for FCCDC through a combination of upfront fees, milestone payments and royalties on production of additional treatment courses.

If we subcontract components of manufacturing and develop our candidate and related drug candidates ourselves, we will hire additional staff as required to manage this greater revenue stream as appropriate. FCCDC will evaluate pursuing additional grant funding and pre-seed investments to continue development up to and through the Animal Rule efficacy studies and Phase 1 clinical safety studies. This path that will increase the value of the company and technology prior to negotiating a commercial agreement or private investment as advancements through the development stages continue.

Pre-seed and seed-stage investment are not revenue, but are sources of working capital. This funding can be obtained through convertible debt or equity investments from early-stage angel and venture investment groups, a number of which are located in the Pennsylvania/New York/New Jersey corridor (Ben Franklin Technology Partners, BioAdvance, Jump Start New Jersey, Mid Atlantic Angel Group, Delaware Crossing, etc.), where many of the major pharmaceutical companies are also located. FCCDC would anticipate generating significant revenues from out-licensing or sale of the technology based upon the well-recognized need for new anti-infective drugs for biodefense applications.

The major source of revenue anticipated for this program is the Neglected Disease Priority Review Voucher offered by the U.S. FDA for new agents to treat the neglected, tropical diseases such as Marburg, Ebola and related viral infections. This voucher could then be sold to a major pharmaceutical company for cash considerations, and in return the acquiring company can use the voucher to reduce the drug approval time for a different therapeutic in their clinical development pipeline.

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PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 02/28/2023

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ❍ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Human Fetal Tissue Section

*Does the proposed project involve human fetal tissue obtained from elective abortions? ❍ Yes ● No

If "yes" then provide the HFT Compliance Assurance

If "yes" then provide the HFT Sample IRB Consent Form

5. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ❍ Yes ● No

If the answer is "Yes" then please answer the following:

*Previously Reported: ❍ Yes ❍ No

6. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

Tracking Number: GRANT13199760 Funding Opportunity Number: PA-20-265 . Received Date: 2020-09-08T12:43:45.000-04:00

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 02/28/2023

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1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	Specific_Aims_Harty_Final.pdf
3. Research Strategy*	Proposal_Harty_Final_2.pdf
4. Progress Report Publication List	Progress_Report_Publication_List_none.pdf
Other Research Plan Section	
5. Vertebrate Animals	VAS_4_combined.pdf
6. Select Agent Research	10._Select_agent_use2_20-240_-_final.pdf
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	Combined_LOI.pdf
9. Letters of Support	Combined_LOS.pdf
10. Resource Sharing Plan(s)	Resource_Sharing_Plan_FCCDC.3.pdf
11. Authentication of Key Biological and/or Chemical Resources	Authentication_for_Harty_combined.pdf
Appendix	
12. Appendix	

Funding Opportunity Number: PA-20-265. Received Date: 2020-09-08T12:43:45.000-04:00

Specific Aims. The ultimate goal of this Phase II application is to discover and develop novel small molecule, broad-spectrum therapeutics against viral infections caused by filoviruses, arenaviruses, and others that depend on the PPxY L-domain motif for virus egress and spread of infection such as Marburg (MARV), Ebola (EBOV), and Lassa fever (LAFV) viruses which are highly pathogenic and classified as Category A bioterror pathogens. In our ongoing studies to identify broad-spectrum small molecule inhibitors of viral egress that can slow the dissemination of both existing and newly arising variants of these potentially lethal viruses in unvaccinated populations, we have discovered several chemical series that block the host Nedd4/virus PPxY complex, a critical regulator of viral transmission. Importantly, we have identified an analog capable of blocking *in vivo* activity in a Marburg virus challenged mouse model, providing essential proof of concept for this novel class of anti-viral therapeutics. As there are no commercially available small molecule therapeutic agents for the treatment of these viral infections, our identification of virus-host inhibitors that prevent virus egress and spread will fill a significant unmet need. The goal of this Phase II STTR grant application is to optimize our lead inhibitor series, exemplified by FC-10696 to generate full-fledged predevelopment drug candidates ready for IND directed studies. This will be accomplished by combining the pharmaceutical and medicinal chemistry expertise of the scientists at the Fox Chase Chemical Diversity Center, Inc. (FCCDC) with the expertise and experience of the Harty Lab at the University of Pennsylvania in the experimental aspects of antiviral therapy, and the lab of Olena Shtanko at Texas Biomedical Research Institute for evaluating compounds against live viruses *in vitro* and *in vivo* using established small animal models of infection under BSL-4 conditions.

Specific Aim 1: Lead optimization medicinal chemistry. Our current generation of PPxY/Nedd4 inhibitors exhibits excellent potency in blocking egress and spread of VLPs and live viruses, and is more metabolically stable than earlier series. We will continue to develop FC-10696 by performing SAR lead diversification using cutting edge methods in medicinal chemistry and drug discovery. This series will serve as the starting point for improved analogs to increase solubility and improve potency, as well as ADME, PK and safety properties suitable for once-daily oral dosing in patients. These data will inform further modifications to advance SAR development.

Specific Aim 2. Evaluate compounds for their ability to specifically inhibit PPxY-mediated virus egress and host interactions. We will determine whether new compounds (Aim 1) block PPxY-mediated budding of VLPs produced by EBOV and MARV VP40, and by LAFV Z proteins using our established budding assays and a bimolecular Nedd4:PPxY complementation (BiMC) approach. We will also evaluate lead candidate inhibitors for potential effects on Nedd4-mediated ubiquitination and ESCRT-associated functions. Lastly, we will use an RNA seq. approach to globally assess the effects of our inhibitors on the host transcriptome, including specific E3 ubiquitin ligases to identify potential off-target effects.

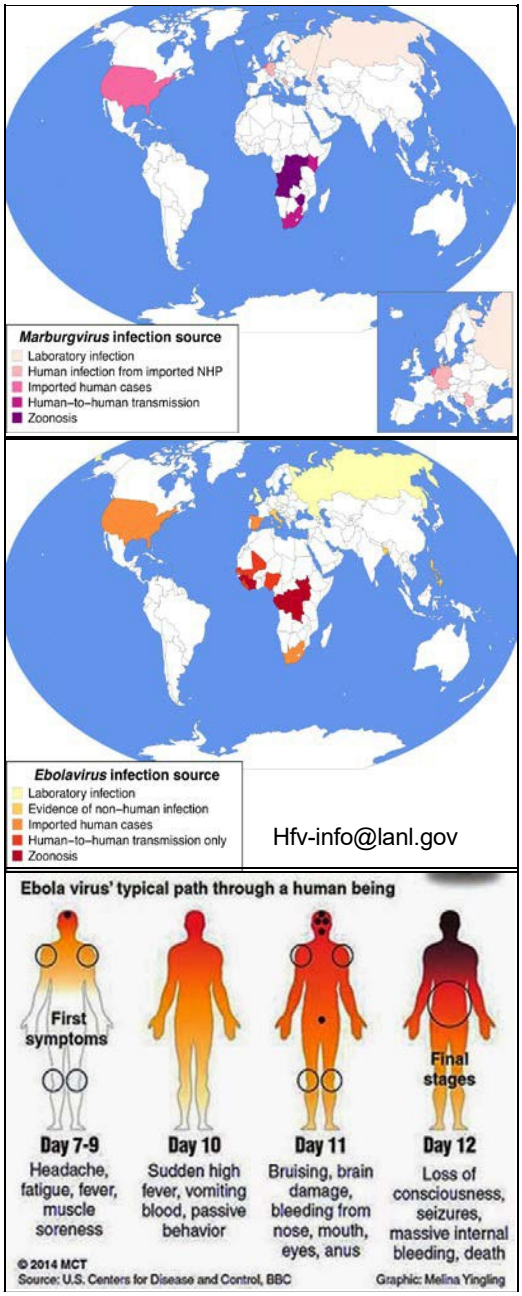
Specific Aim 3: *In vitro* and *in vivo* ADMET evaluation to ensure suitable drug properties and selectivity. We will evaluate up to 15 compounds per year that meet the criteria in Aims 1 and 2 using *in vitro* ADMET assays, including microsomal stability, inhibition of CYP 3A4, solubility, and plasma protein binding. We will evaluate three to six compounds in mouse PK studies via IV and PO administration. The 2-3 most advanced compounds meeting *in vitro* potency and ADME/PK criteria will be evaluated in *in vitro* non-GLP safety assays such as: a) inhibition of cytochrome P450 isozymes (1A2, 2C9, 2D6, 2B6, 2C8, and 2B19, and 3A4-midazolam) most likely to cause variable exposure and drug-drug interactions; b) manual patch clamp assays to assess the hERG channel potential for cardiac arrhythmias; c) Ames testing with/without metabolic activation; and d) PK evaluation in rat, dog and non-human primate. These data will identify the best compounds for *in vivo* testing (Aim 4).

Specific Aim 4: *In vitro* and *in vivo* analysis of lead inhibitors against authentic hemorrhagic fever viruses. We will evaluate the antiviral activity of select lead compounds against live BSL-4 pathogens. In each year, we will evaluate up to 4 compounds developed through studies in Aim 2 for the ability to selectively inhibit EBOV, MARV, and LASV spread and egress in human cell cultures. We will use a mouse model to assess compounds with the highest potency for toxicity, body distribution and accumulation over time. With this invaluable information in hand, we will evaluate two lead compounds per year for antiviral efficacy against filoviruses in a mouse model of MARV or EBOV disease.

Our team is a leader in the development of host-oriented, broad-spectrum inhibitors of RNA virus egress, and we have made tremendous progress toward this goal as described below in our Phase I findings, published manuscripts, and U.S. Patent 10,160,756. Completion of these aims will allow us to choose a predevelopment candidate for IND enabling studies, pursue multiple funding options including additional NIH support; and ultimately leading to clinical evaluation for Marburg and/or Ebola indications, both of which are eligible for an FDA Priority Review voucher currently worth ~$80-100 M.

A. Significance and Impact.

Premise. The ultimate goal of this Phase II application is to develop novel small molecule, broad-spectrum therapeutics against viral infections caused by filoviruses, arenaviruses, rhabdoviruses, and others that depend on the PPxY L-domain motif for virus egress and spread of infection. Some of these viruses, including Ebola (EBOV), Marburg (MARV), and Lassa fever (LAFV) viruses, are highly pathogenic and classified as Category A, bioterror pathogens. The symptoms of these pathogens are often severe and, although these outbreaks largely originate in Africa, they have the ability to spread globally as observed in 2014-2015 (see graphic to the right). In our ongoing studies to identify small molecule inhibitors of viral egress that can slow the dissemination of both existing and newly arising variants of these potentially lethal viruses in unvaccinated populations, we have discovered several chemical series that block the host Nedd4/virus PPxY complex, a critical regulator of viral transmission. Importantly, we have identified an analog capable of blocking *in vivo* activity in a Marburg virus challenged mouse model, providing essential proof of concept for this novel class of anti-viral therapeutics.



We predict that the novel class of anti-viral products targeting EBOV, MARV, and LAFV will be used for treatment of infected individuals as well as in prophylactic treatment of soldiers, healthcare workers, or others at high risk. Emergency administration of such an antiviral therapeutic during an outbreak could potentially inhibit virus dissemination in infected individuals and reduce the efficacy of infection in newly exposed individuals, thus slowing disease progression, allowing for more effective viral clearance by the immune system, and preventing further viral transmission. Moreover, as EBOV and MARV can cross the blood-brain barrier and re-emerge months later in the CNS, semen, and other immunologically privileged sites that are inaccessible to antibody therapy, rapidly diffusible small molecular inhibitors will fill a unique niche in filling the significant unmet need for anti-viral therapeutics against these classes of virus. Finally, as these host-oriented inhibitors are broad-spectrum, they are likely to be effective against newly emerging viruses as well as viral variants. Indeed, we predict that targeting a virus-host interaction necessary for efficient virus egress and dissemination will greatly diminish or eliminate the occurrence of drug resistant viral mutations and may lead to a paradigm shift in the search for better antiviral drugs.

RNA Virus Egress as a Target for Host-Oriented Therapeutics.

