The University of Tennessee Health Science Center
St. Jude Children's Research Hospital
Le Bonheur Children's Hospital

ANNUAL REPORT
2017-2018

The Center for Pediatric Therapeutic Experimental Therapeutics



The Center for Pediatric Experimental Therapeutics
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This is a publication of:
The University of Tennessee
The Center for Pediatric Experimental Therapeutics.

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https://www.uthsc.edu/pharmacy/dcpts/cpet.php

Center for Pediatric Experimental Therapeutics Mission Statement

The mission of the Center for Pediatric Experimental Therapeutics (CPET) is the integration of basic, applied, and clinical sciences towards the development of new treatments for childhood diseases.

Benchmarks for success include:
(1) the number and quality of publications, (2) the quantity and quality of competitive funding to support Center activities, (3) the training opportunities for students, residents and postdoctoral fellows, and (4) the educational offerings by Center investigators to the scientific community. Specific goals:

Education

1. To improve the quality of education by coordinating existing resources and by attracting outstanding nationally and internationally recognized faculty in pediatric experimental therapeutics.
2. To disseminate information resulting from Center research to health professionals and citizens in Tennessee, the Mid South region, and Nation through publications, presentations, participation in professional organizations, and continuing education.
3. To establish the Center as an internationally recognized resource for educational and research training in the area of pediatric experimental therapeutics attracting the very best students and postdoctoral trainees to Tennessee.

Research

1. To coordinate, integrate and enhance pediatric experimental therapeutics research programs, particularly in microbial pathogenesis and in new drug development, to yield highly focused and competitive research.
2. To integrate existing basic research programs and resources, including the Molecular Resource Center (MRC); Regional Bio-containment Laboratory (RBL); other UTHSC COREs; the Departments of Clinical Pharmacy and Translational Science, Microbiology, Immunology, and Biochemistry, and Pediatrics; and St. Jude Children's Research Hospital.
3. To establish the Center as an internationally recognized resources in pediatric experimental therapeutics.

Clinical Care

1. To coordinate pediatric experimental therapeutics research across the Health Science Center, the University, and State of Tennessee into a collaborative program functioning as one program, improving treatments for serious childhood diseases.
2. To recruit talented clinicians of national importance to the Center to broaden the specialized expertise in treating pediatric diseases, particularly infectious diseases and cancer.
3. To serve as a national and international resource for defining optimal pediatric treatment strategies.

Executive Summary

The Center for Pediatric Experimental Therapeutics (CPET) is the only state supported Center of Excellence that includes in its primary mission the health care and treatment of citizens of Tennessee. The University of Tennessee, Health Science Center, has a primary mission to improve human health through education, research, outreach and patient care. The CPET is an example of this effort. The University serves to coalesce programs in affiliated clinical institutions to form a dynamic Center focused on advancing the use of medication in children. The University brings together St. Jude Children's Research Hospital and Le Bonheur Children's Medical Center as both have clinical and laboratory faculty members who are internationally recognized as leaders in their field.

Since receiving accomplished center status in September of 1989, the CPET has not relented in its quest to remain one of the nation's premier centers for the improvement of therapeutics in children. Faculty comprising the CPET have sustained a high level of research productivity during the past year, having authored 55 peer reviewed articles in leading medical or scientific journals. In addition to their original research publications, CPET faculty authored 2 books or book chapters.

During the past year, CPET investigators have made substantial progress in their research programs related to improving the use of medications in children, through a more complete understanding of childhood diseases and more thorough characterization of drug disposition and effect in children. The CPET is dedicated to better understanding of microbial pathogenesis and antiinfectives in children. Productivity is evidenced by the enclosed list of publications. These papers report the results of studies to improve the treatment of childhood infectious diseases. These clinical studies are built on a substantial number of laboratory-based investigations that CPET faculty members are undertaking to define the biochemical and molecular basis for specific pediatric infectious diseases and to discover novel therapeutic targets and therapeutic agents for their treatment.

In the past academic year, the CPET faculty disclosed $8.9 million in NIH grants. This is at a time when NIH funding has never been more competitive. In this time of decreased federal funding shuttering hundreds of laboratories across the nation, CPET NIH funding is quite stable.

Education of students, post-doctoral trainees and visiting investigators continued to be a major priority in the Center. In 2015-2016, the CPET faculty continued to direct the training of sizable numbers of post-doctoral fellows, graduate students, and professional students in the Colleges of Pharmacy and Medicine. In particular, the Center has supported a select group of exceptional students designated as CPET scholars. The hallmark of CPET teaching and research programs continues to be the integration of basic and translational sciences, with the goal of enhancing pharmacotherapeutic strategies for the treatment of pediatric illnesses.

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ABOUT THE CENTER 2017-2018

Directors

P. David Rogers, Pharm.D., Ph.D.
Scientific Co-Director

- First Tennessee Chair of Excellence in Clinical Pharmacy
- Vice-chair for Research
- Director, Clinical and Experiental Therapeutics
- Professor of Clinical Pharmacy and Translational Science,
- Professor, Department of Pediatrics

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Richard E. Lee, Ph.D.
Scientific Co-Director

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- Adjunct Professor, University of Tennessee Health Science Center

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Richard A. Helms, Pharm.D.
Scientific Advisor

- Professor and Chair, Department of Clinical Pharmacy
- Professor, Department of Pediatrics

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James B. Dale, M.D.
Scientific Advisor

- Gene H. Stollerman Professor of Medicine
- Chief, Division of Infectious Diseases

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- Chancellor's Professor Emeritus
- David and Sandra White Endowed Professor of Microbiology, Department of Microbiology, College of Arts and Sciences

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- Assistant Professor, Department of Clinical Pharmacy and Translational Science

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- Gene H. Stollerman Professor of Medicine
- Chief, Division of Infectious Diseases

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- Associate Professor, Department of Clinical Pharmacy and Translational Science

Brian M. Peters, Ph.D.

- Assistant Professor, Department of Clinical Pharmacy and Translational Science

Todd B. Reynolds, Ph.D.

- Associate Professor, Department of Microbiology, College of Arts and Sciences

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Co-Director

- First Tennessee Chair of Excellence in Clinical Pharmacy
- Professor, Departments of Clinical Pharmacy and Pediatrics
- Vice Chair for Research and Director, Clinical and Experimental Therapeutics

Emeritus Faculty

Dennis D. Black, M.D.

- Director, Children's Foundation Research Institute, Le Bonheur Children's Hospital
- Vice-President for Research, Le Bonheur Children's Hospital
- Professor, Departments of Pediatrics and Physiology
- J.D. Buckman Endowed Professorship in Pediatrics at UTHSC

Steven C. Buckingham, M.D.

- Former Associate Professor, Department of Pediatrics, Division of Pediatric Infectious Diseases, Le Bonheur Children's Hospital
  (Dr. Buckingham passed away November 24, 2015.)

Russell W. Chesney, M.D.

- Former Scientific Advisor and Past Director
- Former Professor, Department of Pediatrics, Le Bonheur Children's Hospital Division of Pediatric Nephrology
  (Dr. Chesney passed away April 2, 2015.)

William E. Evans, Pharm.D

- Member, Department of Pharmaceutical Sciences, St. Jude Children's Research Hospital
- Professor, Departments of Clinical Pharmacy and Translational Science, Pediatrics, and Pharmaceutical Sciences
- Endowed Chair in Pharmacogenomics
- Former Scientific Advisor and Inaugural Director

Sheldon B. Korones, M.D.

- Emeritus Professor, Department of Pediatrics, Division of Neonatology, Le Bonheur Children's Hospital
- Past Director, Newborn Center, The Regional Medical Center at Memphis
  (Dr. Korones passed away July 3, 2013.)

John H. Rodman, Pharm.D.

- Former Vice Chair and Member, Pharmaceutical Sciences Department, St. Jude Children's Research Hospital
- Former Professor, Department of Clinical Pharmacy
  (Dr. Rodman passed away April 29, 2006.)

CPET Scholars

Christian DeJarnette

"Identifying Fungal Fatty Acid Biosynthetic Inhibitors Using a Novel Drug Discovery Approach"

Advisor: Glen Palmer, Ph.D.
Integrated Biomedical Sciences

Laura Doorley

"Novel Mechanisms of Fluconazole Resistance in *Candida albicans* and *Candida parapsilosis*"

Advisor: P. David Rogers, Pharm.D., Ph.D.
Integrated Biomedical Sciences

Miranda Jarrett

"Developing Improved Antimicrobial Inhibitors of Folate Biosynthesis Through Evaluation of Sulfonamide Resistance Mechanisms and Exploration of the Cellular Fate of Next-generation Antifolates"

Advisor: Richard Lee, Ph.D.
Integrated Biomedical Sciences

Andrew Nishimoto, Pharm.D.

"Genomic and Transcriptomic Investigation of Azole Antifungal Resistance Mechanisms in *Candida albicans"*

Advisor: David Rogers, Pharm.D., Ph.D.
Pharmaceutical Sciences

Benjamin Patters

“Effects of Ethanol Exposure on Neurotoxic Properties of Exosomes in the Central Nervous System”

Advisor: Santosh Kumar, Ph.D.
Integrated Biomedical Sciences

Parker Reitler

“Commonly Used Drugs Inducing Antifungal Resistance in Candida species".

