PI: **Coleman, Kenneth**	Title: Antibiotics for Recalcitrant Infection	
Received: 12/05/2013	FOA: PA10-123	Council: 05/2014
Competition ID: ADOBE-FORMS-B1	FOA Title: NIAID ADVANCED TECHNOLOGY SBIR (NIAID-AT-SBIR [R43/R44])	
1 R44 AI112187-01A1	Dual:	Accession Number: 3650924
IPF: 10010171	Organization: ARIETIS	
Former Number:	Department:	
IRG/SRG: ZRG1 IDM-U (10)B	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: [redacted] Year 2: [redacted] Year 3: [redacted] Year 4:	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Kenneth Coleman	Arietis Corporation	PD/PI
Kim Lewis	Northeastern University	Consultant
Steven Leonard	Northeastern University	Consultant

Appendices

Sbc 00009717

OMB Number: 4040-0001
Expiration Date: 06/30/2011

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

2. DATE SUBMITTED | **Applicant Identifier**

4. a. Federal Identifier

b. Agency Routing Identifier

5. APPLICANT INFORMATION *** Organizational DUNS:**

* Legal Name: Arietis Corporation
Department: | Division:
* Street1: 650 Albany St.
Street2:
* City: Boston | County / Parish: Suffolk
* State: MA: Massachusetts | Province:
* Country: USA: UNITED STATES | * ZIP / Postal Code: 02118-2518

Person to be contacted on matters involving this application
Prefix: Dr. | * First Name: Michael | Middle Name:
* Last Name: LaFleur | Suffix:
* Phone Number: | Fax Number:
Email:

6. * EMPLOYER IDENTIFICATION *(EIN) or (TIN):*

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:
Antibiotics for Recalcitrant Infection

12. PROPOSED PROJECT:
* Start Date: 07/01/2014 | * Ending Date: 06/30/2018

*** 13. CONGRESSIONAL DISTRICT OF APPLICANT:** MA-008

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION
Prefix: Dr. | * First Name: Kenneth | Middle Name:
* Last Name: Coleman | Suffix:
Position/Title:
* Organization Name: Arietis Corporation
Department: | Division:
* Street1: 650 Albany ST
Street2:
* City: Boston | County / Parish: Suffolk
* State: MA: Massachusetts | Province:
* Country: USA: UNITED STATES | * ZIP / Postal Code: 02118-2518
* Phone Number: | Fax Number:
* Email:

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested	
b. Total Non-Federal Funds	0.00
c. Total Federal & Non-Federal Funds	
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 penalities. (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

Add Attachment | Delete Attachment | View Attachment

19. Authorized Representative

Prefix: Dr. * First Name: Michael Middle Name:

* Last Name: LaFleur Suffix:

* Position/Title: Chief Operating Officer

* Organization: Arietis Corporation

Department: Division:

* Street1: 650 Albany St.

Street2:

* City: Boston County / Parish: Suffolk

* State: MA: Massachusetts Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 02118-2518

* Phone Number: Fax Number:

* Email:

*** Signature of Authorized Representative**: Michael LaFleur

*** Date Signed**: 12/05/2013

20. Pre-application Add Attachment | Delete Attachment | View Attachment

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

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Appendix

Number of Attachments in Appendix: 1

OMB Number: 4040-0010
Expiration Date: 08/31/2011

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: Arietis Corporation

DUNS Number: [redacted]

* Street1: 650 Albany St

Street2: [redacted]

* City: Boston County:

* State: MA: Massachusetts

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 02118-2518 * Project/ Performance Site Congressional District: MA-008

Project/Performance Site Location a ☐ 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: [redacted]

DUNS Number:

* Street1: [redacted]

Street2:

* City: [redacted] County:

* State: [redacted]

Province:

* Country: [redacted]

* ZIP / Postal Code: [redacted] * Project/ Performance Site Congressional District: [redacted]

Additional Location(s) Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED Other Project Information

OMB Number: 4040-0001
Expiration Date: 6/30/2016

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

If no, is the IRB review Pending? ☐ Yes ☐ No

IRB Approval Date:

Human Subject Assurance Number:

2. Are Vertebrate Animals Used? ☒ Yes ☐ No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ☐ Yes ☒ No

IACUC Approval Date: 05/29/2013

Animal Welfare Assurance Number: [redacted]

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 of the United States or partnerships with international collaborators? ☐ Yes ☒ No

6.a. If yes, identify countries:

6.b. Optional Explanation:

7. Project Summary/Abstract 1237-Abstract.pdf Add Attachment | Delete Attachment | View Attachment

8. Project Narrative 1238-Narrative.pdf Add Attachment | Delete Attachment | View Attachment

9. Bibliography & References Cited 1239-Bibliography.pdf Add Attachment | Delete Attachment | View Attachment

10. Facilities & Other Resources 1240-Facilities_Biohazards.pdf Add Attachment | Delete Attachment | View Attachment

11. Equipment Add Attachment | Delete Attachment | View Attachment

12. Other Attachments Add Attachments | Delete Attachments | View Attachments ☒

The goal of the project is to develop a therapeutic capable of sterilizing recalcitrant chronic infections such as deep-seated abscess, osteomyelitis, endocarditis, and biofilms of indwelling devices. Many of these infections are essentially untreatable and lead to substantial morbidity and mortality. In many cases, recalcitrance of an infection is not caused by drug resistance. Rather, a slow-growing biofilm population harbors stationary phase and dormant persister cells that are highly tolerant to killing by antimicrobials. When antibiotic concentration drops, these cells can grow and repopulate the biofilm. In Gram-positive *S. aureus,* it appears that a stationary culture is made mostly of drug tolerant cells which are virtually insensitive to killing by traditional antibiotics. There are many independent, redundant mechanisms of persister formation, and these specialized survivor cells do not have a realistic target which could be exploited for drug development. In order to act, all existing bactericidal antibiotics require active targets which they corrupt. We reasoned that persisters could be killed if a small molecule could simultaneously activate and corrupt a cellular target. We find that acyldepsipeptide (ADEP) activates the ClpP protease in dormant persisters, forcing the cell to self-digest. In order to diminish resistance development, ADEP was combined with rifampicin. The combination completely sterilized a deep-seated biofilm infection of *S. aureus* in a neutropenic mouse model after a single dose. The best conventional antibiotics, alone or in combination, had very little effect. This model emulates the most difficult to treat chronic infection in immunocompromised patients. In this project, we will identify the most promising sterilizing combinations of drugs. Combinations will be evaluated for PK, PD, and efficacy using a deep-seated neutropenic thigh model of MRSA infection, and in an *in vivo* biofilm tissue-cage model. Once validated, the combination therapeutic will enter into preclinical investigation in Phase II, leading to an IND, and subsequent clinical trials of the drug.

The goal of the project is to develop a therapeutic capable of curing currently untreatable chronic infections. Bacterial pathogens are not susceptible to killing by traditional antibiotics when they are in a non-growing, inactive state. This makes it very difficult to treat diseases such as infective osteomyelitis or endocarditis. We have identified a compound capable of killing inactive cells, and we will develop this compound into a therapeutic.

Laboratory: The Arietis laboratory is located in the Biosquare Discovery and Innovation Center of BU Medical School. The 1400 square foot laboratory space and adjacent office space is sufficient for undertaking the work on this project. The laboratory is equipped with biosafety cabinets, anaerobic and microaerophilic chambers, chemical hoods, shakers, incubators, refrigerators, freezers, microscopes, fluorescent and visible spectrophotometers, PCR machines, MicroFill liquid handling dispenser, electroporation device, tissue homogenizer, sonicator, spiral plater with colony enumeration system, photo documentation system with trans-illumination, and small equipment items – gel boxes, balances, water baths, and centrifuges. Common facilities adjacent to the suite include cold rooms, centrifuges, autoclaves, glass washers, incubator space and freezers. Arietis has also negotiated access to the full suite of core facilities available onsite at the BU Medical Center. Core facilities consist of Analytical Instrumentation Core, Biomedical Imaging Center, Biospecimen Archive Research Core, BU Clinical and Translational Science Institute, Cellular Imaging Core, Confocal Facility, Experimental Pathology Laboratory Service Core, Flow Cytometry Core Facility, High Throughput Screening Core, Illumina Sequencing Core Facility, Immunohistochemistry (IHC) Core Facility, LinGA - Linux Genetic Analysis Core, Microarray Resource Core Facility, Molecular Genetics Core Facility, Proteomics Core Facility, Transgenic Center. More information regarding the specific instrumentation at each core facility can be found at http://www.bu.edu/cores/cores/.