Late budding domains (PPxY and PTAP) are highly conserved in the matrix proteins of a wide array of RNA viruses (*e.g.* **filoviruses, arenaviruses, rhabdoviruses, paramyxoviruses, henipaviruses, and retroviruses**) and thus represent broad-spectrum targets for the development of novel antiviral therapeutics [1-11]. Notably, independent expression of filovirus VP40 or arenavirus Z matrix proteins leads to the production and egress of virus-like particles (VLPs) that accurately mimic budding of live virus, and PPxY L-domains within these proteins play a central role in this process [8, 12-18]. Moreover, efficient egress of VLPs depends on viral L-domain mediated recruitment of host proteins required for complete virus-cell separation or pinching-off of virus particles [1-8, 19]. Here we focus on the interaction between Nedd4, a WW-domain containing cellular E3 ubiquitin ligase associated with the host ESCRT1 complex and the viral PPxY L-domain motif. Based on the critical role of this viral-host interaction in efficient budding of filoviruses, arenaviruses, and rhabdoviruses [3, 4, 7, 8, 13, 17, 19-29], we have used a multifaceted approach to identify, develop, and validate several independent series of inhibitors of Nedd4:PPxY complex as potent, broad-spectrum antivirals. For this grant, our team, that has worked together for a half dozen years and composed of experts in the fields of virology and medicinal chemistry, proposes to find a predevelopment candidate for future IND enabling studies by 1) drug property optimization of current of analogs via iterative design and synthesis in **Aim 1**; biological testing for potency and

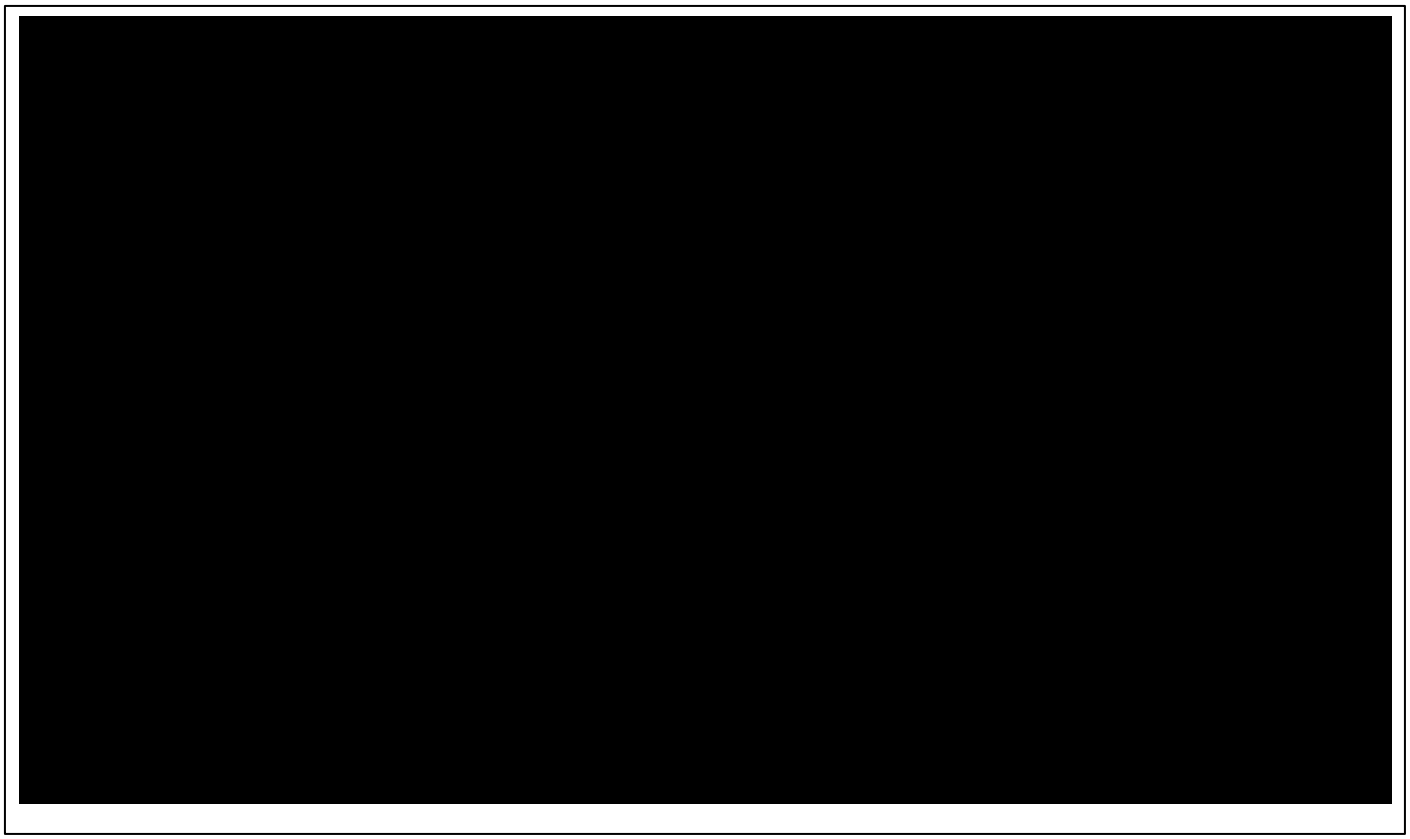
selectivity in **Aim 2**; drug property evaluation for oral activity using state of the art ADME and PK assays in **Aim 3** and finally evaluation of antiviral efficacy under oral administration in a mouse challenged with Marburg and/or Ebola virus in **Aim 4**. Our efforts will result in preclinical antiviral drug candidates for IND enabling studies. We will ensure that all assay experiments will be completed in a rigorous, unbiased manner, and quantitative readouts from virological assays proposed below will be statistically validated as indicated. Our team continues to lead in the area of developing host-oriented, broad-spectrum inhibitors of RNA virus egress, and we have made tremendous progress toward this goal as described below in our Phase I findings, published manuscripts [5, 7, 30-37], and U.S. Patent 10,160,756.

B. Innovation. Development of small molecule therapeutics with potent, broad-spectrum activity against emerging RNA viruses and NIAID Category A pathogens remains a high priority, but also represents a major challenge due to the inherent difficulty of working with a subset of these pathogens under BSL-4 conditions. Several major innovations from our group help to alleviate this obstacle.

1) Our team is a leader in the innovative development of host-oriented, broad-spectrum inhibitors of RNA virus egress, and we have made tremendous progress toward this goal as described below in our Phase I findings, published manuscripts, and U.S. Patent 10,160,756. For example, the Harty lab is a world-leader in functional characterization of VP40-interacting host proteins that facilitate virus egress [31-34, 36, 38, 39] and have developed innovative VLP budding assays that accurately recapitulate authentic live filovirus and arenavirus budding under BSL-2 conditions and state-of-the-art bimolecular complementation (BiMC) approaches to detect and quantitate virus-host protein interactions important for budding in live mammalian cells [37, 40] and to assess the impact of inhibitors on these processes. The innovative use of VSV and VSV recombinants (*e.g.* VSV-M40) as surrogate viruses for BSL-4 pathogens like EBOV, MARV and LASV allows us to test our inhibitors for antiviral activity against live viruses under BSL-2 conditions prior to undertaking these experiments with more dangerous BSL-4 pathogens [19, 41].

2) Our medicinal chemistry experts at FCCDC have successfully used SAR to identify several independent series of novel Nedd4:PPxY inhibitors that target this virus-host interaction, and Dr. Shtanko's team of BSL-4 virology experts demonstrated that our lead inhibitors blocked egress and spread of live authentic viruses in cell culture and protected mice in a MARV challenge model (see below).

3) Overall, our approach is **conceptually innovative** in that it pursues a conserved host:virus complex for the development of broad-spectrum antiviral agents for the treatment of Marburg, Ebola and related virus infections, for which there are no small molecule inhibitors on the market today. The success of our project will validate a new strategy for to the development of antiviral therapy.



C. Progress to Date: Preliminary and Published Findings.

C1. SAR Plan and Design. The primary goal of our Phase I STTR grant was to improve metabolic stability in mouse microsomes and aqueous solubility of our acylurea series of compounds[30] (Fig, 1) for demonstration of proof of concept efficacy in a mouse model of either Marburg or Ebola infection. Our novel agents potently interrupt the host Nedd4: viral PPxY interaction, thereby inhibiting virus budding and spread. Our initial lead series had a quinoxaline scaffold with an acylurea side chain. This subseries was unstable to mouse liver microsomes and also had poor aqueous solubility, resulting in very poor blood levels in mice when administered IV or IP. ***Here we describe the SAR that led to FC-10696, a compound with good antiviral potency, vastly improved overall ADME and PK properties, and demonstrated proof of concept efficacy in a Marburg challenged mouse model.***

Design Plan. We focused our efforts to improve the metabolic stability via both modification of the acyl urea side chain and quinoxaline scaffold (Fig. 1.). Crystallographic studies [42, 43] of compounds that contain the acylurea moiety show that the $CH_2CONHCONHAr$ group exists in a pseudo six membered ring due to internal H-bonding (see Fig. 1). We reasoned that this pseudo six-membered ring may also exist in the bioactive conformation of our acylurea subseries, thus we prepared compounds with covalent six membered rings, as shown in the "Biphenyl subseries" in Fig. 1.

SAR Map. In support of our prediction that elimination of one or more H bonds in this design would improve stability, solubility and overall bioavailability, the biphenyl subseries provided many highly potent and stable analogs (Fig. 1). Over 100 compounds have been prepared in this subseries. We later found that a thiazole amine or thiazole methyl moiety could replace the acylurea in this subseries to afford the "Thiazole benzyl" subseries (Fig. 1), of which over 30 compounds have been prepared.

Table 1 shows *in vitro*, *in silico* ADME and PK data for selected compounds and Fig. 2 shows the structures of the selected compounds in Table 1. The quinoxaline compounds represented by FC-10696 to FC-12004 generally show strong inhibition of Marburg VLP egress activity at 0.1 uM, the lowest concentration tested. These compounds are also potent inhibitors of Ebola VLP egress as well, although less potent than for Marburg egress.

Table 1. In vitro, in silico, ADME and PK data for selected compounds

FC#	Marburg[a]			Ebola[b]		MW	Log D (pH 7.4)	TPSA[b] (Å^2)	MLM[c] stability	HLM[d] stability	P450 3A4 inhibition	Mouse PK, IP administration[f]			
	1 uM	0.3 uM	0.1 uM	0.3 uM	0.1 uM				t1/2 (min)	t1/2 (min)	(uM)	Cmax (ng/ml)	AUC (ng x ml/hr)	$t_{1/2}$ (hrs)	IP Bioavail-bility (%)
FC-10696		*91*	*82*	*57*		*357.5*	*5.9*	*52*	*76.9*	*1670*	*>33*	*253*	*1378*	*1.5*	*42*
FC-10905		92	75			412.4	5.5	52	65.5			188	549	2.6	35
FC-10906		99	90	67		358.5	5.1	52	8.8	73.9	>33	519	597	1.8	22
FC-11017			95	85		403.6	6.0	39	7.7	70.7	>33	513	710	1.2	41
FC-11078		91	87			440.5	5.9	65	87.2			95	269	2.6	16
FC-12004		89				348.5	4.9	26							
FC-11659		100		95	89	350.5	3.8	52	1.0						
FC-11716	99	28				358.5	5.6	33	25.9						
FC-12048		93				372.5	6.0	33	>180						

[a]% Reduction of Marburg VLP at listed compound conc, VLP = virus like particle, in HEK293T cell line. [b]% Reduction of Ebola VLP at listed compound conc.[c]TPSA = total polar surface area. [d]MLM mouse liver microsome. [e]HLM human liver microsome. [f]BalbC mice administered one time 10 mg/kg of drug with plasma collection time points of 0.25, 0.5, 1, 2, and 6 hr. IP Bioavailbility is the ratio of IP AUC to IV AUC x 100. IV PK parameters are not shown.

New Scaffolds. The [redacted] scaffold is exemplified by FC-11659. All members with this scaffold showed high potency; however, they were plagued by poor metabolic stability in mouse microsomes. Very recently we discovered that we could replace the quinoxaline with a [redacted] (FC-11716 and FC-12048) and retain good egress inhibition potency. We found

[redacted]

that the SAR of the sulfur containing side chain and the 3-methyl moiety of this new scaffold is the same as for the quinoxaline scaffold SAR, but the stability to mouse microsomes is superior. Thus, these series will be further pursued.

ADME and PK in Mouse. Five compounds, FC-10696 to FC-11078, were evaluated in a single dose mouse PK experiment under IP administration (Table 1). Compounds containing 6, 7 di-F atoms on the quinoxaline scaffold (FCs 10905 and 11078) showed poor blood levels, despite higher metabolic stability, presumably due to lower plasma solubility, when compared to the des F analogs, possibly resulting in precipitation at the injection site. Three of the five compounds (FC-10696, FC-10906 and FC-11017) that were examined in mouse PK were deemed suitable for live virus and mouse efficacy experiments described in later sections as they showed good stability to human liver microsomes and did not inhibit cytochrome P450 3A4 at concentrations up to 33 uM, thus showing low risk for drug/drug interactions. Of the three analogs, FC-10696 had superior PK properties.

Thus, from our SAR efforts, we have found several series and many potent and stable analogs resulting in good live virus potency and proof of concept in vivo activity in a mouse model as shown below. Our knowledge of the SAR will allow us now to pursue our goals of analogs that are potent and orally available.

C2. In **Aim 2 of Phase I**, we evaluated the compounds described above using VLP, BiMC, and live-virus budding assays to determine their specific inhibition of the PPxY-Nedd4 interaction and PPxY-mediated egress. Below, we highlight representative data to demonstrate that the PPxY-Nedd4 WW/domain interaction is a viable and promising antiviral target.

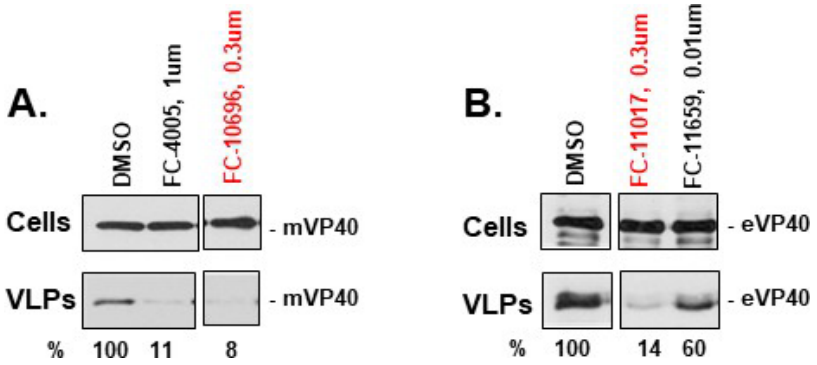


Fig. 3. *Representative Marburg and Ebola VP40 VLP budding assay data.* HEK293T cells were transfected with either mVP40 **(A)** or eVP40 **(B)** plasmids in the presence of DMSO alone, or the indicated budding inhibitors at the stated concentrations. Two of our lead compounds are highlighted in red. mVP40 and eVP40 were detected by Western blot in cell extracts and VLPs at 24 hours post-transfection. mVP40 and eVP40 levels in VLPs were quantified using NIH Image-J software. "%" is percent of VLP after compound treatment relative to DMSO control set at 100%.