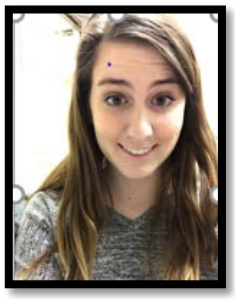
Advisor: Glen E. Palmer, Ph.D.
Integrated Biomedical Sciences

Jeffrey Rybak, Pharm.D.

“Utilizing Next-generation Sequencing to Reveal Novel Mechanisms of Triazole Resistance Among Clinical Isolates of *Aspergillus fumigatus"*

Advisor: David Rogers, Pharm.D., Ph.D.
Integrated Biomedical Science

Olivia Todd



“Mechanisms of Synergistic Virulence during Polymicrobial Intra-Abdominal Infection**”**

Advisor: Brian M. Peters, Ph.D.
Integrated Biomedical Sciences

Faculty Research Activities

P. David Rogers, Pharm.D., Ph.D., FCCP
First Tennessee Endowed Chair of Excellence in Clinical Pharmacy
Vice-chair for Research
Director, Clinical and Experimental Therapeutics
Co-Director, Center for Pediatric Experimental Therapeutics
Professor of Clinical Pharmacy and Translational Science

The overarching long-term goal of the Rogers lab is to improve the safety and efficacy of antifungal pharmacotherapy. My interest in this area is driven by insights gained as an infectious diseases clinical pharmacist into the significant limitations that exist with regard to the treatment of serious fungal infections. Indeed, treatment of such infections is limited to only three antifungal classes. The polyene amphotericin B is effective for many fungal infections, but its use is hampered by significant infusion-related reactions and nephrotoxicity. It is also only available for intravenous administration. The triazole antifungals are effective and in some cases superior, yet much less toxic, inexpensive, and available both orally and intravenously. Unfortunately, resistance has emerged which limits the utility of this antifungal class. The echinocandins, such as caspofungin, are particularly useful for invasive candidiasis, but lack utility against other fungal pathogens and are only available for intravenous administration. Moreover, resistance to this antifungal class has begun to emerge, particularly in the fungal pathogen *Candida glabrata*. It must also be underscored that no new antifungal drug classes are on the horizon. Novel strategies are therefore urgently needed to preserve, improve, and expand the current antifungal armamentarium.

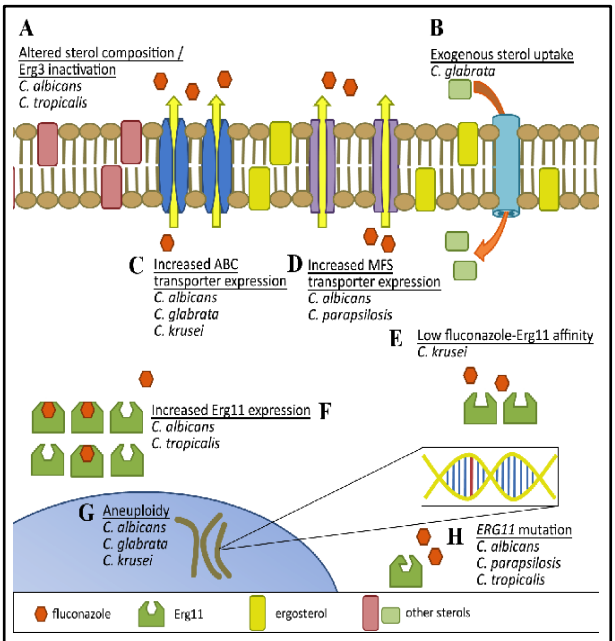


Figure 1. Comparison of documented fluconazole resistance mechanisms in *Candida* species. A) Erg3 inactivation results in utilization of alternative sterols in the yeast membrane. **B)** Uptake of exogenous sterols helps circumvent endogenous sterol production inhibition by fluconazole. Increased production of both **C)** ATP-binding cassette efflux pumps and **D)** major facilitator superfamily transporters reduces intracellular accumulation of azoles. **E)** Inherently low affinity of fluconazole binding to species-specific Erg11 may decrease fluconazole's potential to inhibit the protein. **F)** Increased expression of Erg11 protein can help overcome azole activity and **G)** aneuploidy may promote genetic adaptation to azole exposure. **H)** Mutations in *ERG11* can also result in proteins with reduced affinity for fluconazole binding.

For over a decade our primary focus has been on understanding the molecular and cellular basis of resistance to the triazole class of

antifungal agent in pathogenic fungi (overviewed in **Figure 1**). A long-term interest of my laboratory has been the use of genome-wide technologies to study antifungal stress responses in *Candida* species. We used microarray and proteomic analysis to identify changes in the gene expression and proteomic profiles of these organisms in response to the various classes of antifungal agents. This revealed both general and specific responses, some of which aligned with the mechanisms of action of these agents, and gave insight into factors that influence antifungal susceptibility (such as the azole-induction of the Cdr1 transporter). We also used this approach for genome-wide analysis of azole antifungal proteomic analysis to identify changes in the gene expression and proteomic profiles of these organisms in response to the various classes of antifungal agents. This revealed both general and specific responses, some of which aligned with the mechanisms of action of these agents, and gave insight into factors that influence antifungal susceptibility (such as the azole-induction of the Cdr1 transporter). We also used this approach for genome-wide analysis of azole antifungal resistance in *Candida* species, which has provided insight into this process (1-4).

My laboratory, working in collaboration with the laboratory of Joachim Morschhauser, discovered the transcriptional regulator Mrr1 and demonstrated that activating mutations in this transcription factor gene result in up-regulation of the Mdr1 transporter and fluconazole resistance in clinical isolates of *C. albicans*. In further work we have delineated the regulon of this transcriptional regulator and identified other regulators required for its activity (5-8). Working again in collaboration with the Morschhauser laboratory, we discovered that activating mutations in the transcription factor Upc2 leads to up-regulation of the gene encoding the azole target (*ERG11*), and increased azole resistance in clinical isolates. We have shown that this is a common and important mechanism of resistance among clinical isolates, identified additional regulators required for its activity, and have found it to be essential for azole resistance in clinical isolates exhibiting the major resistance mechanisms (9-12). More recently we have delineated the contribution of the putative lipid translocase Rta3 in azole resistance in this organism (13).

Our work has also explored the problem of triazole resistance in other fungal species. Working in collaboration with the laboratory of Thomas Edlind, we discovered that activating mutations in the transcription factor Pdr1 were responsible for azole resistance in *C. glabrata*. This led to further work by our group elucidating the role of this transcription factor, as well as the transcription factor Upc2, in azole antifungal resistance in this important *Candida* species (14-17). More recently we have begun to dissect this process in other non-albicans *Candida* species as well as the important fungal pathogen *Aspergillus fumigatus* (18, 19). Currently my research program maintains three focus areas: 1) Understanding the genetic and molecular basis of triazole antifungal resistance in *Candida albicans*, 2) Dissecting the Upc2A transcriptional pathway, protein interaction partners, and genetic network to overcome fluconazole resistance in *Candida glabrata*, and 3) Delineating the genetic and molecular basis of triazole resistance in the fungal pathogen *Aspergillus fumigatus*.

1) Novel Azole Resistance Mechanisms in *Candida albicans*

This project has been supported by **NIH/NIAID grant R01 AI058145 (Rogers PI)** since 2005 (through 2022) and has received total funding in the amount of **$5,722,262.**

A critical barrier to progress in overcoming azole antifungal resistance in *Candida albicans* is the lack of a complete understanding of its molecular and genetic basis because the known mechanisms of resistance do not fully explain resistance observed among many clinical

isolates. **Our goal** is to advance the treatment of *Candida* infections by identifying novel azole resistance mechanisms that can be exploited to ultimately overcome this problem. **Our central hypothesis** is that azole resistance in clinical isolates of *C. albicans* is multifactorial and involves complex genetic changes that **1)** alter azole target binding, **2)** activate transcriptional programs that impart resistance, and **3)** reduce azole uptake. **Our objectives** are to **1)** delineate the effects of clinically relevant mutations in *ERG11*, alone and in combination, on the activity of its gene product, fitness, and azole susceptibility, **2)** determine the clinical significance of novel Zn(2)Cys6 transcription factors (ZCFs) that influence azole susceptibility, and **3)** to discover the determinants of reduced azole import and their contribution to azole resistance in clinical isolates. Our preliminary data suggest that different *ERG11* mutations diversely affect sterol demethylase activity, including alterations of catalytic efficiency, target binding kinetics, and reaction velocity. We have also observed that artificial activation of a distinct set of ZCFs in *C. albicans* increases azole resistance. We have identified azole-resistant clinical isolates that exhibit transcriptional profiles consistent with activation of these ZCFs and that contain candidate activating mutations in these ZCF genes. Finally, we have demonstrated that C. albicans takes up fluconazole by energy-independent facilitated diffusion. We have observed that some azole resistant isolates exhibit reduced fluconazole uptake. In Aim 1 of this proposal we will undertake genetic, microbiologic, and biochemical studies to dissect the effects of single and combinatorial mutations in *ERG11* on sterol demethylase susceptibility, substrate affinity, azole binding, catalytic activity, and fitness. In Aim 2 we will undertake genetic and microbiologic studies to determine if and how mutations found in the genes encoding novel ZCFs in resistant clinical isolates result in their activation and increased azole resistance. In Aim 3 we will determine the mechanism of azole antifungal import and its contribution to azole resistance in clinical isolates of *C. albicans*. Our approach is *innovative* as we will determine for the first time precisely how mutations in *ERG11* influence enzyme activity, dissect combinations of mutations, and determine the impact of such mutations on fitness of *C. albicans*. This work also explores novel mechanisms of azole resistance. The *proposed research is significant* as it will provide the understanding needed to ultimately overcome azole resistance through the development of improved azoles, interference with activated ZCFs, and enhancement of azole uptake. By fully understanding the genetic basis of azole resistance it will be possible to eventually develop non-culture based strategies to rapidly and accurately detect azole resistance in clinical isolates.