Animal: The animal studies will be performed by Arietis and take place Laboratory Animal Science Center (LASC) at Boston University, an AAALAC accredited animal care program located on the medical campus comprising Boston University School of Medicine, Boston University School of Public Health, Boston University Goldman School of Dentistry and Boston Medical Center. The animal facilities contain approximately 45,780 sq. feet of animal housing and support space. The Laboratory Animal Science Center is a team of administrative, managerial, technical and professional staff committed to the advancement of science in collaboration with the research community by promoting the humane care and use of animals used in biomedical research and teaching.

The Laboratory Animal Science Center (LASC) oversees veterinary medical care, animal care services, and actively participates in all facility design. Day-to-day care of animals and technical assistance are provided by approximately thirty animal caretakers. The care staff is overseen by Operations Managers and Animal Care Supervisors. In addition, three veterinary technicians are supervised by a Veterinary Services Manager, under the direction of the Attending Veterinarian. Administrative, business and purchasing functions are supported by a Business Manager and three support staff. Standard Operating Procedures and reference materials are available from the IACUC Office for animal use. The animal health program for all Boston University owned laboratory animals is directed by the attending veterinarian, [redacted], and provided by two full-time veterinarians. All studies involving animals will be performed in the Laboratory Animal Sciences Center (LASC). Animals in each LASC room are observed daily for signs of illness by the animal technician responsible for providing husbandry. Medical records and documentation of experimental use are maintained individually for non-rodents and individually or by cage group for rodents. Veterinary technicians under the direction of the attending veterinarian provide routine veterinary medical care to all animals. Animal care and use is additionally monitored for training and compliance issues by the Training and Compliance Manager. The Boston University Medical Campus Animal Welfare Assurance number is [redacted].

Computer: The group has PC computers with internet access and support is available. In addition, researchers at Arietis have full desktop access to: the *Collaborative Drug Discovery* database allowing easy sharing of chemical structure/properties data, with biological screening data populated by collaborator labs; *Pipeline Pilot* which provides the capability for merging and manipulating data from disparate sources; *ChemAxon JChem* scientific software suite allowing library enumeration, searching, and filtering; *CambridgeSoft ChemBioOffice*; and *Statistica* analytical software.

Biohazards: *S. aureus is a Biosafety Level 2 (BSL-2) microorganism associated with a human disease, for which preventive or therapeutic interventions are available. The Arietis Laboratory is BLS-2 certified and all personnel receive BSL-2 training from Boston University and are authorized to work with this pathogen. Training courses include general laboratory, chemical safety and pathogen specific BSL-2 protocols, which are renewed each year. All personnel are monitored annually by the Boston University Occupational Health Center, to which any incidents of exposure are reported. To minimize exposure, guidelines detailed in the Boston University Safety Manual are followed including that all work is carried out in biological safety cabinets (certified annually), all personnel wear personal protective equipment including a laboratory coat, gloves and eye protection, and sterilization procedures are followed for the handling of waste: waste cultures are mixed*

with 10% fresh bleach for 30 minutes; plastic wear is bagged and sealed within the biological safety cabinet before being autoclaved with other biological hazardous waste; glass wear is soaked in 10% fresh bleach for 30 minutes before further washing; all work surfaces are wiped down with 70% ethanol after working and at the end of each day. To clean and contain spills, all personnel are notified to stay away from the area and the spill is covered with paper towels, soaked with 10% fresh bleach for 30 minutes before being wiped up from the edges towards the center. Safety is monitored by all laboratory members including the PI and Laboratory Safety Officer. Review of protocols and procedures is carried out by the Arietis Institutional Biosafety Committee to ensure that practices are in compliance with Boston University standards and good laboratory practices. All strains are logged in the Arietis Laboratory Database, a copy of which is also held by the Boston University Health and Safety department. On-site environmental health and safety consulting services are available through Boston University.