VLP Budding Assay. The well-established and validated filovirus VLP budding assay that recapitulates live virus budding under BSL-2 conditions [4-7, 12] is our primary assay used to evaluate the antiviral potency of all new compounds (see Table 1 and Fig. 2). Representative results are shown in Fig. 3. Briefly, we transfected HEK293T cells with either mVP40 or eVP40 expression plasmids in the absence (DMSO alone) or presence of the indicated compounds at the stated concentrations (Fig. 3). Expression levels of mVP40 and eVP40 were detected in cell extracts and VLPs by Western blotting and quantified by Image-J software as described previously (Fig. 3) [30, 36]. Here we used FC-4005, one of our early "acylurea inhibitors [30], at **1uM** as our positive control. Notably, we observed robust inhibition of mVP40 and eVP40 VLPs in the presence of two of our leading candidate inhibitors (FC-10696 and FC-11017 highlighted in red) at **0.3uM** compared to DMSO alone (set at 100%) (Fig. 4). These results were consistent in multiple independent budding assays (data not shown), and highlight the enhanced antiviral potency and broad-spectrum nature of these newly identified compounds.

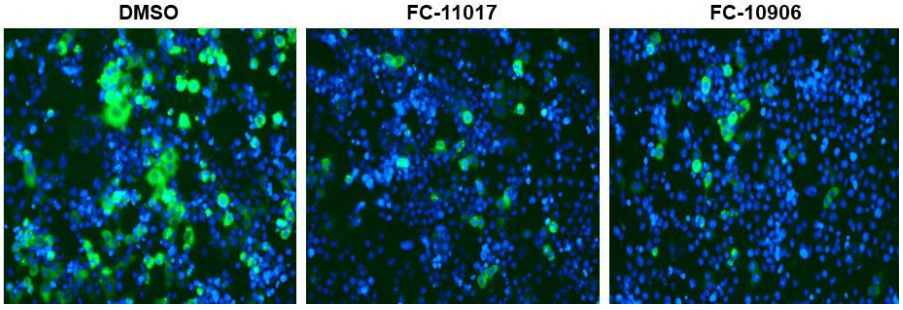
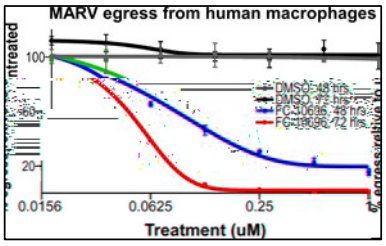


Fig. 4. *Bimolecular Complementation (BiMC) Assay data.* HEK293T cells were co-transfected with equal amounts of NYFP-Nedd4 + CYFP-mVP40 in the presence of DMSO alone, or 0.5uM concentration of FC-11017 or FC-10906. Representative cell images (out of ~100 each) are shown for each condition and were obtained at 24 hrs post-transfection using fluorescence microscopy. NucBlue = stained nuclei. Green = a Nedd4/mVP40 interaction.

BiMC Assay. We used our well-established and validated BiMC approach [30, 37] to detect and visualize a MARV VP40-Nedd4 interaction in live mammalian cells in the absence (DMSO alone) or presence of FC-11017 or FC-10906 (Fig. 4). Briefly, HEK293T cells were co-transfected with plasmids expressing NYFP-Nedd4 and CYFP-mVP40 in the presence of vehicle (DMSO) alone, or PPxY inhibitors FC-11017 and FC-10906 at a concentration of 0.5 µM (Fig. 4). Total cell counts based on NucBlue staining confirmed equal numbers of cells in all assay samples. YFP fluorescent complementation (*i.e.* green cells) is indicative of a PPxY-mediated interaction between mVP40 and host Nedd4, and fluorescent cells were visualized and quantified using an inverted Leica Sp5-II confocal microscope and MetaMorph software, as described previously [30, 36] (Fig. 4). In multiple independent experiments, we observed a significant decrease in the relative number of YFP-positive cells in samples treated with either FC-11017 and FC-10906 compared to that in vehicle-alone control samples (Fig. 4). As we published previously [36], a PPxY L-domain mutant of mVP40 that does not interact with Nedd4 served as a valid negative control (data not shown). These results demonstrate that our lead candidate inhibitors specifically block the PPxY-mediated interaction between mVP40 and Nedd4 in mammalian cells and, together with data provided below, provide compelling evidence for their further optimization in Phase II.

Table 2. IC_{50} and SI_{50} values (in uM) for replication-competent EBOV and MARV egress at 48 h

	Cell type	CC_{50}	EBOV IC_{50} egress	EBOV SI_{50} egress	MARV IC_{50} egress	MARV SI_{50} egress
FC-10696				< 1		11.7
[illegible]	[illegible]	[illegible]	[illegible]	0.9	[illegible]	9.7
[illegible]		[illegible]	[illegible]	0.5	[illegible]	10
C-10696	Human macrophages	4.8	> 10	< 0.5	0.18	26.7
C-11017		2.6	> 10	< 0.3	<0.016	> 162.5
FC-10906		> 10	2.4	> 4.2	<0.016	> 625



Cytotoxic properties of FC-11017, FC-10696, and FC-10906 were tested in HeLa cell line and human monocyte-derived macrophages using CellTiter-Glo kit. Cells grown in 96-well plates were left untreated, treated with 11 2-fold serially diluted concentrations of the compounds or equal concentrations of the solvent, DMSO, for 48 or 72 hrs in triplicate. The number of metabolically active cells was determined by measuring luciferase activity. For each compound, non-linear regression analysis was performed using GraphPad 8 software to select a non-toxic concentration range for antiviral tests. In the virus tests, HeLa cells or macrophages were challenged with either EBOV or MARV at multiplicity of infection (MOI) of 0.01 for 1 hr to allow binding, then washed, and incubated with new medium containing 7 2-fold serially diluted concentrations of the compounds, equal concentrations of DMSO, or no treatment, for 48 or 72 hrs. To assess virus egress, cell supernatants were titrated on Vero cells. Infected cells were detected by staining with highly specific anti-EBOV glycoprotein (GP) antibody (clone 4F3) [12] or anti-MARV VLP antibody [13], and nuclei were stained with Hoechst dye. Samples were subsequently photographed using a Nikon automated system and analyzed by CellProfiler software as we described [13, 21] to quantify infected cells and nuclei. Selectivity index (SI_{50}) for each compound was calculated as CC_{50}/IC_{50}. Only data for 48 hr time point are shown. The insert illustrates MARV egress inhibition curves for FC-10696 in human macrophages.

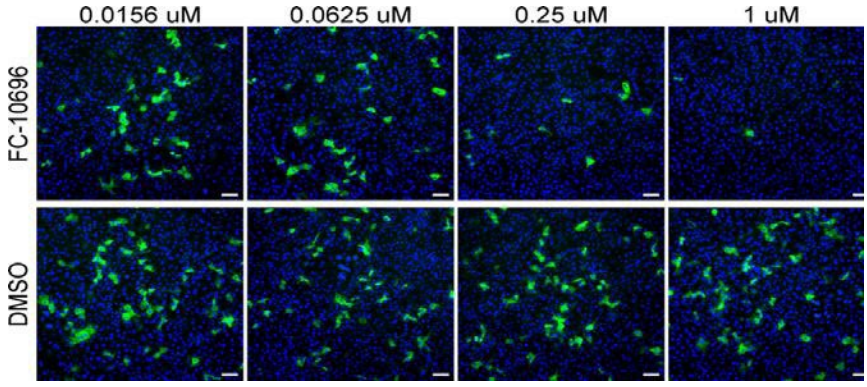


Fig. 5. *FC-10696 inhibits MARV egress from primary human macrophages.* Supernatants of primary human macrophages challenged with MARV and treated with indicated concentrations of FC-10696 or equal amount of DMSO were titrated on Vero cells for 24 hrs. Samples were treated with anti-MARV VLP antibody to detect infected cells (green) and Hoechst dye to detect nuclei (blue) and subsequently photographed using a Nikon imaging system. The bar in each image is 100 um.

***In Vitro* Live Filovirus Assays**. To assess safety of the three lead compounds, we first assessed their cytotoxicity in a HeLa cell line, routinely used in anti-filoviral screens, and primary human macrophages, the initial targets of filovirus infection in the host [44, 45]. The compounds appeared to be more cytotoxic to HeLa cells than to macrophages, with FC-10696 being the least toxic among the three. Assessment of efficacy revealed that all compounds inhibited both MARV and EBOV replication; albeit inhibition of MARV was more efficient. We determined CC_{50} and IC_{50} values for all time points for each virus using non-linear regression analysis using GraphPad 8 software and listed select values in Table 2 and images of MARV

in Fig. 5. Notably, the selectivity index (SI_{50}, calculated as CC_{50}/IC_{50}) for each compound was ≥10 in MARV virion egress assay, signifying antiviral potency and selectivity of these compounds against this virus.

***In Vivo* Toxicity Studies**. Since BALB/c mice will be used in efficacy studies with live virus, we used this mouse strain to assess toxicity of our lead compounds at two different concentrations: 1) the highest resuspendable concentration of each compound (20 mg/kg for FC-10696, 10 mg/kg for both FC-11017 and FC-10906) and 2) a concentration which was 5-fold lower. Dosing with FC-10696 resulted in the smallest overall weight loss and only transient clinical signs of toxicity (Fig. 6). Based on these toxicity data and *in vitro* data (Table 2), we chose to test FC-10696 for protection against MARV disease in a suitable mouse model.

Efficacy studies using a mouse model of MARV disease. BALB/c mice are highly susceptible to infection with mouse-adapted MARV, developing disease symptoms and high viremia approximately three days post-infection, and succumbing to the disease by day 6. Treatment with 20 mg/kg twice daily for 10 days delayed the onset of mortality (p=0.0182; Fig. 7A), weight loss (Fig. 7B), and virus load in serum (p=0.0255; Fig. 7D), providing strong support for this class of compounds for further development into potent antivirals against filoviruses and possibly other viruses that require the PPxY/Nedd4 interaction for productive infection. These ground-breaking findings represent the first proof-of-concept in vivo activity for our lead host-oriented PPxY inhibitor.

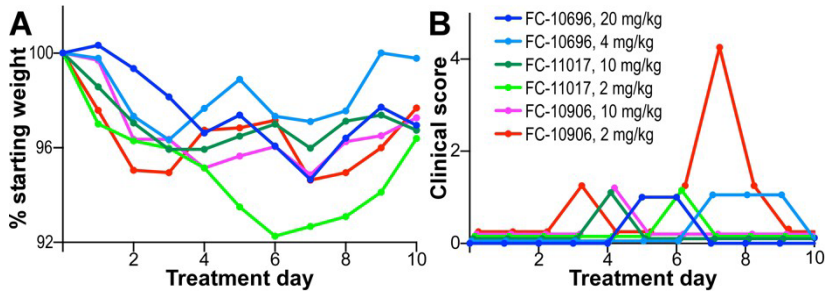


Fig. 6. *In vivo* toxicity studies. Compounds were resuspended in 30%PEG400/ 2%DMSO/14%kleptose HPB parenteral grade (Roquette) a formulation at two different concentrations: 20 and 4 mg/kg (FC-10696); 10 and 2 mg/kg (FC-11017, FC-10906), and administered to groups of five 4-week old female BALB/c mice twice daily (BID) via the intraperitoneal route (IP) for a period of 10 days. Animals were monitored daily for signs of treatment-associated toxicity: weight loss **(A)**; rough hair coat, discharge from eyes and nose, diarrhea, decreased food intake and activity **(B)**; and mortality. Clinical scores for each group were recorded as a sum of all observations in the group.

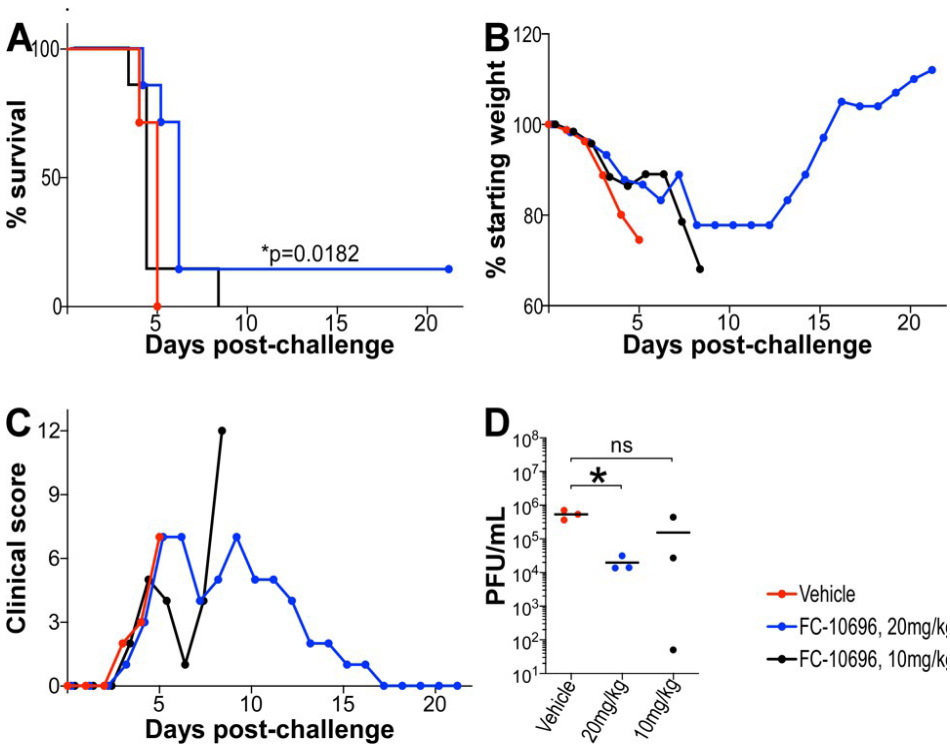


Fig. 7. *Efficacy studies of FC-10696 treatment in a mouse model of MARV disease.* Three groups of 10 4-week old female BALB/c mice were challenged with 1,000 plaque-forming units (PFUs, as determined on Vero cells) of mouse-adapted MARV. IP dosing by vehicle or FC-10696 at either 20 or 10 mg/kg started 6 h post-challenge and continued BID for 10 consecutive days. Animals were observed daily for mortality **(A)**, weight loss **(B)**, and clinical signs of disease **(C)** for 21 days post-infection. Clinical scores for each group were recorded as a sum of all observations in the group, and if a score of ≥12 was recorded for an individual animal, it was considered terminally ill and euthanized. On day 3 post-challenge, 3 animals/group were euthanized to collect serum for virus load assessment by the neutral red plaque assay **(D)**. The remaining 7 mice/group were used to determine animal survival.