2) Upc2A: A Central Regulator and "Achilles' Heel" of Fluconazole Resistance in *Candida glabrata*

This project is supported **NIH/NIAID grant R01 AI131620 (Rogers PI) since** 2017 (through 2022) and has received total funding in the amount of **$2,651,896.**

There is a **significant gap** in knowledge concerning the molecular and cellular underpinnings of triazole resistance in the important fungal pathogen *Candida glabrata* and how such resistance might be overcome. Our **long-term goal** is to improve the treatment of *Candida* infections by overcoming resistance to the triazole class of antifungals. Our **overall objective** in the present application is to identify the target genes directly regulated by this transcription factor, its protein interaction partners, and the genes that interact with *UPC2A* in the pathogenic fungus *Candida glabrata*. Our preliminary data demonstrate that loss of Upc2A function in both wild-type and triazole resistant isolates results in increased susceptibility to sterol biosynthesis inhibitors, including a reduction in fluconazole minimum inhibitory and

minimum fungicidal concentrations and enhanced fluconazole activity by time-kill analysis. Our findings indicate that Upc2A is a key regulator of ergosterol biosynthesis as well as other unknown processes and is essential for resistance to fluconazole in *C. glabrata*. The Upc2A pathway therefore represents a potential co-therapeutic target for enhancing fluconazole activity against this inherently resistant species and restoring and preserving this class of antifungal for the treatment of invasive Candidiasis. In **Aim 1** we will identify Upc2A target genes using transcriptional profiling (RNA-seq) and ChIP-seq, and we will then determine which target genes influence susceptibility to fluconazole by targeted gene disruption. In **Aim 2** we will identify Upc2A interaction partner proteins using tandem affinity purification (TAP) and will determine which of these are essential for Upc2A activity under fluconazole exposure and which of these influence fluconazole susceptibility using targeted gene disruption. In **Aim 3** of this proposal we will undertake screens of a transposon insertion mutant library as well as a recently developed deletion mutant library for genes that interact with, and are required for, Upc2 activation by sterol biosynthesis inhibition in order to identify and characterize the Upc2A genetic interaction network. The proposed studies are *innovative* as they uniquely focus on interference of activity of the transcription factor Upc2A as a strategy for circumventing fluconazole resistance in *C. glabrata*. Moreover, our approach is *innovative* as we will for the first time make use of a comprehensive set of genomic tools and techniques designed for yeast research and apply them to clinical isolates of the fungal pathogen *C. glabrata*. The *proposed research is significant* as it will provide new knowledge that can ultimately be exploited to overcome triazole resistance in this inherently resistant species of *Candida* and restore and preserve the use of this antifungal class for serious Candida infections.

3) Novel Mechanisms of Triazole Resistance in *Aspergillus fumigatus*

A **critical barrier** to overcoming triazole resistance in *Aspergillus fumigatus* is the significant lack of understanding of its genetic and molecular basis because the known mechanisms of resistance do not fully explain resistance observed among many clinical isolates. **Our goal** is to advance the treatment of *Aspergillus* infections by understanding the genetic and molecular basis of triazole resistance, which will ultimately be exploited to overcome this problem. Our central hypothesis is that triazole resistance in clinical isolates of *A. fumigatus* is multifactorial and involves complex genetic changes that alter 1) triazole target enzyme binding and enzyme function, 2) sterol biosynthesis and its transcriptional activation, and 3) triazole efflux and import. **Our objectives** are to **1)** delineate the effects of clinically relevant mutations in cyp51A on the activity of its gene product and on triazole susceptibility, **2)** identify genetic and molecular determinants that influence triazole susceptibility through altered sterol biosynthesis and its transcriptional activation, and **3)** identify the efflux and import transporters that participate in triazole resistance and determine how they are regulated. Our preliminary data suggest that mutations in *cyp51A* among triazole resistant clinical isolates are common, and that some influence susceptibility to multiple triazoles, whereas others do not. We have also observed mutations in sterol biosynthesis genes among resistant isolates that were not observed in susceptible isolates. Our preliminary data implicate these genes as contributing to this phenotype. Six isolates in our collection exhibit up-regulation of *cyp51A* that cannot be explained. Our preliminary data point to mutations that activate the *srbA* transcriptional pathway. Finally, our preliminary data implicate several transporters that may contribute to non-Cyp51A-medited triazole resistance through either increased triazole efflux or reduced triazole import. In Aim 1 we will undertake genetic, microbiologic, and biochemical studies to dissect the effects of mutations in *cyp51A* on sterol

demethylase activity, susceptibility, and fitness. In Aim 2 we will undertake genetic and microbiologic studies to determine if triazole resistance is due in part to genetic mutations that alter sterol biosynthesis and its transcriptional activation. In Aim 3 we will determine whether non-Cyp51A-mediated triazole resistance is mediated in part by altered expression of drug transporters. Our approach is *innovative* as we will determine for the first time the genetic and biochemical effects of clinically relevant mutations in *cyp51A* on triazole susceptibility and fitness. Our proposed work also explores novel mechanisms of triazole resistance in this pathogen such as alteration in, and upregulation of, sterol biosynthesis, increased triazole efflux mediated by novel transporters, and reduced triazole import. The *proposed research is significant* as it will provide the understanding needed to ultimately design the next generation of triazoles and sterol biosynthesis inhibitors and will make possible the development of strategies to overcome triazole resistance, preserve the utility of the triazole antifungals, and more efficiently predict clinical treatment failure and success.

Current Lab Members:

P. David Rogers, Pharm.D., Ph.D., FCCP – Principal Investigator
Qing Zhang – Laboratory Manager
Sarah G. Whaley, Pharm.D. – Graduate Student, Pharmaceutical Sciences
Andrew T. Nishimoto, Pharm.D. - Graduate Student, Pharmaceutical Sciences
Jeffery M. Rybak, Pharm.D. – Graduate Student, Integrated Biomedical Sciences
Laura Doorley – Graduate Student, Integrated Program in Biomedical Sciences
Yu Li – Graduate Student, Integrated Program in Biomedical Sciences

Key Collaborators:

Joachim Morschhäuser, Ph.D. - Universität Würzburg
Steven Kelly, Ph.D., D.Sc. – Swansea University
Scott Moye-Rowley, Ph.D. – University of Iowa
Damian Krysan, M.D., Ph.D. – University of Rochester
Theodore White, Ph.D. – University of Missouri Kansas City
Nathan Wiederhold, Pharm.D. – University of Texas Health Science Center
Martine Raymond, Ph.D. – Université de Montréal

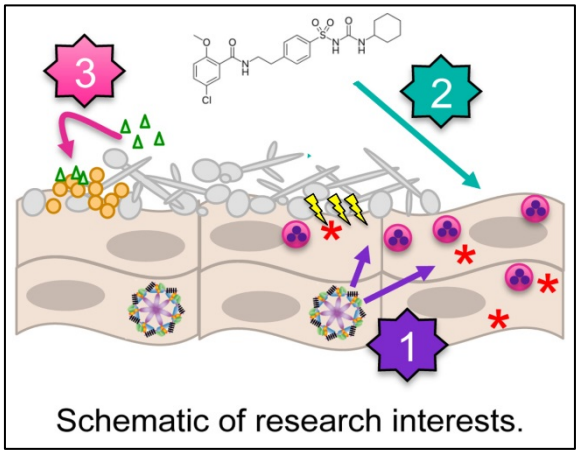
Brian M. Peters, Ph.D.
Assistant Professor of Clinical Pharmacy and Translational Science

The Peters lab has two main foci of research: **1)** the host and fungal molecular mechanisms responsible for the immunopathogenesis of vulvovaginal candidiasis and **2)** quorum sensing and toxin regulation during fungal-bacterial intra-abdominal infection.

Immunopathogenesis of vulvovaginal candidiasis:

Candida albicans, an opportunistic human fungal pathogen, is the leading causative agent of vulvovaginal candidiasis (VVC) and presents major quality of life issues for women worldwide. It is estimated that nearly every woman of childbearing age will be afflicted by VVC at least once in her lifetime. Although these treatments are typically effective at reducing organism burden, static function of azole activity, fungal recalcitrance to clearance, and lack of comprehensive understanding of disease pathology necessitates further insight into the host and fungal factors that contribute to vaginitis immunopathology.