SBIR.gov SBC Registration Control ID Form

SBC CONTROL ID	SBC_000097171

FIRM INFORMATION					
Company	ARIETIS				
Address	650 Albany St				
City	BOSTON	State	MA	Zip	02118-
TIN/EIN		DUNS			
Company URL					
Number of Employees:					
Is this SBC majority-owned by multiple venture capital operating companies, hedge funds, or private equity firms?					No
What percentage (%) of the SBC is majority-owned by multiple venture capital operating companies, hedge funds, or private equity firms?					0%

SBC CONTROL ID	SBC_000097171

OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. * First Name: Kenneth Middle Name:
* Last Name: Coleman Suffix:
Position/Title: Department:
Organization Name: Arietis Corporation Division:
* Street1: 650 Albany ST
Street2: [redacted]
* City: Boston County/ Parish: Suffolk
* State: MA: Massachusetts Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 02118-2518
* Phone Number: [redacted] Fax Number:
* E-Mail: [redacted]

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

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

Degree Type:
Degree Year:

***Attach Biographical Sketch** 1234-Biosketch_KC.pdf [Add Attachment] [Delete Attachment] [View Attachment]
Attach Current & Pending Support [Add Attachment] [Delete Attachment] [View Attachment]

PROFILE - Senior/Key Person 1

Prefix: * First Name: Kim Middle Name:
* Last Name: Lewis Suffix:
Position/Title: Professor Department: Biology
Organization Name: Northeastern University Division:
* Street1: 360 Huntington Ave
Street2: 134 Mugar Hall
* City: Boston County/ Parish:
* State: MA: Massachusetts Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 02115-0000
* Phone Number: [redacted] Fax Number:
* E-Mail: [redacted]

Credential, e.g., agency login:

*** Project Role:** Consultant **Other Project Role Category:**

Degree Type:
Degree Year:

***Attach Biographical Sketch** [Add Attachment] [Delete Attachment] [View Attachment]
Attach Current & Pending Support [Add Attachment] [Delete Attachment] [View Attachment]

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 2

Prefix: | * First Name: Steven | Middle Name:

* Last Name: Leonard | Suffix:

Position/Title: Assistant Professor | Department: Pharmacy

Organization Name: Northeastern University | Division:

* Street1: 140 The Fenway, R225

Street2:

* City: Boston | County/ Parish:

* State: MA: Massachusetts | Province:

* Country: USA: UNITED STATES | * Zip / Postal Code: 02115-0000

* Phone Number: [redacted] | Fax Number:

* E-Mail: [redacted]

Credential, e.g., agency login:

*** Project Role:** Consultant | **Other Project Role Category:**

Degree Type:

Degree Year:

***Attach Biographical Sketch**	1236-Leonard_Biosketch.pdf	Add Attachment	Delete Attachment	View Attachment
Attach Current & Pending Support		Add Attachment	Delete Attachment	View Attachment

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE
Coleman, Kenneth	Principal Investigator
eRA COMMONS USER NAME (credential, e.g., agency login) [redacted]	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of London, London, UK	B.Sc.	1970	Microbiology/Chemistry
Univ. of Nottingham, Nottingham, UK	Ph.D.	1974	Microbiology

A. Personal Statement

I have a broad background in microbiology and chemistry, with specific training and expertise in key research areas for this application, and have worked in the anti-infective area in small and large pharmaceutical companies for the past 35 years. For most of that time I have been focused on lead optimization of antibacterial and antifungal compounds, but I have also been a member of development project teams and have managed numerous discovery and development projects myself. While working in the UK, I held a Home Office experimental animal license for 20 years and ran many *in vivo* anti-infective models, including the murine staphylococcal thigh model and the tissue cage model. Due to a strong anti-vivisection group in the UK, company policy discourages publication of in vivo work, so there is little evidence of this capability in my publication list. While at GSK, I contributed to the development of the two clavulanic acid drugs, Augmentin and Timentin, and to the quinolone, gemifloxacin.

In my last position, as Chief Scientific Officer of Novexel SA (Romainville, France) I oversaw the biology and chemistry research departments and the Safety Assessment department of the company and was on the steering team for three development projects, two antibacterial (NXL103 and NXL104) and one antifungal (NXL201). We advanced NXL104 (avibactam), a novel β-lactamase inhibitor, through a successful Phase II clinical trial, after which the Company was acquired by Astra Zeneca.