Highlights of what we have achieved with our analogs are listed below.

- Low nanomolar functional activity (<100 nM in VLP Marburg and Ebola cell assay);
- Low nM target engagement (<500 nM, inhibition of Nedd4/VP40 PPxY binding in cellular BiMC assay);
- Inhibition of live virus cellular egress (<100 nM, Marburg);
- Little to no cytotoxicity at effective antiviral concentrations;
- Stable in human liver microsomes (low clearance and long *in vivo* half-life);
- No inhibition of CYP 3A4 (lower probability of drug-drug interactions);
- Suitable ADME and PK properties for IP administration in mice;
- Tolerability in mice at doses up to 20 mg/kg;
- **Proof of concept *in vivo* activity in a mouse model of Marburg virus disease; and**
- Novelty in structure, resulting in composition of matter patent protection.

Based on these favorable properties, additional analogs will be prepared and tested, with the goals of maintaining and improving favorable ADMET and PK properties using pharmaceutical industry standard *in vitro* and *in vivo* metrics, maintaining and improving VLP and live virus potency, and showing efficacy in a mouse model of Ebola or Marburg virus disease under **oral administration**. Medicinal chemists have routinely improved drug properties that overcome many issues including designing and identifying analogs with oral activity so we fully expect to accomplish the goals below.

Criteria (Go/No Go) for analogs to move forward to preclinical candidate status include:

- Low nM (<100 nM) potency for VLP and BiMC *in vitro* comparable to our current leads, FC-10696;
- Inhibition of live virus proliferation (IC_{50} <100 nM);
- Minimal compound effects on other cellular Nedd4 activities;
- Little or no cytotoxicity in mammalian cell lines at 50-fold higher than minimum effective concentrations;
- Suitable ADME and PK properties for once a day oral administration;
- **In vivo activity in a mouse model of Ebola or Marburg virus disease under oral administration (PO)**;
- Composition of matter patent protection worldwide.

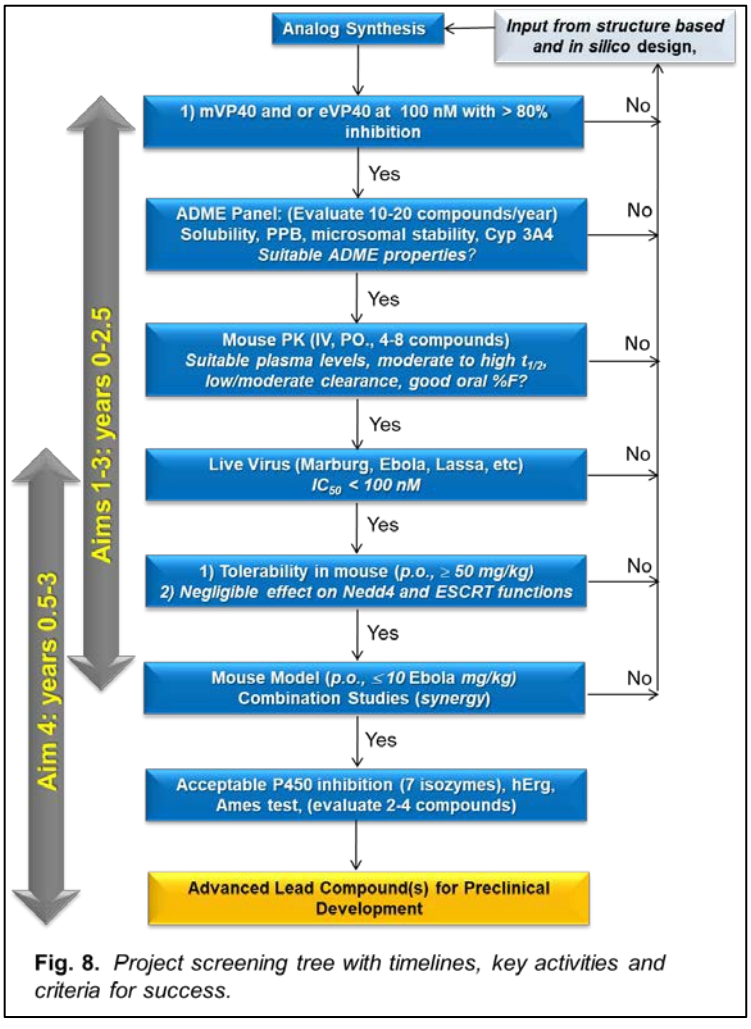


Fig. 8. *Project screening tree with timelines, key activities and criteria for success.*

D. Experimental Approach:

Specific Aim 1: Lead optimization medicinal chemistry. Our goal is to design and synthesize novel analogs based on FC-10696 and to identify 2-3 that meet the above criteria. We will pursue acceptable ADME and PK properties suitable for PO administration and identify one or more of these compounds to move forward to IND-enabling studies. We will accomplish these objectives via an iterative SAR approach using medicinal chemistry with parallel synthesis methods according to the work flow diagram and timelines in Figure 8. We will use *in silico* property calculations and pharmacophore analysis to monitor ADME drug properties, such as Log D, solubility and permeability using Chem Axon and QikProp from the Schrodinger Small-Molecule Drug Discovery Suite. Orally administered drugs must maintain good aqueous solubility by maintaining lipophilicity (cLog D, cLogP) and topological polar surface area (tPSA) values <5 and < 120 $Å^2$, respectively. These values predict optimal clearance properties, good cell-based activity and avoidance of off-target

effects associated both with high molecular weight and lipophilicity. All compounds will be synthesized, purified and characterized at FCCDC labs. FCCDC has the capacity to prepare up to 250 g of any chemical analog. We will scale up, in multigram amounts, analogs that meet the criteria on the flow scheme for *in vivo* or other non-GLP assays. In addition, we will synthesize possible metabolites found during the metabolite identification processes.

Our mandate is to improve aqueous solubility of our analogs while continuing to improve their potency, therapeutic index, ADME, PK and safety properties. We will focus on the following four areas:

1) Understand stereospecificity of FC-10696. Of our three lead compounds, FC-10696 has the best microsomal stability and solubility. Thus, we will synthesize both R and S enantiomers from the readily available starting materials S and R 1-[1,1'-biphenyl]-4-ylethanol, respectively. These will be evaluated for potency, stability and solubility to determine the most promising enantiomer for further studies. We will also determine IV and PO PK in mice of the best enantiomer to determine if it could be an early front runner in our pursuit of a preclinical candidate.

Fig. 9. *Quaternary methylene analogs of FC-10696*

2) Quarternary methylene analogs of FC-10696. Analogs **C** and or **D** in Fig. 9 avoid the chiral center of FC-10696 altogether and we expect good potency in our primary assays based on previous SAR, as well as good stability in mouse microsomes. Our previous method of preparing compounds of this type utilized 4-(1-bromo-1-methyl-ethyl)-biphenyl to alkylate a quinoxaline thiol and led to FC-12004 in Table 1 among others, which gave poor yields of desired products (< 5%). However, using a different synthetic approach outlined in Figure 9, much better yields are expected. The starting materials **A** [46] and **B** [47] should be easily synthesized from readily available commercial chemicals.

3) Water soluble prodrugs of FC-10696. Despite our SAR studies to date, we have been unable to add appropriate basic or acidic heteroatoms or sp^3 carbons that might increase water solubility, and also retain good potency. However, FC-10696 has a 2-NH_2 moiety, which we could link to a water-soluble prodrug group, to give FC-10696 (or the active enantiomer of FC-10696) greater water solubility to aid intestinal absorption following oral administration. The prodrug moiety of the absorbed drug would be removed by plasma or liver esterases, amidases or phosphatases (depending on prodrug type) to provide good plasma levels of parent compound (FC-10696) [48-50]. FCCDC has considerable experience with prodrugs of riluzole, which contains a similar NH_2 moiety from which the prodrug group emanates [51, 52]. One of our riluzole prodrug derivatives is currently in Phase II clinical trials. Some potential water-soluble prodrugs are shown in Fig. 10.

Fig. 10. *Potential water-soluble prodrugs of FC-10696.*

4) New quinoxaline analogs. In a further effort to increase water solubility of quinoxaline analogs of FC-10696, we plan to find an appropriate area on our molecules to append a water-soluble group (WSG) as outlined in Fig. 11. We know from our wide-ranging SAR that the quinoxaline ring or 2-methyl moieties cannot tolerate extensive modifications. However, the two phenyl rings of the biphenyl side chain moiety can tolerate substitutions. Therefore, our plan is to prepare analogs such as **A** and **B** that walk the WSG around the two rings to identify the best position to accept these groups. In addition, we know the middle ring of the "biphenyl like" side chain of our analogs can be a pyridine ring. Thus, we will prepare the two pyridyl analogs **C** and **D** in Fig. 11, and this should provide additional solubility to common formulations when compared with FC-10696.

Fig. 11. *Quinoxaline analogs with water soluble groups and pyridyl moieties.*

5) New scaffolds. As a backup compound strategy, we will pursue alternative scaffold compounds, such as the [redacted] scaffold, that yielded the promising analog FC-12048 (Table 1, Figure 2). In addition, we will prepare the close analog [redacted] **A** shown in Fig. 12, which will increase the basicity and ability to form salts with simple acids to increase aqueous solubility. This compound should be readily synthesized from [redacted] and [redacted]. Other scaffolds we will pursue that fit the current SAR profile and have been vetted for synthetic feasibility include: [redacted] respectively which can be prepared from readily available appropriately substituted [redacted] (for **B**) or [redacted] (for **C**) and [redacted].

[redacted]

Fig. 12. *Potential New Scaffolds*

All new analogs will be evaluated in our filovirus VLP MARV budding assay (Aim 2) [4-6, 12] to determine the antiviral potency, and those meeting potency criteria will be further assessed as described the screening tree in Fig. 8 and in Aim 3 and 4.

Specific Aim 2. Evaluate compounds for their ability to specifically inhibit the PPxY-Nedd4 interaction and subsequent virus egress. All of the assays, reagents, and expertise are in place and readily available for these studies [30, 36]. We will use a three-pronged approach under BSL-2 conditions to identify the best compounds to be advanced to the more challenging and hazardous studies in the BSL-4 laboratory. First, we will use our well-established filovirus and arenavirus VLP budding assays, which recapitulate live-virus budding [4-7, 12] (see Fig. 4), to determine the antiviral potency of the best compounds identified in Aim 1. For EBOV and MARV, we will also include co-expression of NP and GP (along with VP40) in our VLP budding assays to more closely mimic budding of authentic EBOV and MARV. We will quantify egress of VLPs, focusing initially on mVP40-WT or PPxY mutants of mVP40 as controls (*e.g*. mVP40-AAAA or mVP40-ΔPY), in the presence of each compound, and identify those that robustly block PPxY-dependent VLP egress in a dose-dependent manner. Once we identify inhibitors having activity against PPxY-mediated mVP40 VLP budding, we will then test these compounds for broad-spectrum activity using both EBOV VP40 and LAFV Z VLP budding assays [30, 36].

Second, we have implemented a BiMC approach [37] to detect and visualize mVP40-Nedd4 interactions in live mammalian cells [30] . We will use BiMC to evaluate the relative ability of analogs identified in Aim 1 to disrupt mVP40-Nedd4 interactions (see Fig. 3). NYFP-Nedd4 and appropriate CYFP-WT or PPxY mutants of mVP40 will be co-expressed in HEK293T cells, and we will use an inverted Leica Sp5-II confocal microscope to evaluate the ability and potency of inhibitors to block fluorescent complementation of mVP40-Nedd4 complexes. Quantitative analysis of complementation will be achieved by scoring individual cells and the rate, localization, intensity, and kinetics of fluorescence accumulation to determine drug potency. A PPxY L-domain mutant of mVP40 that does not interact with Nedd4 will be utilized as a negative control [36], and MTT cell viability assays will be performed in triplicate for all potent analogs tested, as described previously [34, 36].