[1] We are interested in exploring virulence mechanisms utilized by *C. albicans*, including the fungal toxin Candidalysin, to activate inflammasome signaling at the vaginal mucosa. We are also focused on determining the downstream signaling events relevant to disease pathogenesis, including the protective role of innate IL-17 signaling at the vaginal mucosa.
[2] We are also currently testing sulfonylurea drugs as repurposed adjunctive therapeutic agents to more quickly arrest symptomatic disease. Using a forward genetics approach, we are also interested in understanding how host genetic determinants alter symptoms of vaginal disease.



Schematic of research interests.

Polymicrobial intra-abdominal infection:

[3] Microorganisms rarely exist as single species communities but instead exist within multi-species consortia where mutually beneficial, parasitic, and antagonistic interactions may develop. However, relatively little is known about the functional consequences of these interactions as they relate to health and disease.

We aim to determine the complex inter-microbial signaling events that mediate infectious synergism observed during intra-abdominal infection with the ubiquitous bacterial pathogen *Staphylococcus aureus* and the fungus *C. albicans*. Current studies are focused on identifying activation of the *S. aureus* agr-quorum sensing system and downstream toxins as key pathways contributing to lethal infection. This polymicrobial intra-abdominal infection serves as an excellent model system for determining microbe-microbe induced virulence gene regulation in vivo. Identification of virulence determinants may serve as rationale for selection of vaccine candidates to reduce lethality clinically associated with fungal-bacterial intra-abdominal infection.

Current Lab Members:

Dr. David Lowes (Research Associate)
Dr. Emily Sansevere (Postdoctoral fellow)
Dr. Marjoleine Willems (Postdoctoral fellow)
Olivia Todd (PhD Student, Integrated Biomedical Sciences Program)

Glen E. Palmer, Ph.D.
Associate Professor of Clinical Pharmacy and Translational Science

Title: Antifungal drug discovery and mechanisms of resistance.

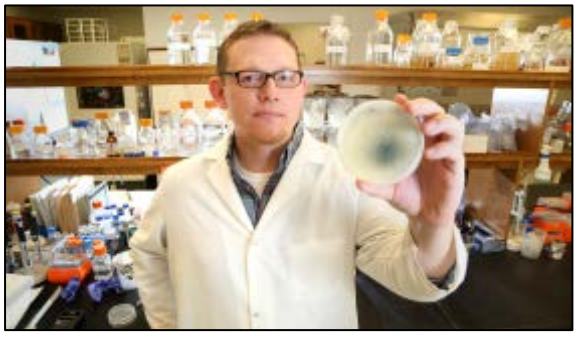
An estimated 1.5 million people die each year from invasive fungal infections, and many millions more are afflicted by debilitating mucosal and subcutaneous mycoses. Current antifungal therapies have serious deficiencies including poor efficacy, limited spectrum of activity, patient toxicity and the emergence of resistant fungi. Consequently, mortality rates are disturbingly high. A major obstacle to developing effective new antifungal drugs is the fundamental similarity between the cells of these eukaryotic pathogens and their mammalian host. This presents a challenge in devising therapeutic agents with pathogen selective toxicity. A major long-term goal of my research program is to identify and validate new target proteins that can provide a basis to develop efficacious new antifungal therapies. Current investigations within my lab include the discovery and development of new classes of antifungal agents that target either: 1). The integrity of a sub-cellular organelle called the fungal vacuole; 2). Fungal fatty acid biosynthesis; and 3) aromatic amino acid biosynthesis. As part of these studies we have devised several high-throughput (HTP) chemical screening assays to identify compounds that target these cellular functions. This includes a new and broadly applicable type of target based whole-cell screen (TB-WCS) that combines the benefits of both traditional target-based and cell-based chemical screens into a single HTP assay. We anticipate our TB-WCS approach to chemical screening will greatly enhance the speed and efficiency with which new pre-therapeutic leads, with a defined mechanism of action can be identified. Through these efforts, I have become increasingly excited about the enormous potential of applying yeast based systems (which are highly amenable to HTP approaches) to the discovery of new pharmacotherapies that target human disease related proteins.

Current Lab Members:

Lab manager - Tracy Peters M.S.
Postdoctoral researchers – Helene Tournu Ph.D; Arielle Butts Ph.D; Arturo Luna-Tapia Ph.D
Research Associate – Kathy Barker Ph.D
Graduate Students – Christian DeJarnette B.S.; Parker Reitler B.S.

Jarrod R. Fortwendel, PhD.
Associate Professor of Clinical Pharmacy and Translational Science

Control of Antifungal Drug Tolerance through the *Aspergillus fumigatus* Kinome



Invasive aspergillosis, caused mainly by *A. fumigatus*, is the most prevalent invasive mold infection of immunocompromised individuals and is associated with mortality rates of 35-90%. Therapy options are extremely limited for invasive aspergillosis and resistance to the triazole class of antifungals is on the rise. Although more than a decade of research has focused on characterizing the emerging threat of triazole resistance in *A. fumigatus*, strategies for preventing or circumventing this increasingly grave phenomenon remain elusive. Significant knowledge gaps concerning how *A. fumigatus* adapts to drug-induced stress (i.e., drug tolerance) impair our ability to improve antifungal therapy and halt the rise in resistance. Without new resources to hasten discovery, progress in these areas is unlikely. In turn, we risk losing the most important class of drugs with anti-*Aspergillus* activity and endanger the lives of the increasing at-risk population. Our long-term goal is to improve antifungal therapy and to ensure the sustained clinical utility of the triazole class for treatment of invasive aspergillosis. The objective of this proposal is to delineate novel phospho-regulatory events utilized for antifungal tolerance by the major mold pathogen, *Aspergillus fumigatus*.

Reversible protein phosphorylation regulates the majority of eukaryotic cellular processes. A robust history of published research, including a recent functional analysis of the entire *Cryptococcus neoformans* kinome, implicates multiple protein kinases in the regulation of antifungal tolerance. To delineate novel roles for the *A. fumigatus* kinome in triazole tolerance, we have completed a preliminary phospho-proteomics analysis of the response to voriconazole treatment. Our preliminary data reveal significant changes in the phosphorylation status of ~1400 proteins, implying extensive kinase-mediated adaptation to drug-induced stress. Filtering this dataset for proteins known to mediate triazole tolerance, we identified voriconazole-induced phosphorylation state changes in HapB. The HapB protein is a subunit of the heterotrimeric CCAAT-binding complex (CBC), a crucial transcriptional repressor of ergosterol biosynthesis genes. Interestingly, clinical triazole resistance in select *A. fumigatus* isolates has been ascribed to mutations that alter functionality of the CBC. Therefore, delineation of CBC regulatory mechanisms could lead to novel interventions targeting antifungal tolerance and/or resistance. To identify kinases required for phosphorylation of the CBC, and to explore roles for *A. fumigatus* kinase networks in drug tolerance, an in-depth investigation of the *A. fumigatus* kinome is needed. A *critical barrier* to performing large-scale analysis of sizeable gene families in *A. fumigatus* has been the notoriously low homologous recombination rates of wild type strains. *We have overcome this barrier* by successfully adapting a novel CRISPR/Cas9-based mutational approach. We will utilize this facile system to test our hypothesis that the CBC is phospho-regulated in response to triazole stress through modification of the HapB subunit and to interrogate all 131 *A. fumigatus* protein kinases for networks modifying HapB and/or supporting triazole tolerance.

We are working now to test the working hypothesis that CBC-mediated regulation of triazole tolerance is controlled by differential phosphorylation of the HapB subunit. Unbiased and targeted phosphoproteomics analyses will first be completed using multiple *Aspergillus*-

active triazoles to confirm HapB phosphorylation state changes. Using directed mutagenesis combined with triazole sensitivity and Nanostring gene arrays, we will then systematically test each putative HapB phosphorylation event for regulation of CBC function in triazole tolerance and repression of ergosterol biosynthesis gene expression. Then, we aim to identify novel protein kinases supporting antifungal fitness in *A. fumigatus*. Using our novel CRISPR/Cas9 mutational approach, we will generate barcoded libraries of tetracycline-repressible and overexpression mutants representing each protein kinase in *A. fumigatus*. These libraries will be employed in pooled competitive fitness analyses to identify important components regulating fitness during stress induced by voriconazole. Kinase mutants with altered fitness will be further subjected to targeted phosphoproteomic analyses to identify the kinase(s) regulating HapB in response to inhibition of ergosterol biosynthesis.

Our expected outcome is that we will uncover multiple, novel contributions of protein kinases to *A. fumigatus* antifungal fitness. In addition, we will potentially delineate a phospho-regulatory mechanism controlling the CBC, a crucial transcriptional regulator of the triazole stress response. As they are considered the second largest class of proteins currently functioning as drug targets, identification of the protein kinases crucial to triazole tolerance could reveal novel targets for use in new stand-alone or combination therapies. Therefore, the potential ***impact*** of this work is the improvement of antifungal therapy and significant advance towards the sustained clinical utility of triazole antifungals against *Aspergillus*.

Systematic Functional Analysis *Aspergillus fumigatus* Kinases

To cause invasive disease, *A. fumigatus* must be able to sense and utilize tissue-specific nutrient sources and effectively handle host-induced stress. A strong history of published research implicates protein kinases as essential for orchestration of a wide variety of nutrient sensing/utilization and stress response networks in pathogenic fungi. Reversible protein phosphorylation regulates almost all eukaryotic processes and, on average, about 30% of cellular proteins are modified by phosphorylation. Although no systematic analysis has yet been accomplished in *A. fumigatus*, the relatively few protein kinases that have been characterized play diverse roles in cellular stress responses and virulence. Furthermore, *kinases are considered the second largest protein class currently functioning as drug targets*, as their inhibition can be readily accomplished by small molecules. Unfortunately, the vast majority of *A. fumigatus* protein kinases remain unstudied. We have successfully adapted a novel CRISPR/Cas9-based mutational approach for use in wild type strains of *A. fumigatus*. Our preliminary data show that this facile system increases the typically low levels of gene targeting in wild type *A. fumigatus* to as high as 90%. With this new tool, we propose to systematically delete and functionally analyze all putative protein kinases in the wild type *A. fumigatus* genetic background, Af293. The CRISPR/Cas9 components are being designed based on our preliminary results and purchased from commercial vendors. The necessary ribonucleotide complexes will be assembled via a short, *in vitro* reaction and then mixed with microhomology repair templates before protoplast transformation. Repair templates will be designed to incorporate signature tags into each kinase mutant, barcoding the strains for competitive fitness analyses. Essential kinases will be confirmed by tetracycline-regulatable promoter replacement using a modification our CRISPR/Cas9 approach. We will then perform competitive fitness analyses employing signature-tagged pools of kinase mutants to identify novel roles for the kinome during pathogenic growth. To identify kinases that may regulate fitness in response to host immune status, we will utilize two highly characterized models of invasive aspergillosis that re-capitulate the immune dysfunction in both neutropenic and non-neutropenic hosts. We will also perform *in vitro* competitive fitness assays using culture conditions that mimic pathobiologically relevant stress. A subset of the

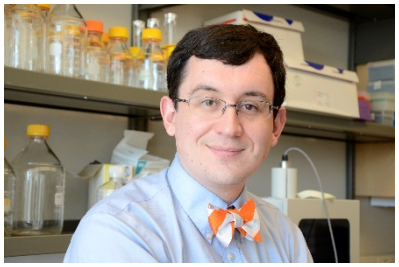
least-fit mutants from both *in vivo* and *in vitro* studies will be complemented and employed in single infection/inoculation studies to confirm roles in fitness. We expect to discover multiple, novel contributions of protein kinases to the pathobiology of invasive aspergillosis. The information generated by completion of this work will support future applications exploring novel aspects of *A. fumigatus* virulence.

New Tools for Aspergillus fumigatus Molecular Analyses: CRISPR/Cas9 gene editing unsing *in Vitro*-Assembled Cas9-Guide RNA Ribonucleoproteins Coupled with Microhomology Repair Templates

Tackling the multifactorial nature of virulence and antifungal drug resistance in *A. fumigatus* requires the mechanistic interrogation of a multitude of genes, sometimes across multiple genetic backgrounds. Classical fungal gene replacement systems can be laborious and time-consuming and, in wild-type isolates, are impeded by low rates of homologous recombination.CRISPR (clustered regularly interspaced short palindromic repeat)-Cas9 is a novel genome-editing system that has been successfully established in *Aspergillus fumigatus*. However, the current state of the technology relies heavily on DNA-based expression cassettes for delivering Cas9 and the guide RNA (gRNA) to the cell. Therefore, the power of the technology is limited to strains that are engineered to express Cas9 and gRNA. To overcome such limitations, we developed a simple and universal CRISPR-Cas9 system for gene deletion that works across different genetic backgrounds of *A. fumigatus*. The system employs *in vitro* assembly of dual Cas9 ribonucleoproteins (RNPs) for targeted gene deletion. Additionally, our CRISPR-Cas9 system utilizes 35 to 50 bp of flanking regions for mediating homologous recombination at Cas9 double-strand breaks (DSBs). As a proof of concept, we first tested our system in the Δ*akuB* (Δ*akuB*^{ku80}) laboratory strain and generated high rates (97%) of gene deletion using 2 µg of the repair template flanked by homology regions as short as 35 bp. Next, we inspected the portability of our system across other genetic backgrounds of *A. fumigatus*, namely, the wild-type strain Af293 and a clinical isolate, *A. fumigatus* DI15-102. In the Af293 strain, 2 µg of the repair template flanked by 35 and 50 bp of homology resulted in highly efficient gene deletion (46% and 74%, respectively) in comparison to classical gene replacement systems. Similar deletion efficiencies were also obtained in the clinical isolate DI15-102. Taken together, our data show that *in vitro*-assembled Cas9 RNPs coupled with microhomology repair templates are an efficient and universal system for gene manipulation in *A. fumigatus*. Our simple and universal CRISPR-Cas9 system for gene manipulation generates efficient gene targeting across different genetic backgrounds of *A. fumigatus*. We anticipate that our system will simplify genome editing in *A. fumigatus*, allowing for the generation of single- and multigene knockout libraries. In addition, our system will facilitate the delineation of virulence factors and antifungal drug resistance genes in different genetic backgrounds of *A. fumigatus*.

Theodore J Cory, Pharm.D., Ph.D.
Assistant Professor of Clinical Pharmacy and Translational Science

To determine methods to increase antiretroviral concentrations in macrophage subsets



Viral persistence is a critical barrier to the eradication of HIV-1 in infected individuals. One hypothesis is that HIV resides in cells in locations with subtherapeutic antiretroviral concentrations, which are insufficient to fully inhibit viral replication, making elimination of the virus from these sites impossible. These sites include the brain, lymph nodes, and secondary lymphoid tissues. While CD4+ T cells are the primary target of HIV, macrophages are infected early, and remain an important infected cell population. These two host cells interact in lymph nodes and secondary lymphoid tissue. Macrophages exist in two phenotypically dissimilar polarized subsets, the classically activated (M1) phenotype, which is pro-inflammatory and involved in the destruction of intracellular pathogens, and the alternatively activated (M2) phenotype, which is anti-inflammatory and involved in tissue repair. The role of these two subsets of macrophages in HIV is uncertain, as is the disposition of antiretrovirals in the cells. Our goal is to define the mechanisms by which intracellular antiretroviral concentrations are altered in macrophage subsets, and the effect of this on viral replication and spread, and do develop strategies to increase antiretroviral concentrations in the macrophage reservoir of HIV. Additionally we are interested in how drugs of abuse including nicotine and alcohol influence concentrations of the drugs used in HIV inside of cells, and are aiming to develop new strategies to increase the concentrations of these drugs inside of cells.

Current Lab Members:

Graduate Student: Ying Mu, M.S.

Recent publications:

1. He H, Buckley M, Mu Y, Kumar S, and **Cory TJ**. Polarized macrophage subsets differentially express the drug efflux transporters MRP1 and BCRP, resulting in altered HIV production. *Antiviral Chemistry and Chemotherapy*. 2018:26, 1-7
2. Midde NM, Gong Y, **Cory TJ,** Li J, Meibohm B, Li W, and Kumar S. Influence of Ethanol on Darunavir Hepatic Clearance and Intracellular PK/PD in HIV-infected Monocytes, and CYP3A4-Darunavir Interactions Using Inhibition and in Silico Binding Studies. *Pharmaceutical Research*. 2017
3. Deckman JM, Kurkjian CJ, McGillis JP, **Cory TJ**, Birket SE, Schutzman LM, Murphy BS, Garvy BA, and Feola DJ. Pneumocystis Infection Alters the Activation State of Pulmonary Macrophages. *Immunobiology*. 2017:222(2):188-197.
4. **Cory, TJ**, He H, Winchester LC, Kumar S, Fletcher CV. Alterations in P-Glycoprotein Expression and Function between Macrophage Subsets. *Pharmaceutical Research*. 2016:33(11):2713-2721.

Santosh Kumar, Ph.D
Associate Professor of Pharmaceutical Sciences

Research Interests

Our research program is at the intersection of **HIV-1, drugs of abuse, and cytochrome P450**. Drugs of abuse, especially alcohol drinking and tobacco smoking are highly prevalent in HIV-1-infected individuals. Known enhancers, Alcohol and tobacco, augment HIV-1 replication and reduce the response to antiretroviral therapy. However, the mechanism by which this known occurrence is inadequate. We propose that alcohol- and tobacco-mediated increase in HIV-1 replication and decrease in the response to antiretroviral therapy occur through cytochrome P450 (CYP) pathway. Our ongoing projects are to study the "role of CYP in alcohol- and tobacco-mediated HIV-1 pathogenesis and antiretroviral therapy". The alcohol project is NIH/NIAAA R01 grant funded. Our group is the first one to show a potential role of CYP pathway in context to drugs of abuse mediated HIV pathogenesis. This provides a novel target to treat HIV-infected drugs of abuser effectively. In fact, we are already in the process of developing a novel inhibitor of CYP2E1, which potentially prevents alcohol, analgesic, and HIV-mediated toxicity and HIV replication.