Third, we will test lead analogs for their ability to block PPxY-mediated budding of live BSL-2 viruses VSV-WT, VSV-M40, and PPxY mutants of each (21). While results of the VLP budding and BiMC assays will identify novel potent budding inhibitors, the ultimate test of efficacy will be their ability to inhibit live-virus budding. We will test lead compounds at various concentrations for their ability to block live virus budding and spread in cell culture, using confocal microscopy and virus titration assays to quantify our findings. Lead compounds with robust activity in VLP, BiMC, and VSV budding assays will be prioritized by their anti-budding potency and used to test for antiviral activity against authentic BSL-4 pathogens at TBRI as described in Aim 4. We do not anticipate any insurmountable pitfalls with these well established and routinely used experimental approaches.

Evaluate lead candidate inhibitors for potential effects on Nedd4-mediated ubiquitination and ESCRT-associated functions. Nedd4 is a member of the HECT family of E3 ubiquitin ligases that mono-ubiquitinates ESCRT proteins as well as viral matrix proteins to regulate their functions [3, 4, 7, 8, 13, 17, 19-29]. Since our budding inhibitors target Nedd4 interactions, it is important to determine whether these inhibitors adversely affect the normal function(s) of Nedd4, which includes trafficking and degradation of cell surface receptors, as well as cytokinesis [54-58]. Cells proliferate normally in the presence of these inhibitors and the presence of a number of potentially compensatory HECT E3 ubiquitin ligases suggests that global cellular ubiquitination will be maintained. However, to verify that there is no inhibitor-induced defect in global cellular ubiquitination, we will assess total cellular

ubiquitination profile by Western analysis using HA-tagged ubiquitin in HEK293T cells treated with either DMSO alone or with working concentrations of our lead inhibitors. As Nedd4 regulates the ESCRT protein Tsg101-dependent turnover and degradation of transferrin receptor 1 (TfR1) and epidermal growth factor receptor (EGFR) [54, 55, 59-61], we will determine whether our lead PPxY inhibitors alter Nedd4-mediated protein trafficking events at concentrations that block virus budding by measuring total cellular and surface expression levels of TfR1 and EGFR using immunofluorescence and confocal microscopy. DMSO treated cells will be used as a negative control.

Second, we will assess potential global effects on total cellular RNA expression in HEK293T cells treated with either DMSO alone or with working concentrations of our lead inhibitors using RNAseq in collaboration with Dr. Dan Beiting (letter attached). mRNA-seq libraries will be prepared using the TruSeq Stranded mRNA LT Kit (Illumina), and all sequencing will be carried out in-house on our Illumina NextSeq 500 (base calling accuracy of >99.9%). All data processing and analyses will be carried out using the R programming language (Version 3.2.2) and the RStudio interface (Version 0.99.489). We do not anticipate that our inhibitors will impact gene expression and our preliminary findings using RNA seq. revealed no significant difference in expression levels of the Nedd4 gene in the presence of DMSO alone vs. 1μm of FC-11017 (data not shown), suggesting that the effects of our inhibitors are not due to decreased Nedd4 expression and instead work by blocking Nedd4 interactions with viral proteins.

Specific Aim 3. *In vitro* and *in vivo* ADMET evaluation to ensure suitable drug properties and selectivity**.** FCCDC will evaluate potential drug suitability issues as another filter to choose the best compounds for *in vivo* study and preclinical candidate selection. An estimated10-15 compounds per year that meet activity criteria in the screening tree (high potency and suitable *in silico* property criteria; Fig. 8) will be evaluated in *in vitro* ADMET assays (performed by the CRO Alliance Pharma, Inc.). Specifically, we will use microsomal stability and plasma protein binding assessment as reliable indicators of clearance and half-life *in vivo.* In addition, measurement of CYP 3A4 inhibition will give us an early indication as to whether our compounds may have variable exposure *in vivo* or a risk of drug-drug interactions. Aqueous solubility and permeability will also be assessed, because low solubility/stability in assay medium is detrimental to development of a sound SAR and will also hamper our ability to formulate analogs for *in vivo* studies. We will move compounds forward that have acceptable values according to standard drug property criteria (see Fig. 13). We will also evaluate the **5-8 best compounds** for PK properties in male and female mice following IV and PO administration (performed by the CRO Alliance Pharma, Inc.) to assess PK properties for eventual study in our mouse model (Aim 4). We will evaluate each sex separately to determine whether sex is a biological variable. If there is no difference, we will use both sexes in later studies. We expect to see low to moderate clearance, moderate volume of distribution, moderate to long half-life and good oral bioavailability. This last point is important, because we envision that the ultimate clinical use of our agents will involve oral administration alone or in combination with other antiviral drugs. The **2-4 most advanced compounds** that have met potency and selectivity criteria and have acceptable *in vitro* ADME and PK properties will be evaluated further in the following non-GLP safety assays:

- IC_{50} values will be determined for the seven [redacted] most likely to cause exposure variability and drug-drug interactions (Alliance Pharma).
- Manual patch clamp assays will be performed to assess hERG (human Ether-à-go-go-Related Gene product) potential for cardiac arrhythmias (Cyprotex).
- Ames testing will be performed with and without metabolic activation to assess mutagenic potential (Cyprotex).
- Safety panel of 44 primary molecular targets that detects major potential adverse activity (Eurofins).

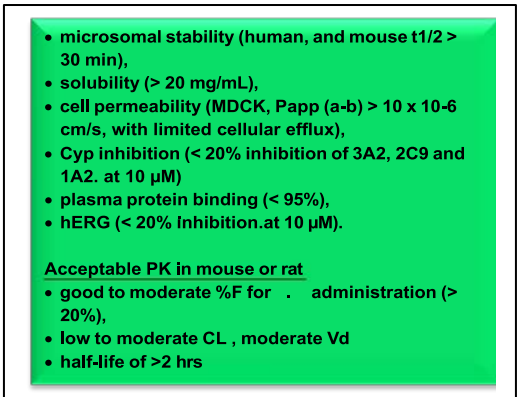


Fig. 13. *Standard drug property criteria*

Compounds that have successfully completed the *in vivo* live virus mouse model (Aim 4), will be evaluated for PK properties in male and female rats and dogs following IV and PO administration (performed by the CRO Absorption Systems, Inc.) in preparation for evaluation in IND enabling studies. The best overall compounds will also be incubated in mouse and human microsomes under phase I and II conditions to generate and identify metabolites.

These industry-standard preclinical ADMET/PK studies will ensure that the selected analogs have the best chance to complete the drug development process. We are confident that at least one preclinical candidate will ultimately proceed through IND-enabling, preclinical development studies prior to human clinical evaluation. For a detailed description of the activities and timeline surrounding our push to an IND application, please refer to the Commercialization Plan.

Specific Aim 4: *In vitro* and *in vivo* analyses of lead inhibitors against authentic hemorrhagic fever viruses. All experiments with replication-competent EBOV, MARV, and LASV will be performed in the BSL4 laboratory of Texas Biomedical Research Institute (TBRI) by Dr. Olena Shtanko (letter of collaboration is attached). We will first determine the cytotoxic properties of lead compounds identified in Aim 2. HeLa cells will be left untreated or treated with the compounds at ≥12 2-fold serially diluted concentrations or DMSO for 24, 48, or 72 hrs. The number of metabolically active cells will be determined using a CellTiter-Glo kit as in Table 2 and described [62, 63]. We will determine the CC_{50} value for each compound at each time point using non-linear regression analysis to select a non-toxic concentration range for antiviral tests.

To test whether the compounds affect egress and spread of EBOV, MARV, and LASV (virus stocks available at TBRI repository), we will incubate HeLa cells with virus at MOI=0.01 for 1 hr to allow binding, then wash, and overlay with new medium containing compounds at ≥7 2-fold serially diluted concentrations, DMSO, or no treatment, for 24, 48, or 72 hrs to cover several cycles of virus replication. We will detect infected cells by treatment with EBOV GP antibody, MARV VLP antibody, or LASV GP antibody (clone 83.6; Dr. Martinez-Sobrido's letter is attached) [64], and nuclei by staining with Hoechst dye. We will photograph samples using a Nikon automated system and use CellProfiler software to quantify virus spread. To assess virus egress, we will titrate cell supernatants at each time point on Vero cells. Virus infection and inactivation studies will be performed in BSL4 using approved protocols. All post-infection analysis will be conducted in BSL2. The IC_{50} (virus spread, egress) values for each compound will be determined by non-linear regression analysis. We will use the CC_{50}/IC_{50} (selectivity index, SI_{50}) to assess antiviral potential of the compounds; those with $SI_{50}\geq10$ in egress assays will be tested for antiviral activity in primary human macrophages, the initial and clinically relevant target cells of filoviruses and arenaviruses [44, 45, 65].

Human monocytes will be obtained from peripheral blood, with a typical yield of ≥15-20 million cells per a pint of blood from each donor and monocyte-derived macrophages (MDMs) will be obtained by differentiation in autologous serum [66, 67] to more closely mimic the physiological environment. We will assess compound cytotoxicity and antiviral properties at 24, 48, and 72 hrs time points as described above and, for those with $SI_{50}\geq10$ in egress assays, determine the IC_{95} value to be used in the design of a treatment regimen in animals.

For each experiment, ≥3 independent tests in triplicate will be performed. Experiments with human MDMs will be performed from ≥6 different healthy adult donors (50:50 female:male ratio). We expect the same outcome trend among donors, although there may be a difference in the magnitude of the response. All data will be analyzed for statistical significance using either a Student *t*-test to compare averages of 2 groups or one-way ANOVA with Tukey's test to compare averages of >2 groups, with $p\leq0.05$ being considered significant. We will inspect data for normality. Where deviations from normality are seen, we will use appropriate transformations, or non-parametric tests.

In vivo analysis of toxicity and accumulation of lead inhibitors. All ABSL2 experiments in mice will be performed at the TBRI rodent vivarium, which is staffed with full-time veterinarians and a large number of support personnel. Up to 4 compound candidates will be assessed for toxicity in BALB/c mice each year. Due to their susceptibility to filoviruses infection and pathology [44, 68], these mice have been used extensively in efficacy studies. Males and females randomly assigned to groups of 5 animals will receive dose formulation containing compounds at 2 different doses or vehicle PO, up to 2 times a day (BID) for 10 consecutive days. The starting dose level and dosing frequency for each compound will be selected based on the findings of the PK study (Aim 3) and IC_{95} data, above, to continuously maintain blood concentrations sufficient to block virus replication. A lower concentration will be needed to assess whether any observed toxicity is dose-dependent. The following parameters and end points will be evaluated in each group: **(i)** mortality; **(ii)** clinical signs, including weight change, food consumption, decreased motor activity, eye and nasal discharge, dyspnea, and unresponsiveness; **(iii)** serum biochemistry, including aspartate aminotransferase, alanine transarninase, alkaline phosphatase, total and direct bilirubin, urea, and creatinine levels, at the end of the study; **(iv)** post-mortem gross examination of the external features of the carcass as well as cranial, thoracic, and abdominal cavities, at the terminal necropsy; and **(v)** post-mortem histopathological examination of major organs, including liver, kidney, spleen, brain, heart, lungs, and adrenal glands (to be performed by TBRI Pathology Core personnel). We will use 5

mice/experimental condition as determined by power calculation. A Student *t*-test (with proper data transformation if needed) will be used to compare the difference between two groups. A sample size of 5 mice/experimental condition will provide at least 80% power to detect a 3 standard deviation difference at each condition between two groups at a significance level of 0.01 (up to 5 multiple comparison adjustments). Multiple group comparisons will be analyzed by one-way ANOVA with Tukey's test. A $p \leq 0.05$ will be considered significant. The experiments will be performed at least 2 times to ensure experimental reproducibility. To evaluate any sex-specific differences in response to treatment, groups of both male and female animals will be used. We will require 540 mice total [5 mice per group x 9 treatments (vehicle, 4 compounds at 2 different concentrations) x 2 sexes x 2 (repetitions) x 3 (years)] to complete these studies.

Based on the results of the toxicity studies, we will select up to 2 compounds per year to assess body distribution and accumulation over time. Treated mice will be used to collect blood and organs critical for filovirus replication (liver, kidney, spleen) on days 1, 3, 5, 7, and 11. Blood samples will be centrifuged to separate the plasma fraction and, together with tissues, shipped to Alliance Pharma, Inc. to quantify compound levels. We will determine whether the compounds (i) circulate systemically and (ii) are present at concentrations necessary to inhibit ≥95% of virus replication. In these tests, 3 animals are required to show uniform outcome by a Student *t*-test or one-way ANOVA. We will repeat each study at least once. These experiments will require 360 mice [3 mice per time point x 5 time points x 2 compounds x 2 sexes x 2 (repetitions) x 3 (years)].