Our other project is on **HIV, drugs of abuse, and exosomes.** Exosomes are clinically relevant in developing biological markers and novel therapeutics is many diseases. The known role of exosomes in context to drugs of abuse and HIV is below par. We propose that exosomes derived from macrophages and microglia play important role in drugs of abuse, especially smoking and alcohol, mediated effects on HIV pathogenesis and neuronal damage. We also propose that exosomal CYP, antioxidant and pro-oxidant enzymes, and cytokines, as well as miRNA that regulate these enzymes play important role in such effects. This project is NIH/NIDA R21 grant funded.

Finally, we have been developing new projects related to drugs of abuse, including alcohol, tobacco, and cocaine and their effects on exosomes-mediated neuroinflammation and neuronal damage. We essentially focus on how different dose of these drugs of abuse alter the antioxidant and inflammatory characteristics of exosomes that are secreted from CNS cell; microphages, microglia, and astrocytes. We then study how these exosomes act as neuroprotective or neurotoxic agent.

Our group is comprised of one post-doctorate fellow, six graduate students, and one research supervisor. Our research program benefits from several strong intra-and inter-university collaborations. We have extensively published in the past five years (>45 papers), with a total of over 75 publications in my scientific career.

Current Lab Members:

Sunitha Kodidela, Post-Doctorate Fellow
Namita Sinha, Research Supervisor
Mohamad A. Rahman, Graduate Student
Sabina Ranjit, Graduate Student

Benjamin Patters, Graduate Student
Yuqing Gong, Graduate Student
Sanjana Haque, Graduate Student
Kelly Gerth, Pharm D/PhD Student

Kirk E. Hevener, Pharm.D., Ph.D.
Assistant Professor of Pharmaceutical Sciences

Title: Narrow-Spectrum Antibacterial Target Validation & Drug Discovery

Every year in the United States, over 2 million people are infected with drug-resistant bacteria and over 23,000 people die as a direct result of these infections. The overuse of broad-spectrum antibacterial agents has been linked to the alarming rise in drug-resistant bacteria we are currently seeing. Further, we are continuing to understand the role of the human microbiome in health and disease and the adverse effects on human health that can result from the disruption to the microbiome caused by broad spectrum antibacterials. Therefore, there is an urgent need to validate and characterize novel antibacterial targets, particularly those that may result in a narrow-spectrum antibacterial effect against pathogenic, invasive organisms that can spare the human microbiota, and to develop therapeutic agents that affect these validated targets. The Hevener laboratory is currently investigating two such targets: the enoyl-acyl carrier protein (ACP) reductase enzyme (FabK) in *Clostridioides difficile* & *Porphyromonas gingivalis*, and the topoisomerase I enzyme in *Streptococci*. FabK is an essential enzyme in the bacterial fatty acid synthesis pathway (FAS-II) of certain pathogenic organism, such as *C. difficile* and *P. gingivalis*, which are responsible for GI and oral infections. FabK is a unique isozyme at this essential step that is distinct from the FabI isozyme found at this step in many of the non-pathogenic gut organism, which makes it an attractive target for narrow-spectrum antibacterial design. The type 1A topoisomerase found in Streptococci presents another potential narrow-spectrum antibacterial target as many non-pathogenic organisms express additional, redundant topoisomerase enzymes that pathogenic species of Streptococci do not. My laboratory is using a variety of microbiological, biochemical and structural biology approaches to validate and characterize these targets and is concurrently using structure-based design strategies to identify novel and potent inhibitors of these targets for further use as chemical probes and potential drug discovery leads.

Current Lab Members:

Graduate Students – Jesse A. Jones, Pharm.D.

Pharmacy Students – Rebecca Wahrmund, B.S.; Hoang P. Nguyen, B.S., B.A.; Kristiana Watson, B.S.

DIRECTION OF THE CENTER 2017-2018

Annual Report 2017-2018

Goals and Future Plans

In the coming year the CPET will continue to refine its focus on the overarching theme of Pediatric Antiinfective Pharmacotherapy. We will expand our work specifically in the areas of fungal pathogens, HIV/AIDS, respiratory viruses, and anti-infective drug discovery and development. CPET investigators will compete for new extramural funding within these domains and continue to facilitate discovery and generate and disseminate new knowledge. We hope to expand our expertise with the recruitment of new faculty to the UTHSC campus as well as to the CPET. We will continue to train elite graduate students in the biomedical and pharmaceutical sciences with the support of the CPET Scholars Program. Dissemination of our discoveries and sharing and exchange of new ideas will be facilitated through CPET support of events such as the annual UTHSC Fungal Pathogens Group Research Conference, the annual CPET Research Day, and the CPET Seminar Series.

Schedule 7

CENTERS OF EXCELLENCE ACTUAL, PROPOSED, AND REQUESTED BUDGET

Institution: UNIVERSITY OF TENNESSEE HEALTH SCIENCE CENTER **Center:** PEDIATRIC PHARMACOKINETICS

	FY 2017-18 Actual			FY 2018-19 Proposed			FY 2019-20 Requested		
	Matching	Appropr.	Total	Matching	Appropr.	Total	Matching	Appropr.	Total
Expenditures									
Salaries									
Faculty	$1,261,352	$17,746	**$1,279,098**	$1,184,039	$17,746	**$1,201,785**	$1,184,039	$17,800	**$1,201,839**
Other Professional	$290,369	$34,068	**$324,437**	$217,718	$14,716	**$232,434**	$227,718	$14,500	**$242,218**
Clerical/ Supporting	$214,512	$0	**$214,512**	$258,387	$0	**$258,387**	$358,387	$0	**$358,387**
Assistantships	$45,900	$135,049	**$180,949**	$161,758	$157,504	**$319,262**	$161,758	$155,000	**$316,758**
Total Salaries	**$1,812,133**	**$186,863**	**$1,998,996**	**$1,821,902**	**$189,966**	**$2,011,868**	**$1,931,902**	**$187,300**	**$2,119,202**
Longevity (Exclude from Salaries and include in Benefits)	$7,000	$403	**$7,403**	$7,480		**$7,480**	$7,960		**$7,960**
Fringe Benefits	$452,102	$23,187	**$475,289**	$421,606	$11,819	**$433,425**	$421,606	$11,000	**$432,606**
Total Personnel	**$2,271,235**	**$210,453**	**$2,481,688**	**$2,250,988**	**$201,785**	**$2,452,773**	**$2,361,468**	**$198,300**	**$2,559,768**
Non-Personnel									
Travel	$20,466	$11,082	**$31,548**	$0	$5,000	**$5,000**	$0	$5,000	**$5,000**
Software	$0	$0	**$0**	$0	$0	**$0**	$0	$0	**$0**
Books & Journals	$0	$0	**$0**	$0	$0	**$0**	$0	$0	**$0**
Other Supplies	$0	$12,824	**$12,824**	$0	$15,000	**$15,000**	$0	$58,370	**$58,370**
Equipment	$30,391	$5,121	**$35,512**	$0	$15,000	**$15,000**	$0	$0	**$0**
Maintenance	$0	$4,075	**$4,075**	$0	$5,000	**$5,000**	$0	$0	**$0**
Scholarships	$0	$0	**$0**	$0	$0	**$0**	$0	$0	**$0**
Consultants	$0	$0	**$0**	$0	$0	**$0**	$0	$0	**$0**
Renovation	$0	$0	**$0**	$0	$0	**$0**	$0	$0	**$0**
Other (Specify):	$0	$0	**$0**	$0	$0	**$0**	$0	$0	**$0**
Media Processing	$1,658	$1,175	**$2,833**	$0	$4,099	**$4,099**	$0	$0	**$0**
Communication	$4,815	$352	**$5,167**	$0	$3,000	**$3,000**	$0	$0	**$0**
Professional Services & Memberships	$3,136	$14,572	**$17,708**	$0	$9,000	**$9,000**	$0	$0	**$0**
Insurance & Interest	$0	$6,631	**$6,631**	$0	$6,000	**$6,000**	$0	$0	**$0**
Grants & Subsidies	$0	$1,335	**$1,335**	$0	$0	**$0**	$0	$0	**$0**
Contractual & Special Services	$17,689	$7,524	**$25,213**	$0	$20,000	**$20,000**	$0	$0	**$0**
Other Services & Expenditures	$0	$8,524	**$8,524**	$0	$40,000	**$40,000**	$0	$0	**$0**
Total Non-Personnel	**$78,155**	**$73,215**	**$151,370**	**$0**	**$122,099**	**$122,099**	**$0**	**$63,370**	**$63,370**
GRAND TOTAL	**$2,349,390**	**$283,668**	**$2,633,058**	**$2,250,988**	**$323,884**	**$2,574,872**	**$2,361,468**	**$261,670**	**$2,623,138**
Revenue									
New State Appropriation	$0	$244,518	**$244,518**	$0	$249,235	**$249,235**	$0	$261,670	**$261,670**
Carryover State Appropriation	$0	$113,799	**$113,799**	$0	$74,649	**$74,649**	$0	$0	**$0**
New Matching Funds	$2,349,390	$0	**$2,349,390**	$2,250,988	$0	**$2,250,988**	$2,361,468	$0	**$2,361,468**
Carryover from Previous Matching Funds	$0	$0	**$0**	$0	$0	**$0**	$0	$0	**$0**
Total Revenue	**$2,349,390**	**$358,317**	**$2,707,707**	**$2,250,988**	**$323,884**	**$2,574,872**	**$2,361,468**	**$261,670**	**$2,623,138**

YEAR-IN-REVIEW 2017-2018

Annual Report 2017-2018

Program Overview and Accomplishments

The Center for Pediatric Experimental Therapeutics (CPET) has been continuously funded for over 30 years. It achieved accomplished status early, and has been among the best Centers statewide when one considers return on investment. The CPET is among the smallest Centers by total annual appropriations, but consistently brings grant and contract dollars in excess of $9 million per year to the Health Science Center (HSC), its affiliated programs, and the State of Tennessee. The Center has been multidisciplinary, interprofessional, multi-institutional, multi-college and multi-departmental from its beginning, and has had translational science at its core (from bench-top to patient and back again). It is the only state-funded Center of Excellence with improvement in children's health as its primary mission. The CPET has accomplished its mission over the years through research, education, outreach, and patient care.

Extramural funding and research publications from faculty supported by the Center are outlined in the following pages. In addition to this grant support and research productivity, the Center supports graduate education through the CPET Scholars Program. Exceptional students enrolled in graduate education at UTHSC under the direction of Center faculty have been selected for partial support from the center (See CPET Scholar section).

This year the Center was instrumental in supporting the 3rd Annual UT Fungal Pathogens Group which was held at Evins Mill in Smithville, TN. David Andes, M.D., William Craig Professor and Chief of Infectious Diseases at the University of Wisconsin, and Ted White, Ph.D., Marion Merrell Dow Professor and Dean, UMKC School of Biological Sciences, were this year's First Tennessee Distinguished Visiting Professors and delivered the keynote lectures. The UT Fungal Pathogens Group is an integral part of the UT Center for Pediatric Experimental Therapeutics and consists of the NIH funded laboratories of Drs. Jeff Becker and Todd Reynolds at UT Knoxville, and of Drs. Glen Palmer, Brian Peters, Jarrod Fortwendel, and Dave Rogers at UTHSC. In the 2017-18 academic year the group brought in over $2.9 million in NIH funding, published over 30 unique manuscripts, and graduated three graduate students. Collectively this group continues to make key advances in the understanding of serious fungal infections and towards improving their treatment.

In the coming year the CPET will continue to direct its efforts to the focus of pediatric infectious diseases and finding ways to overcome them. Infectious diseases are a leading cause of death in the pediatric population world-wide. This has been complicated by increases in resistance to existing antimicrobial agents. New therapeutic strategies are desperately needed. The CPET has evolved to include leading investigators focused on the bacteria, fungi, and viruses that cause many of the most significant infectious diseases including tuberculosis, pneumonia, blood steam infections, HIV/AIDS, and fungal infections. We expect the next few years to be filled with novel and important research, thus invigorating CPET faculty, transforming the care of patients, and building new connections with the communities we touch. The CPET serves as a unifying force for scientists within these domains and connects the resources and efforts of our faculty through pivotal relationships with Le Bonheur Children's Medical Center and St. Jude Children's Research Hospital. In addition to our efforts in the laboratory, CPET scientists, clinicians, and educators have developed professional curriculum course materials, innovative interprofessional

educational programs, scientific seminars and conferences, and train the next generation of pediatric biomedical scientists through our graduate and postdoctoral training programs.

In our continuing story of innovative science, education, and patient care, CPET members have added an update to the previous chapters of their stories found in last year's annual report. These are examples of evolving science under the guidance of CPET-supported scientists, and are indicative of our continued evolution. Combined with our established investigators, the CPET is a potent force in improving the health of children in Tennessee, the country, and the world.

Extramural Funding

Federal Including NIH Funding

Investigator: **Becker, JM**
Title: The Quiesome and Signalosome of A Model GPCR
Source: NIH/NIGMS
R01 GM112496
Dates: Not Disclosed
Total Direct:
Year Direct: $276, 260

Investigator: **Dale JB**
Title: Structure-Based Design of Broadly Protective Group A Streptococcal Vaccines
Source: NIH (National Institutes of Health)
R01 AI 132117
Dates: 7/1/16 - 6/30/21
Total Direct: $2,768,850
Year Direct: $610,142

Investigator: **Dale JB**
Title: Vaccine Prevention of Group A Streptococcal Infections
Source: NIH (National Institutes of Health)
R01 AI10085
Dates: 11/1/11 – 10/31/17
Total Direct: Not disclosed
Year Direct: $250,000

Investigator: **Dale JB**
Title: Group A Streptococcal Vaccine Contining Immunogenic Peptides of Streptolysin S
Source: NIH (National Institutes of Health)
R21 AI 116808
Dates: 4/1/15 – 3/31/18
Total Direct: Not Disclosed
Year Direct: $150,000

Investigator: **Dale JB**
Title: Administrative Supplement
Source: NIH (National Institutes of Health)
R01 AI 10085
Dates: 11/1/13 – 103/31/17

Total Direct: Not Disclosed
Year Direct: $135,000

Investigator: **Fortwendel J**
Title: Fungal Ras-mediated Invasive Growth Mechanisms
Source: NIH/NIAID (National Institute of Allergy and Infectious Diseases)
R01 AI106925
Dates: 02/2014 - 01/2019
Total Direct: $1,515,000
Year Direct: $287,034

Investigator: **Fortwendel, J**
Title: Systematic Functional Analysis of the *Aspergillus* Fumigatus Kinome
Source: NIH/NIAID
R21 AI139388
Dates: 5/17/2018 - 4/30/2020
Total Direct: $418,000
Year Direct: $209,000

Investigator: **Kumar S, Cory T**
Title: Role of Cytochrome P450 in Alcohol-Mediated HIV-1 Pathogenesis and Antiretroviral Therapy
Source: NIH/NIAAA (National Institute on Alcohol Abuse and Alcoholism)
R01AA022063
Dates: 06/01/2014 – 08/31/2018
Total Direct: $960,000
Year Direct: $250,000

Investigator: **Kumar S**
Title: Exosomes in Tobacco- and HIV-Mediated Neurotoxicity
Source: NIH/NIDA (National Institute of Drug Abuse)
R21DA042374
Dates: 06/01/2016 – 05/31/2018
Total Direct: $275,000
Year Direct: $125,000

Investigator: (Duke Sub) **Lee RE**
Title: Transdisciplinary Program to Identify Novel Antifungal Targets and Inhibitors
Source: NIAID (National Institute of Allergy and Infectious Diseases)
5 P01AI104533-03
Dates: 6/25/2015 - 5/31/2020
Total Direct: $279,020
Year Direct: $279,020

Investigator: (White), **Lee RE**
Title: Training in the Design and Development of Infectious Disease Therapeutics
Source: NIAID
5 T32AI106700-03
Dates: 7/01/2015 – 6/30/2020
Total Direct: $161,719
Year Direct: $161,719

Investigator: **Lee RE**
Title: Bactericial Antibiotic for Vancomycin Resistant Enterococci
Source: NIH/NIAID (National Institute of Allergy and Infectious Diseases)
1 R44AI122426-01A1 ARIETIS SBIR
Dates: 03/15/2016 - 02/20/2019
Total Direct: $382,930
Year Direct: $382,930

Investigator: **Lee RE, Meibohm B**
Title: Development of Aminospectinomycins for Biodefense
Source: NIH/NIAID (National Institute of Allergy and Infectious Diseases)
R01AI111449 Subcontract to St. Jude Children's Research
Dates: 06/2014 - 05/2019
Total Direct: $1,237,511
Year Direct: $250,000

Investigator: **Lee RE**
Title: Development of Novel Proteins Synthesis Inhibitors for MDR Tuberculosis YR6
Source: NIAID
2 R01AI090810-06
Dates: 7/06/10 – 2/28/2023
Total Direct: $3,997,590
Year Direct: $799,518

Investigator: **Lee RE**
Title: Broad Spectrum Antifungals Targeting Fatty Acid Biosynthesis
Source: NIAID
1 R21AI127607-01
Dates: 12/01/2017 – 11/30/2021
Total Direct: $75,000
Year Direct: $75,000

Investigator: **Lee RE**
Title: Mechanisms of Susceptibility and Resistance of Mycobacterium Tuberculosis to Isoxyl and Thiacetazone
Source: NIH

5 R21AI130929-02 CO STATE
Dates: 2/10/2017 – 1/31/2019
Total Direct: $21,267
Year Direct: $21,267

Investigator: Lewis K, **Lee RE**
Title: Developing Therapeutics to Treat Chronic Infections
Source: NIH/NIAID (National Institute of Allergy and Infectious Diseases)
5 R01AI110578-05
Dates: 03/01/2014 - 02/28/2019
Total Direct: $1,215,645
Year Direct: $243,129

Investigator: Li W, Miller DD, **Meibohm B,** Hamilton D
Title: Selective Targeting Survivin for Cancer Therapy
Source: NIH/NCI (National Cancer Institute)
R01CA193609
Dates: 04/2016 - 04/2021
Total Direct: $1,913,635
Year Direct: $380,127