In vivo treatment efficacy studies. Based on the data obtained from the compound toxicity/accumulation studies above, we will test up to 2 different treatments each year for protection against filovirus challenge using a mouse model of MARV disease. TBRI maintains an experienced and trained staff of veterinarians and veterinary technicians available to perform rodent studies in the ABSL4 laboratory. Groups of 10 BALB/c mice will be challenged with a lethal dose of 1,000 PFU of mouse-adapted MARV by IP. PO dosing will start 6±2 hrs post-challenge and will continue for 10 days at a frequency determined above. Animals will be closely monitored at least twice daily for signs of viral disease (ruffled hair coat, hunch back, inappetence, weight loss, and decreased movement) and mortality for 21 days post-challenge. Group clinical scores will be recorded as the sum of all clinical observations for the group. If a clinical score of ≥12 is recorded for an animal, it will be considered terminally ill and euthanized. Three animals from each group will be euthanized on day 3 post-challenge to collect blood and organs (liver, spleen, and kidneys) to determine virus titer by a plaque assay and load by qPCR as we described [69] and in Fig. 7D. Based on our data, BALB/c mice challenged with 1,000 PFU of mouse-adapted MARV become ill at 3 days and succumb to disease 5-6 days post-challenge (Fig. 7A). Assuming that we aim to protect 70% of animals from the virus disease, a power calculation comparing two independent proportions (significance=0.05 and power=0.8) indicates that 6-7 animals would be required for the survival study. Since we require 3 animals to be euthanized on day 3 for evaluation of virus burden, the group size is set to 10. To evaluate any gender-specific differences in response to treatment, groups of both male and female animals will be used. Viral burden will be analyzed using a Student *t*-test or one-way ANOVA Tukey's test, and survival analysis will be performed using a Log-rank (Mantel-Cox) test, with $p \leq 0.05$ considered significant in all analyses. The efficacy studies will require 180 mice [10 mice per group x 3 treatments (vehicle, 2 dosings) x 2 sexes x 3 (years)].

Summary. Our success in Phase I of identifying potent, in vivo active, filovirus egress and spread inhibitors has positioned us to execute an expanded novel drug discovery program in Phase II. Notably, the success of inhibitor **FC-10696** in protecting mice against MARV disease upon IP administration highlights the tremendous potential for this class of host-oriented compounds to be further developed into potent broad-spectrum antivirals. In Phase II, we seek to further transition one or more full-qualified PPxY: Nedd4 complex inhibitors into more detailed IND-directed pharmacokinetic, pharmacodynamic and toxicity studies suitable for once-daily PO administration in the clinic. Successful completion of these goals will provide a comprehensive package to support advanced development, efficacy testing in nonhuman primates and an IND submission for Phase I clinical safety testing in normal volunteers. Our ultimate goal is to provide and oral small molecule pharmacotherapy to patients who may be infected with Marburg, Ebola or related viruses in the future. Such viral hemorrhagic fever diseases are listed as being a focus of interest for SBIR/STTR funded research in the 2020 omnibus SBIR/STTR solicitation. Clinical development would be simplified by the use of the animal rule, in which Phase II/III efficacy studies are not required in human patients as FDA approval would be based upon efficacy in relevant animal models followed by acceptable safety and tolerability in Phase I. Drug approvals for Marburg and Ebola would both qualify for a saleable Neglected Disease Priority Review Voucher, currently worth [redacted]

Progress Report Publication List

None

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001

Expiration Date: 02/28/2023

Use of Human Specimens and/or Data

Does any of the proposed research in the application involve human specimens and/or data *	● Yes ○ No
Provide an explanation for any use of human specimens and/or data not considered to be human subjects research.	
Are Human Subjects Involved	● Yes ○ No
Is the Project Exempt from Federal regulations?	○ Yes ● No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Other Requested Information	

Human Subject Studies

Study#	Study Title	Clinical Trial?
1	Development of Host-Oriented Small Molecule Therapeutics for Filoviruses and Arenaviruses	No

Section 1 - Basic Information (Study 1)

OMB Number: 0925-0001

Expiration Date: 02/28/2023

1.1. Study Title *

Development of Host-Oriented Small Molecule Therapeutics for Filoviruses and Arenaviruses

1.2. Is this study exempt from Federal Regulations * ❍ Yes ● No

1.3. Exemption Number ❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8

1.4. Clinical Trial Questionnaire *

1.4.a. Does the study involve human participants?	● Yes	❍ No
1.4.b. Are the participants prospectively assigned to an intervention?	❍ Yes	● No
1.4.c. Is the study designed to evaluate the effect of the intervention on the participants?	❍ Yes	● No
1.4.d. Is the effect that will be evaluated a health-related biomedical or behavioral outcome?	❍ Yes	● No

1.5. Provide the ClinicalTrials.gov Identifier (e.g. NCT87654321) for this trial, if applicable

Section 2 - Study Population Characteristics (Study 1)

2.1. Conditions or Focus of Study

- Develop host-oriented therapeutics targeting filoviruses and arenaviruses

2.2. Eligibility Criteria

Must be 18-50 years old- Must weigh at least 110 pounds- Cannot be pregnant- Cannot be on a medication- No illness within 10 days

2.3. Age Limits	Min Age: 18 Years	Max Age: 50 Years
2.3.a. Inclusion of Individuals Across the Lifespan	20-240_Inclusion_Across_Lifespan.pdf	
2.4. Inclusion of Women and Minorities	20-240_Inclusion_Women_Minorities.pdf	
2.5. Recruitment and Retention Plan	20-240_Recruitment_and_Retention_Plan.pdf	
2.6. Recruitment Status	Not yet recruiting	
2.7. Study Timeline	20-240_Study_Timeline.pdf	
2.8. Enrollment of First Participant	04/01/2021	Anticipated

Inclusion of Individuals Across the Lifespan

Women and men between the ages of 18-50 years old will be enrolled onto the study.

The venipuncture of healthy children (less than 18 years old) adds concerns and risks beyond those seen with adults. At the present time, there is no reason to believe that children would represent a unique population compared to adults in this study. The amount of blood required for the requisite number of cells essential for an experiment would be proportionally higher in children due to their decreased size. Anatomically, children have significantly smaller veins presenting increased challenges of drawing blood in a laboratory setting. The discomfort and initial pain of the blood draw procedure while minimal in adults would be more likely to incite increased psychological trauma and fear in children. Thus, the inclusion of children at the present time is not justified given the additional risks for the procedure. With additional data obtained from adults, in the future a new study directly comparing peripheral blood mononuclear cells from adults and children may be warranted.

Venipuncture of aged adults (over 50 years old) adds concerns and risks, particularly of infection, beyond those seen with adults. Thus, the inclusion of aged adults at the present time is not justified given the additional risks for the procedures. It is well established that cells from aged and adult individuals exhibit different inflammatory phenotypes. It is beyond the scope of the current proposal to compare responses to aged and adult cells, these complex studies are better suited for dedicated studies once responses in adults have been clearly defined.

The study team is well experienced in working with adults (18-50 years old) and facilities for working with these individuals are in place and being utilized for other studies. The developmental nature of this study does not require assessing the impact of aging on Marburg, Ebola, and Lassa virus infection. It is critical to develop *in vitro* models with adult cells prior to conducting studies directly comparing cells from adults to children and aged adults.

Inclusion of Women and Minorities

There will be no discrimination based on gender, race, or ethnicity. To ensure that the gender distribution is equal, recruitment will be directed at acquiring approximately 50% females and males. For venipuncture, 9 females and 9 males will be recruited. Women who are currently pregnant will be excluded from the study, due to health concerns associated with donating blood. Recruitment will be monitored to assure that minority populations are represented in this cohort of subjects. The target percentages of racial minorities are based on the racial distribution in San Antonio, TX. The population of San Antonio, TX is 62.7% Hispanic/ Latino, 26.6% Caucasian, 6.8% African-American, 2.4% Asian, and less than 0.4% American Indian/Alaska Native, and, thus the study population may reflect a slight bias toward Hispanic/Latino to reflect the population demographics. These demographics are acceptable for studying macrophage responses during EBOV infection. If the usual recruitment methods yield only Caucasian volunteers, notices will be placed on bulletin boards where larger numbers of minority students are likely to see them, such as the Afro-American Culture Center. The notices could also be expanded to include a statement that “women and minority students are especially encouraged to apply.”

Recruitment and Retention Plan

Recruitment

Subjects are recruited from among healthy laboratory and office personnel, other Texas Biomed employees; students at the Colleges and Universities in San Antonio, including UT Health San Antonio and University of Texas San Antonio (UTSA); hospitals, including the San Antonio Metropolitan Health District; and the community. Subjects are largely recruited through flyers that are posted throughout the community, schools, hospitals, and Texas Biomed, as well as word of mouth. Recruitment activities also involve community events. Recruitment will be monitored to assure that minority populations are represented in this cohort of subjects. The target percentages of racial minorities are based on the racial distribution in San Antonio, TX. The population of San Antonio, TX is 62.7% Hispanic/Latino, 26.6% Caucasian, 6.8% African-American, 2.4% Asian, and less than 0.4% American Indian/Alaska Native, and, thus the study population may reflect a slight bias toward Hispanic/Latino to reflect the population demographics. If the usual recruitment methods yield only Caucasian volunteers, notices will be placed on bulletin boards where larger numbers of minority students are likely to see them, such as the Afro-American Culture Center. The notices could also be expanded to include a statement that “women and minorities are especially encouraged to apply.”

Retention

Subjects are allowed to donate no more than 480 ml of blood in an 8-week time span. They will be allowed, but not expected, to participate multiple times, as long as they donate no more than 480 ml of blood in an 8-week time span, or only once.

Study Timeline

Recruiting for the study will start on 04/01/21, following the approved UT Health San Antonio IRB protocol #HSC20180013H. Recruitment is expected to occur throughout the entire three year duration of the proposal.

2.9. Inclusion Enrollment Reports

IER ID#	Enrollment Location Type	Enrollment Location
Study 1, IER 1	Domestic	Texas Biomedical Research Institute

Inclusion Enrollment Report 1

1. Inclusion Enrollment Report Title* : Development of Host-Oriented Small Molecule Therapeutics for Filoviruses and Arenaviruses

2. Using an Existing Dataset or Resource* : ❍ Yes ● No

3. Enrollment Location Type* : ● Domestic ❍ Foreign

4. Enrollment Country(ies): USA: UNITED STATES

5. Enrollment Location(s): Texas Biomedical Research Institute

6. Comments:

Planned

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	0
Asian	0	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	1	1	0	0	2
White	2	2	6	6	16
More than One Race	0	0	0	0	0
Total	3	3	6	6	18

Cumulative (Actual)

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/ Alaska Native	0	0	0	0	0	0	0	0	0	0
Asian	0	0	0	0	0	0	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	0
Black or African American	0	0	0	0	0	0	0	0	0	0
White	0	0	0	0	0	0	0	0	0	0
More than One Race	0	0	0	0	0	0	0	0	0	0
Unknown or Not Reported	0	0	0	0	0	0	0	0	0	0
Total	0	0	0	0	0	0	0	0	0	0

Section 3 - Protection and Monitoring Plans (Study 1)

3.1. Protection of Human Subjects	20-240_Human_Subjects_-_final.pdf
3.2. Is this a multi-site study that will use the same protocol to conduct non-exempt human subjects research at more than one domestic site?	❍ Yes ● No ❍ N/A
If yes, describe the single IRB plan	
3.3. Data and Safety Monitoring Plan	
3.4. Will a Data and Safety Monitoring Board be appointed for this study?	❍ Yes ● No
3.5. Overall structure of the study team	

PROTECTION OF HUMAN SUBJECTS

1. Risks to Human Subjects

Human Subjects Involvement, Characteristics, and Design

The proposed research involves use of human subjects as a source of blood obtained by venipuncture for the isolation of peripheral blood mononuclear cells. Studies in primary human macrophages, initial targets of MARV, EBOV, and LASV, are important to understand whether antiviral treatment affects virus replication and spread in clinically relevant cells. Subjects will be limited to normal healthy adult (18-50 years old) volunteers without symptoms of recent infections. Both genders will be studied and there will be no discrimination of race or ethnicity. To ensure that the gender distribution is equal, recruitment will be directed at acquiring a 50% female:male ratio. Participant recruitment will also be monitored to assure that minority populations are represented in this cohort of subjects. Individuals under the PI's supervision, including students or staff, will be excluded. We will obtain venous blood samples from 6 volunteers/year for a total pool of 18 individuals (see *Targeted/Planned Enrollment Tables*).

All of the risks described below will be explained to the subject by the principal investigator or an appropriate physician representative. The subject will be given the opportunity to ask questions about the procedures and the risks. They will then be asked to sign a written consent form which summarizes the risks and also states that the subject is free to withdraw at any time. Special groups (pregnant females, fetuses, the elderly, children, prisoners and the cognitively impaired, etc.) will be excluded from this protocol. All studies will be conducted at Texas Biomedical Research Institute.

Study Procedures, Materials, and Potential Risks

Peripheral blood cells will be obtained from venipuncture, following standard procedures from healthy subjects. The date on which blood was given and the amount of blood withdrawn will be recorded in a notebook to be kept locked in the medical personnel's research office. This information and the peripheral blood are the only things collected from the human subjects. Specimens and data will be labeled with a numerical code. The subjects' name will not appear on any specimens and participation will remain confidential. The identity of the subjects will not be revealed in any publications, slides, presentations or other related materials. The subject's name, phone number, and subject numerical designation will be recorded in a notebook to be kept in the medical personnel's research office. This office is kept locked and only laboratory personnel have access to the human subject notebook.

The possible risks associated with participating in this research project are as follows: most people experience transient pain on insertion of the needle. Occasionally, a small bruise will develop at the site of the blood drawing. Transient lightheadedness can develop and on rare occasions, fainting may occur.

2. Adequacy of Protection against Risks

Informed Consent and Assent

Consent will be obtained at Texas Biomedical Research Institute, room L117 by lab personnel. All risks described above will be explained to the subject. The subject will be given the opportunity to ask questions about the procedures and the risks. They will then be asked to sign a written consent form which summarizes the risks and also states that the subject is free to withdraw at any time.