Investigator: Lowe T, Chaum E, **Meibohm B,** Hamilton D
Title: Nanogels for Drug Delivery Across the BRB to Treat Diabetic Retinopathy
Source: NIH/NEI
R01EY023853
Dates: 09/2016 - 08/2021
Total Direct: $1,900,000
Year Direct: $380,000

Investigator: **Meibohm B**, Gonzales-Juarrero M, Hickey A, Braunstein M
Title: Aerosol spectinamide-1599 Therapy Against Tuberculosis
Source: NIH/NIAID
R01AI120670
Dates: 06/2016 - 05/2021
Total Direct: $3,734,418
Year Direct: $848,545

Investigator: **Meibohm B**
Title: Development of Aminospectinomycins for Biodefense
Source: NIH/NIAID (Subcontract to St. Jude Children's Research)
R01 AI111449
Dates: 05/2014 - 04/2019
Total Direct: $771,768
Year Direct: $90,950

Investigator: **Palmer G, Meibohm B, Lee RE**
Title: Broad Spectrum Antifungals Targeting Fatty Acid Biosynthesis
Source: NIH/NIAID (National Institute of Allergy and Infectious Diseases) 1R21AI127607-01
Dates: 12/8/16 – 11/30/2018
Total Direct: $449,622
Year Direct: $152,000

Investigator: **Palmer GE**
Title: Molecular and Chemical Validation of the Vacuole as a New Antifungal Target
Source: NIH/NIAID (National Institute of Allergy and Infectious Diseases) 5 R01AI099080-04
Dates: 05/20/14 – 04/30/19
Total Direct: $1,804,354
Year Direct: $377,577

Investigator: Reiter L, **Palmer GE**
Title: An in Vivo Chemical Screen for Seizure Suppression in Duplication 15q Syndrome
Source: NIH/NIAID (National Institute of Allergy and Infectious Diseases)
Dates: 04/01/17 – 03/31/18
Total Direct: $418,000
Year Direct: $228,000

Investigator: **Peters B**
Title: A Novel Role for the Inflammasome in the Immunopathogenesis of *Candida* Vaginitis
Source: NIH/NIAID (National Institute of Allergy and Infectious Diseases) 1 K22AI110541-01A1
Dates: 1/01/2016 – 12/31/2018
Total Direct: $270,000
Year Direct: $108,000

Investigator: **Peters B**
Title: Sulfonylureas as Repurposed Agents Against Vulvovaginal Candidiasis
Source: NIH/NIAID 1R21AI127942-01A1
Dates: 1/01/2018-12/31/2019
Total Direct: $418,000
Year Direct: $228,000

Investigator: **Peters B**
Title: Host and Microbial Factors Promoting Synergistic Mortality During Polymicrobial Intra-abdominal Infections with *Candida albicans* and *Staphylococcus aureus*
Source: NIH/NIAID (R01AI116025-01)
Dates: 12/01/2015-11/30/2019

Total Direct: $301,196
Year Direct: $91,954

Investigator: **Reynolds T**
Title: Screen for Phosphatidylserine Synthase Inhibitors: Antifungals & Lipid Probes
Source: NIAID
R01 AI105690-01
Dates: 02/01/2013-01/31/2018
Total Direct: Not Disclosed
Year Direct: No Cost Entension

Investigator: **Reynolds T**
Title: Identification of CDP-DAG and Serine Binding Sites in *Candida albicans* Phosphatidylserine Synthase, an Antifungal Drug Target
Source: NIAID
1R21AI130895
Dates: 01/16/17 – 12/31/18
Total Direct: Not Disclosed
Year Direct: $233,457

Investigator: **Rogers PD**
Title: Novel Azole Resistance Mechanisms in *Candida albicans*
Source: NIH/NIAID (National Institute of Allergy and Infectious Diseases)
4 R01AI058145
Dates: 06/21/17 – 05/31/18
Total Direct: $2,122,820
Year Direct: $454,854

Investigator: **Rogers PD**
Title: Upc2A: A Central Regulator and "Achilles' Heel" of Fluconazole Resistance in *Candida glabrata*
Source: NIH/NIAID
R01 AI131620
Dates: 06/21/17 – 05/31/18
Total Direct: $2,706,949
Year Direct: $589,278

Investigator: White S**, Lee RE**
Title: Development of novel DHPS Inhibitors
Source: NIH/NIAID (National Institute of Allergy and Infectious Diseases)
Dates: 12/2012 - 11/2017
Total Direct: $1,139,528
Year Direct: $227,900

Investigator: White, Lee RE
Title: Training in the Design and Development of Infectious Disease Therapeutics

Source:	NIH/NIAID
Dates:	7/2015-6/2020
Total Direct:	$161,719
Year Direct	$32,344

Foundation/Industry Funding

Dollar amounts are not always disclosed due to foundation/industry request.

Investigator: **Dale J**
Title: Strengthening the Health System Response to Rheumatic Heart Disease: Developing Evidence-Based Strategies For Prevention
Source: AHA Strategically Focused Research Network Award
Dates: 7/1/16 - 6/30/20
Total Direct: $1,000,000
Year Direct: $199,008

Investigator: **Fortwendel, J**
Title: Systematic Functional Analysis of the *Aspergillus* Fumigatus CAMK Kinome
Source: UT College of Pharmacy
Dates: 11/1/2017 – 6/30/2018
Total Direct: $15,000
Year Direct: $15,000

Investigator: Jackowski, **Lee, RE**
Title: Small Molecule Modulators of Pantothenate Kinase
Source: COA Therapeutics Inc.
Dates: 5/01/2017 – 4/30/2019
Total Direct: $649,444
Year Direct: Not Disclosed

Investigator: **Kumar S**
Title: Exosome Cathepsins in Alcohol-Mediated Neurotoxicity
Source: UT College of Pharmacy
Dates: 01/01/2018 – 06/30/2018
Total Direct: $15,000
Year Direct: $15,000

Investigator: **Palmer, GE**
Title: Identifying Chemical Modulators of the UBE3A Ubiquitin Ligase as a Therapeutic Strategy to Treat Epileptic Seizures and HPV Related Cancers
Source: UTHSC Cornet Award
Dates: 06/01/2017 – 5/31/2018
Total Direct: $47,824
Year Direct: $47,824

Investigator: **Palmer, GE**
Title: Targeting the Aromatic Amino Acid Synthesis Pathway to Develop a New Class of Broad Spectrum Antimicrobial Agent.
Source: UTHSC Special Cornet Award (SRI)
Dates: 8/01/2017 – 7/31/2019
Total Direct: $100,000
Year Direct: $50,000

Investigator:	**Peters, B**
Title:	Genetic Mechanisms of Candidalysin Regulation in the Fungal Pathogen *Candida albicans*
Source:	UTHSC College of Pharmacy (Seed Grant)
Dates:	11/2017 – 6/2018
Total Direct:	$14,620
Year Direct:	$14,620

Investigator:	**Peters B, Fortwendel J**
Title:	The GentleMACS Octo Dissociator for Standardized and Reproducible Tissue Preparation in Molecular and Immunological Analyses
Source:	UTHSC College of Pharmacy (Equipment Grant)
Dates:	11/2017-06/2018
Total Direct:	$11,835
Year Direct:	$11,835

Publications

Books / Book Chapters

Lukka PB, Wagh S, **Meibohm B**. Translational Considerations in Developing Bispecific Antibodies: What Can We Learn from Quantitative Pharmacology? In: Zhou H, Mould DR (eds). Quantitative Pharmacology and Individualized Therapy Strategies in Development of Therapeutic Proteins for Immune-Mediated Inflammatory Disease, Wiley 2018 (accepted)

Meibohm, B. Pharmacology. In: Chyka P, Parker RB et al. (eds). APhA Complete Review for the Foreign Pharmacy Graduate Equivalency Examination (FPGEE), 2nd ed. American Pharmacists Association, Washington 2018 (accepted)

Journal Articles

Al Abdallah Q, Ge W and **Fortwendel JR.** A Simple and Universal System for Gen Manipulation in Aspergillus Fumigatus: in Vitro-Assembled Cas9-guide RNA Ribonucleoproteins Coupled with Microhomology Repair Templates. *mSphere*. 2017. 2(6). pii: e00446-17. doi: 10.1128/mSphere.00446-17.

Al Abdallah Q, Martin-Vicente A, Souza ACO, Ge W, and **Fortwendel JR**. C-terminus Proteolysis and Palmitoylation Cooperate for Optimal Plasma Membrane Localization of RasA in Aspergillus Fumigatus. *Frontiers in Microbiology*. 2018. 9:562 doi: 10.3389/fmicb.2018.00562.

Brown Gandt A, Griffith EC, Lister IM, Billings LL, Han A, Tangallapally R, Zhao Y, Singh AP, **Lee RE**, LaFleur MD. In Vivo and In Vitro Effects of a ClpP Activating Antibiotic Against Vancomycin Resistant Enterocccoci. *Antimicrob Agents Chemother*. 2018 May 21. pii: AAC.00424-18. doi: 10.1128/AAC.00424-18. [Epub ahead of print]. PMID: 29784838

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