Protections Against Risk

Several procedures will be employed in order to minimize the risks. Venipuncture will be performed by a physician or a qualified technician. The antecubital vein will be used and ordinary sterile technique will be observed. The subject will be in a sitting or supine position. Following withdrawal of blood, firm pressure will be applied to the site for several minutes. The usual amount of blood will vary from 30-200 ml, and any single adult subject will be limited to a maximum of 480 ml in any two-month period. The identity of subjects will not be revealed in any publications, slides, presentations or other related materials. The date on which blood was given and the amount of blood withdrawn will be recorded in a notebook to be kept locked in the research office. The subjects' name will not appear on any specimens and participation will

remain confidential. Specimens and data will be labeled with a numerical code.

Due to the nature of the study, no incidental findings are expected to occur.

Vulnerable subjects

Special groups (pregnant females, fetuses, the elderly, children, prisoners and the cognitively impaired, etc.) will be excluded from this protocol.

3. Potential Benefits of the Proposed Research to Research Participants and Others

For study participants, both the anticipated risks and benefits are very low. Society is expected to gain a long-term benefit from the results of this study, related to discovery of novel countermeasures against filoviruses and arenaviruses.

4. Importance of the Knowledge to be Gained

There will be no direct benefits to the individual subject, but expected long-term benefits to society include improved treatment of patients with filovirus and arenavirus infection. Thus, the potential benefits to society are high.

Section 4 - Protocol Synopsis (Study 1)

4.1. Study Design

4.1.a. Detailed Description

4.1.b. Primary Purpose

4.1.c. Interventions

Type	Name	Description

4.1.d. Study Phase

Is this an NIH-defined Phase III Clinical Trial? ❍ Yes ❍ No

4.1.e. Intervention Model

4.1.f. Masking ❍ Yes ❍ No

❑ Participant ❑ Care Provider ❑ Investigator ❑ Outcomes Assessor

4.1.g. Allocation

4.2. Outcome Measures

Type	Name	Time Frame	Brief Description

4.3. Statistical Design and Power

4.4. Subject Participation Duration

4.5. Will the study use an FDA-regulated intervention? ❍ Yes ❍ No

4.5.a. If yes, describe the availability of Investigational Product (IP) and Investigational New Drug (IND)/ Investigational Device Exemption (IDE) status

4.6. Is this an applicable clinical trial under FDAAA? ❍ Yes ❍ No

4.7. Dissemination Plan

Delayed Onset Studies

Delayed Onset Study#	Study Title	Anticipated Clinical Trial?	Justification
The form does not have any delayed onset studies			

VERTEBRATE ANIMALS: MOUSE

Texas Biomedical Research Institute

1. Description of animals and how they will be used

Suppliers
Wild-type BALB/c mice for *in vivo* treatment toxicity, compound concentration and accumulation studies, and infections will be obtained from The Jackson Laboratory.

Strain choice
BALB/c mice are being generated and widely supplied by The Jackson Laboratory for research purposes. Wild-type BALB/c mice have been shown to be susceptible to infection with mouse-adapted Marburg virus and develop symptoms that are consistent with human infections.

Age choice
We will use 4-5-week old mice.

Sex differences
We will use a 50:50 female:male ratio distribution of sex to ensure our results are not confounded by sex differences.

Experimental needs
Experiments in this proposal require:

1. Oral administration (PO) of compounds twice daily (BID) over a period of 10 days, followed by post-mortem gross examination and tissue isolation for *ex vivo* analysis for any pathobiological changes, to determine if the treatments have adverse effect in the host (270 males + 270 females = 540 animals).
2. BID administration of compounds by PO and subsequent plasma and organ isolation (after 1 treatment; 3, 5, 7, or 10 days of treatment) to determine compound distribution and concentration over time (180 males + 180 females = 360 animals).
3. Challenge with mouse-adapted MARV by intraperitoneal route and subsequent BID treatment by PO for 10 consequent days to follow the course of infection and isolation of tissues for *ex vivo* analysis (90 males + 90 females = 180 animals).

The safety operations procedure (SOP) for filovirus infection and other procedures specified in this application have been filed and approved by Texas Biomed Biohazard & Safety Committee. The IACUC application #1706MU detailing procedures in this application has been approved by Texas Biomed Institutional Animal Care and Use Committee. The Texas Biomedical Research Institute animal assurance welfare number is D16-00048 (A3082-01) under file with the NIH.

A total of 1,080 mice (540 males + 540 females) is requested.

2. Justification for the use of animals

The use of animals is justified by the fact that the course of MARV infection and affect of antiviral treatment on the disease outcome cannot yet be reliably predicted by *in vitro* assays or computer simulations. BALB/c mice have been shown to be susceptible to infection with mouse-adapted Marburg virus and develop symptoms that are consistent with human infections. Because of this sensitivity, the high degree of similarity between the murine and human immune systems, and the large number of reagents available to study mice, the species used in the study would provide the most complete information possible. The small size of the animal requires smaller amounts of treatment reagents injected, thus maximizing reagents that may be in short supply. Because of the limitation of ABSL4 space, the smaller model facilitates proof-of-concept testing at a fraction of the cost of experiments in larger animals. The inbred nature of the mice also reduces variation in response to treatments and virus, allowing for limiting the size of test groups.

3. Provisions to minimize discomfort, distress, pain, and injury

To help minimize pain and distress, as well as minimize risk for care staff, all mice will be sedated with isofluorane for virus challenge or ketamine+xylazine for terminal blood collections. After challenge, animals will be closely monitored for signs of: (i) ruffled hair coat, (ii) hunch back, (iii) inappetence, (iv) weight loss, and (v) decreased movement. If the animal fulfills 2 of the above criteria, where animals are in distress as result of disease or treatment, it will be euthanized. If at any time the animal experiences labored breathing, the animal will be euthanized immediately. If at any time in the responsible veterinarian's judgment, unnecessary suffering is occurring, the animal will be euthanized. Based on our preliminary data, BALB/c mice challenged with mouse-adapted MARV become ill at 3 days and die 5-6 days post-challenge.

Point 1: Description of animals and how they will be used

A total of 3 Sprague Dawley rat, male and female, aged approximately 6-8 weeks and weighing 200-400 grams will be used for each test article for the studies. Rats for pharmacokinetic evaluation will be serially bled over a 24 hour period. Route of administration for the test article will be intravenous (IV) at a dose TBD. Dosing of the test article will occur once via the corresponding dose route. Animals will be fasted prior to dosing with water provided *ad libitum.* All blood samples will be collected via tail vein and/or mandibular vein and then placed in tubes with anticoagulant. All samples will be transferred to the Absorption Systems Analytical Department for analysis. The blanket IACUC protocol approval number for this study is ASLP16-01 and was approved February 12, 2016.

Point 2: Justifications for use of animals

There are no known acceptable alternatives to the use of live animals to accomplish the purpose of this study. The test article is a novel drug and the study does not unnecessarily duplicate any previous work. The rat is a standard model for non-clinical studies, for which there is a large historical database, and has been found to be a suitable model in previous investigations with similar test articles. Examination of pharmacokinetics and distribution studies is critical for drug development. This study was designed to use the fewest number of animals possible consistent with the scientific objectives of the study, the goals of the sponsor, contemporary scientific standards, and in consideration of applicable regulatory requirements. The number of animals used is considered the minimum to make meaningful biological comparisons, and for statistical calculations.

Point 3: Provisions to minimize discomfort, distress, pain and injury

All animals will be observed by professional technicians trained in lab animal care and use. An attending veterinarian is available at all time for emergencies. In the event that any aspect of this study causes more than normally expected brief pain or distress to the animals, the study director shall determine, in consultation with the attending veterinarian, if administration of appropriate sedatives, analgesics, or anesthetics, or other therapies would be contraindicated by the objectives of the study and document the resultant course of action. Animals that experience severe or chronic pain or distress that cannot be relieved will be euthanized.

Point 4: Euthanasia

Animals will typically be sacrificed after the last clinical observation noted or blood sample has been collected. All rats will be euthanized by cervical dislocation under isoflurane anesthesia. Isoflurane ensures that the rats are unconscious, while dislocation ensures quick death. This minimizes animal distress, is effective, and efficient; it is consistent with the recommendations of the AVMA Guidelines on Euthanasia.

VERTEBRATE ANIMALS SECTION (VAS)

1 Description of procedures

In vivo pharmacokinetics (PK) assessment will be performed to determine the dose and dosing schedule for additional *in vivo* studies. PK work will be performed at Alliance Pharma (Malvern, PA), a CRO specializing in drug metabolism and PK (DMPK). C57Bl/6 mice will be used.

2 Justifications

The proposed animal experiments are critical to determine PK parameters after intravenous (IV) and intratracheal (IT) drug administration, including half-life, peak plasma and lung tissue concentration (C_{max}), and the integral of the concentration-time curve in both plasma and lung tissue (AUC, a measure of drug exposure) to determine the dosing schedules to be used in future studies, and to enable an understanding of how drug exposure relates to efficacy in models of lung disease.

To assess the PK profile of each compound, mice will be dosed IV and/or IT with each compound, and plasma concentrations will be determined at up to eight time-points over 24 hours (n=3 mice per time-point) using LC-MS/MS. Total number of mice to be used for PK studies is 192 over a two year period.

3 Minimization of Pain and Distress

All procedures are designed to minimize discomfort and pain. Mice will be fed and watered appropriately throughout the entire protocol. Each cage also contains a nestlet for enrichment purposes. Levels of pain/distress and endpoints are discussed by the IACUC as an integral part of the protocol review process. The IACUC ensures that the procedures described in the protocol are categorized appropriately. As an IACUC member, the Attending Veterinarian reviews all animal care and use protocols with an emphasis on those that require any anesthetics or analgesics. The AV and investigators discuss the appropriate selection and regimen for anesthetics and analgesics for the animal procedures in the protocol. The recommendations are then transferred into the protocol for IACUC review and approval.

4 Euthanasia

Any mice exhibiting pain or discomfort lasting longer than a few seconds that cannot be instantly relieved will be immediately euthanized. No animals are euthanized in the presence of their conspecifics (same room). Animals are placed in a clear, plexiglass chamber of known size and CO2 is introduced at a flow rate of 10-30% (based on chamber size) via a calibrated flow meter. When animals are euthanized, death is assured by direct observation to ensure an absence of respiration. In addition, the chest is palpated to ensure an absence of a heartbeat. Methods of euthanasia used at Alliance are in compliance with the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition.

SELECT AGENTS

Texas Biomedical Research Institute (Texas Biomed)

BSL4 agents licensed for use in this proposal. Low passage human isolates of Ebola virus variant Mayinga, Marburg virus strain Musoke, Lassa virus strain Josiah as well as mouse-adapted Marburg virus strain Angola will be used in this study and are available in the repository at Texas Biomed. Work with replication competent viruses will be performed in the Texas Biomed ABSL4 facility by Dr. Olena Shtanko, Ms. Gloria Rodriguez (Research Assistant), and ABSL4 veterinary staff. Dr. Shtanko, Ms. Rodriguez, and veterinary staff are authorized for work with these agents and have appropriate Homeland Security and CDC approval for this work. Dr. Shtanko is a CDC-registered PI and has been working with high-containment viruses for >9 years. Ms. Rodriguez has been working with filoviruses for >5 years. Animal work (mouse, guinea pigs and non-human primate) with Ebola, Marburg, and Lassa viruses has been performed at Texas Biomed for >15 years. The Biohazard & Safety Committee, Animal Care & Use Committee, and the Institutional Review Board to protect the rights and welfare of human research subjects have approved the protocols detailing the work described herein.

Registration status of all entities where Select Agent(s) will be used. Select agent work will only be conducted at Texas Biomed. Texas Biomed is a select agent registered entity with Health and Human Services (HHS), Centers for Disease Control and Prevention (CDC) and U.S. Department of Agriculture, Animal Plant Health Inspection Service, National Select Agent Program. Texas Biomed has been inspected by the CDC National Select Agent Program for use of HHS Select Agents and Toxins, Overlap Select Agents and Toxins and USDA Select Agents and Toxins. Per the requirements of 42 CFR 73, the most recent certificate of registration was issued on November 20, 2017 for use of select agents at BSL2, 3, and 4 and ABSL3 and 4. Texas Biomed registration number is 20171120-1976. Texas Biomed has a Responsible Official (RO) and an Alternate Responsible Official.

Facilities where the Select Agents will be used. We have one ABSL4 full-suit lab which is registered for CDC and USDA select agent work on human and animal pathogens. Access to the ABSL4 area is secured via three successive magnetic card/keypad controlled airlock passages: The first is located at the entry foyer to the BSL3 suite, the second at the entry foyer to the outer changer room, and the third at the entry foyer to the ABSL4 area. The ABSL4 area is equipped with an entry and exit decontamination airlock and an emergency exit/decontamination chamber. The latter also serves as an equipment decontamination chamber to allow for the repair, removal and/or replacement of faulty equipment without requiring a complete laboratory decontamination process. All supply and exhaust air for the ABSL4 passes through dual (and tandem) HEPA filters. The laboratory contains 3 x class IIB biological safety hoods, 4 x water-jacketed CO_2 incubators, low-, high- and ultra-speed centrifuges equipped with both analytical and preparative scale rotors, a microfuge, 2 x 4□C refrigerators, 2 x liquid nitrogen storage systems, and 3 x -80□C freezers. The ABSL4 also has 2 CytoSMART Lux systems, an optical ELISA plate reader, MAGPIX multiplexing 96-well format unit, luminometer, Innova 4000 environmental shaker, microplate shaker, BioFlo III fermenters with chillers, Evos fluorescent microscope, pass- through autoclave, dunk tank, facsimile machine and networked computers.

Housing for animals in the ABSL4 space includes 2 rodent racks able to accommodate 36 cages for guinea pigs or up to 480 mice. Non-human primate (NHP) housing consists of squeeze back cages to house up to 12 marmosets and 24 macaques which are placed within a microisolated enclosure. Vetscan and Procyte hematology and blood chemistry Abaxis analyzers are available in the space for analysis of blood collected from infected animals. Coagulation parameters can be measured in the lab with Idexx analyzers. Specialized equipment for monitoring biopotentials of NHPs in the ABSL4 includes the DSI (Data Science International) Telemetry System with analysis software. A dedicated procedure area is located within the lab to perform routine animal sampling and full necropsies.

Critical parameters (air supply and exhaust flow rates, pressure differentials, normal and emergency breathing air, decontamination systems, etc.) are continuously monitored. Personnel within the lab communicate, and are actively monitored by outside personnel, through a scrambled UHF communication system worn inside the laboratory suit. The 500 sq. ft. ABSL4 laboratory staging area includes an air locked suit change room, two inner change rooms with pass-through showers, and an outer “clean” change room equipped with sinks,

lockers, toilets, etc. Critical mechanical support devices are located in an attached 3,000 sq. ft. mechanical support complex. All critical equipment and devices (valves, etc.) are redundant, including duplicate air-supply and exhaust systems, decontamination shower systems, as well as the compressors, pumps, valves, heater cores, etc. that comprise the liquid waste decontamination system. All critical mechanical devices operate on a system with automatic emergency electricity backup generators. The ABSL4 area (including mechanical support rooms) is equipped with an elaborate intrusion detection system that includes electronic door and roof hatch sensors, motion detectors, etc.

Texas Biomed maintains an experienced and trained staff of scientists, veterinarians, research technicians and veterinary technicians available to perform studies at high biocontainment. These individuals have demonstrated proficiency at conducting in vitro and animal studies with the agent identified in the proposal. The ABSL4 Operations and Safety Manuals specify policies, procedures, and standard operating procedures (SOPs) for the safe handling of biological materials in biosafety laboratories. The policies, procedures, and SOPs comply with applicable Federal, State, and municipal regulations and with the guidelines “Biosafety in Microbiological and Biomedical Laboratories” issued by the CDC and the National Institute of Health (NIH). Employees are trained from these manuals on each facility mechanical systems, biosafety, biocontainment and security. Employees are also trained according to project specific and Departmental SOPs. These procedures apply to all that use, generate, store, or dispose of potentially infectious materials in Texas Biomed biosafety laboratories and to persons who must enter these laboratories to perform services.

Select agent use, transfer or possession is forbidden without the permission of the Responsible Official (RO) and until the required forms filed and written approval received from the CDC Select Agent Program. Upon approval, ABSL4 investigators desiring to work on a BSL4 project must also submit an application to the Biohazard and Safety Committee for approval. The Committee is responsible for evaluating the facility, equipment, and staff capabilities to perform work in a safe manner.

Infectious cultures and inventory stocks are stored inside the ABSL4 laboratory in locked refrigerators and freezers. An electronic Inventory (eInventory) system is used to document usage of infectious stocks. All infectious material stored in refrigerators or freezers is properly labeled and stored in containers capable of withstanding thermal shock of freezing and thawing. Each container is labeled with the identity of the infectious agent, the date of the preparation, and a barcode that links the material to the more inclusive information contained in the inventory database.

When work is completed, all infectious cultures are removed from workbenches and cabinets and stored in a designated refrigerator or freezer. Materials to be discarded are placed in a sealable container filled with a suitable disinfectant. The container is placed in a discard pan containing the disinfectant. Discard pans are placed on a cart and transported to the autoclave. Labware containing infectious liquids are stored and transported in leak-proof containers large enough to contain the fluid in case of leakage.

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RESOURCE SHARING PLAN

Fox Chase Chemical Diversity Center Inc. is committed to rapidly sharing data, following NIH guidelines as noted in the document “NIH Grants Policy Statement and the Principles and Guidelines for Recipients of NIH Research Grants and Contracts on Obtaining and Disseminating Biomedical Research Resources”. After the filing of patents if appropriate, data will be prepared and submitted for publication in scholarly journals. Peer reviewed publications will be submitted to publicaccess@nih.gov according to and within the time parameters required. Project resources will also be made available as per NIH policy. For example, we will distribute any materials requested to collaborators and will fill requests in a timely fashion.

Sharing model organisms: No model organisms will be generated.

Genome-wide association studies: No genome-wide association studies will be performed.

AUTHENTICATION OF KEY BIOLOGICAL AND CHEMICAL RESOURCES

FOX CHASE CHEMICAL DIVERSITY CENTER, INC.

Medicinal Chemistry. FCCDC will synthesize all new chemical entities and scale-ups at its Doylestown, PA or King of Prussia, PA facilities using modern methods of synthetic organic chemistry and established method of analysis for chemical identity and purity (e.g. ≥95% by ^{1}H NMR and LC/MS). Record keeping and compound registration and management are delineated by our standard operating procedures described below.

Laboratory Notebook Maintenance and Records Retention:

1. Ideas, calculations and experimental results are entered into the notebook as soon as possible, preferably the same date they occur, so that the laboratory notebook becomes a daily record of the inventor's activities.
2. All notebook pages are signed and dated by the researcher on a daily basis.
3. All entries are made in the notebook in permanent ink to be as legible and complete as possible.
4. Entries are made in the notebook without skipping pages or leaving empty spaces at the bottom of a page.
5. NMR spectral data is archived onto a primary server, and periodically downloaded onto a back-up server.
6. ACD software is used when printing out NMR data for patent applications and publications, with chemical shifts and coupling contents added in.

Compound Registration and Management:

1. We require ≥95% purity by one electronic measure (e.g. NMR, LC/MS). If purity is <95%, then the structural assignment and data package is reviewed by FCCDC management, and the apparent purity is indicated in a separate comments field.
2. Once approved, the structure, notebook page number, comments, and project number are entered into an electronic database for registration and assignment of a unique FCCDC number in Instant J Chem.
3. Upon receipt of FCCDC number, this number is recorded in the laboratory notebook on the specified page.
4. Compounds are stored under refrigeration in well-marked vials in a separate compound repository.
5. Compounds prepared for one project are not cross-screened for a different project, without the express written consent of the original collaborator(s).

CROs and Vendors, We will seek authentication of reagents, starting materials, antibodies, and biological results from well-established vendors via their product specifications, work descriptions and statistical analyses, and we will independently check or verify these if issues arise.

University of Pennsylvania - Authentication of Key Biological Resources

We will use the following antibodies in this proposal: anti-eVP40, anti-Flag (to detect Flag-tagged mVP40, and LAFV-Z proteins), anti-Nedd4, and anti-VSV M (Abcam, Cell Signaling Technology, Santa Cruz Biotechnology, Cocalico). Each manufacturer will authenticate these antibodies and detailed specification sheets will be provided upon receipt or available online. Upon receipt and when we use them after a long period of storage (>6 months), we will authenticate these antibodies by Western blot using HEK293T cells overexpressing the corresponding gene product or HEKs in which corresponding genes have been silenced by gene-specific shRNAs.

The following cell lines will be used in this proposal: human embryonic kidney epithelial 293T cells (HEK293T), baby hamster kidney 21 cells (BHK-21), Vero-E6 cells, HeLa cells. These cells were either purchased from ATCC or from the Univ. of Penn. High Throughput Screening (HTS) Core where they have been certified to be free of mycoplasma. Cells purchased from ATCC have been authenticated by ATCC using Short Tandem Repeat (STR) profiling. We will continuously monitor every 10th passages or 12-month of use, whichever comes first for mycoplasma contamination.

The following BSL-2 viruses will be used in this proposal: vesicular stomatitis virus (VSV, Indiana serotype) wild type and various recombinants expressing foreign viral sequences (L-domains). These viruses will be authenticated by plaque purification and subsequent sequencing to confirm their identity and insertions.

The other key reagents to be used in this proposal will include expression plasmids. We will authenticate all expression plasmids in this proposal by direct sequencing. In subsequent amplifications and when we distribute them to the interested parties, we will verify the identity of each construct by appropriate restriction enzyme digest, followed by gel electrophoresis, or direct DNA sequencing.

Scientific Rigor and Statistical Analysis . We will statistically evaluate data from all immunoblot assays and virus titration experiments (above) as follows. P-values less than 0.05 will be interpreted as statistically significant. Briefly, the normality of the data will be assessed by visualizing the distribution of each dataset using probability plots and carrying out Shapiro-Wilks tests. Data that are not normally distributed will be compared using Mann-Whitney U test, and the results of this test will be interpreted in combination with evaluation of the distribution of scores for the groups of interest. Data that are not normally distributed (Shapiro Wilks t-test p-value <0.05) will be log transformed and reassessed for normality. If raw data or transformed data are normally distributed, then we will use an F-test to determine equality of variance. For those comparisons in which equality of variance is not met (F-test p-value <0.05), we will compare data using Welch's t-test. Data that do not violate the assumption of homoscedasticity will be compared using Student t-test.

AUTHENTICATION OF KEY RESOURCES PLAN

Texas Biomedical Research Institute

Virus. This project will use low passage, replication competent wild-type EBOV variant Mayinga, MARV strain Musoke, and LASV strain Josiah, all available in Texas Biomed's repository. The virus stocks have been purified by ultracentrifugation through a sucrose cushion, and their titer is 10^7 focus-forming units (FFUs) per mL on Vero cells using the fluorescent focus assay. The mouse-adapted MARV strain Angola at a titer of 10^7 plaque-forming units (PFUs) per mL on Vero cells is also available in the repository. All virus stocks are currently stored at ≤-65°C in the ABSL4 laboratory. The stocks have been confirmed mycoplasma- and endotoxin-free by MycoSEQ mycoplasma detection and LAL endotoxin kits, respectively. Virus genome of the stocks was either deep- sequenced by Illumina sequencing technology or sequenced by PCR to determine that the stocks were identical to those reported for NCBI accession numbers:

EBOV variant Mayinga	NC_002549
MARV strain Musoke	NC_001608
LASV strain Josiah	HQ_688672, HQ_688674
Mouse-adapted MARV strain Angola	KM_261523

Cell lines. Vero cell line will be used for generation of virus stocks and virus stock titering. HeLa cell line will be used to test whether small-molecule compounds affect EBOV, MARV, and LASV replication and spread. The cells will be obtained from ATCC where authentication of cells is now routinely performed. Cells will be routinely checked for mycoplasma contamination.

Human macrophages. The proposed research will use macrophages to study EBOV, MARV, and LASV spread in the presence of small-molecule compounds. Macrophages will be derived from peripheral blood mononuclear cells isolated from human subjects by venipuncture. Subjects will be limited to normal healthy adult (18-50 years old) volunteers without symptoms of recent infections. Both genders will be studied and to ensure that the gender distribution is equal, recruitment will be directed at acquiring a 50% female:male ratio. We will obtain venous blood samples from 6 volunteers/year for a total pool of 18 individuals.

Peripheral blood cells will be obtained from venipuncture, following standard sterile procedures from healthy subjects, according to the approved UT Health IRB protocol #HSC20180013H. The date on which blood was given and the amount of blood withdrawn will be recorded in a notebook to be kept locked in the medical personnel's (phlebotomist's) research office. This information and the peripheral blood are the only things collected from the human subjects. Specimens and data will be labeled with a numerical code. The subjects' name will not appear on any specimens and participation will remain confidential.

Monocyte-derived macrophages (MDMs) will be prepared at Texas Biomed as we described. Briefly, heparinized human peripheral blood will be layered on a Ficoll-Paque cushion to collect peripheral blood mononuclear cells. Subsequently, the cells will be cultured in suspension in medium supplemented with autologous serum to differentiate monocytes into macrophages. Macrophages will be harvested and adhered to tissue culture dishes while lymphocytes will be washed away. Such macrophage monolayers are 99% pure, viable and highly susceptible to infection with viruses to be used in this study.

A typical MDM yield is over 15-20 million cells/pint of blood from each donor, which is sufficient to complete the proposed studies. We will utilize MDMs differentiated in autologous serum to more closely mimic the physiological environment. This eliminates artifactual or well-established inflammatory cellular responses that may arise from exposure to human AB or bovine sera.

Mouse macrophages. The proposed studies will use primary peritoneal macrophages isolated from wild-type Balb/c mice. These cells will be isolated using well-established, published protocols. Isolated cell populations will be characterized visually for the morphology and by immunofluorescence for surface staining of antigens specific for macrophages.

Antibodies, Chemicals, siRNAs, PCR primers. The reagents will be selected based on published literature and then purchased from reputable commercial vendors in quantities sufficient to complete the proposed studies. If it becomes necessary to obtain additional batches, we will purchase only products with identical specifications from the same vendors.

Lymphocytic choriomeningitis virus (LCMV) mouse monoclonal anti-GP antibody (83.6) which cross-reacts with LASV GP2 will be provided by Dr. Luis Martinez-Sobrido (Texas Biomed; letter of collaboration is attached).

Rigor and Reproducibility. For each virus infection experiment, ≥3 independent tests in triplicate will be performed. Experiments with human MDMs will be performed from ≥6 different healthy adult donors (50:50 female:male ratio) per year. We expect the same outcome trend among donors, although there may be a different magnitude of response. *In vivo* studies will be repeated at least once and will use ≥5 mice/experimental condition for both sexes. All data will be analyzed for statistical significance using either a Student *t*-test to compare averages of 2 groups or one-way ANOVA to compare averages of >2 groups, with $p<=0.05$ being considered significant. We will inspect data for normality. Where deviations from normality are seen, we will use appropriate transformations, or non-parametric tests.