I joined Arietis in 2010. We are an early startup Biotech focused on narrow-spectrum agents and compounds capable of treating dormant pathogens in chronic infections. An ADEP4 combination is our first candidate for a systemic sterilizing antimicrobial. It comes out of a successful collaboration with Kim Lewis's group at NU, and I feel that we have a good chance to develop this into a therapeutic for curing currently untreatable infections.

B. Positions and Honors

Positions and Employment

1971 - 1974	Lecturer in Microbiology, Trent Polytechnic, Nottingham, UK
1974 - 2001	Assistant Director, SmithKline Beecham Pharmaceuticals
2001 - 2008	Director, Anti-Infective Biosciences, AstraZeneca R&D Boston
2008 - 2010	Chief Scientific Officer, Novexel SA, Romainville, France
2010 -	Chief Scientific Officer, Arietis Corp., Boston, MA
2004 –	Editorial Board, Antimicrobial Agents & Chemotherapy

2009 – 11 CLSI Subcommittee on Susceptibility Testing of Human Mycoplasmas

C. Selected Peer-reviewed Publications

1. **K. COLEMAN, D. R. J. GRIFFIN AND PAULINE A. UPSHON.** (1991). Pharmacokinetic studies and renal dehydropeptidase stability of the new ß-lactamase inhibitor BRL 42715 in animals. *Antimicrobial Agents and Chemotherapy*, **35**: 1748 - 1752.
2. **K. COLEMAN, M. ATHALYE, M. DAVISON, D. PAYNE, C. PERRY & I. CHOPRA.** (1993). Bacterial resistance mechanisms as therapeutic targets. *Journal of Antimicrobial Chemotherapy,* **33**: 1091 - 1116.
3. **K. COLEMAN.** (1995)**.** An update on ß-lactamases and ß-lactamase inhibitors. *Expert Opinion in Investigational Drugs,* **4:** 693 – 704
4. **J. BROSKEY, K. COLEMAN et al.** (2000). Efflux and target mutations as quinolone resistance mechanisms in clinical isolates of *S. pneumoniae. Journal of Antimicrobial Chemotherapy,* **45, S1**: 95 – 99.
5. **K. COLEMAN.** (2004)**.** Recent Advances In The Treatment Of Gram-Positive Infections. *Drug Discovery Today: Therapeutic Strategies, .* **1**: 455 – 460
6. **K. COLEMAN.** (2006)**.** Extending The Life Of β-Lactam Antibiotics: New β-Lactamase Inhibitors. *Drug Discovery Today: Therapeutic Strategies, .* **3**: 183-188
7. **M. BLACK and K. COLEMAN.** (2009). New inhibitors of bacterial topoisomerase GyrA/ParC subunits. *Current Opinion In Investigational Drugs,* **10** (8), 804-810*.*
8. **K. COLEMAN.** (2011). Diazabicyclooctanes (DBOs): a potent new class of non-β-lactam β-lactamase inhibitors. *Current Opinion in Microbiology.* **14**: 550-555.
9. **LAFLEUR MD, SUN L, LISTER I, KEATING J, NANTEL A, LONG L, GHANNOUM M, NORTH J, LEE RE, COLEMAN K, DAHL T, LEWIS K.** (2013). Potentiation of azole antifungals by 2-adamantanamine. *Antimicrob Agents Chemother.* **57:**3585-92.
10. **CONLON, B. P., E. S. NAKAYASU, L. E. FLECK, M. D. LAFLEUR, V. M. ISABELLA, K. COLEMAN et al.** (2013) Activated ClpP kills persisters and eradicates a chronic biofilm infection. *Nature* **503**: 365-370.

D. Research Support (ongoing or completed in past 3 years)

06/15/2012-05/31/2014. Compounds to treat *Helicobacter pylori* infection
Principal Investigator: Dr Ken Coleman (Dr R. E. Lee Co-Investigator)
ARIETIS CORP
Agency: NIH 1 R43AI098327-01A1
In this study we propose to develop novel and selective anti *H. pylori* therapeutics.

07/01/2012-06/30/2016. Narrow-spectrum Agents Acting against *Helicobacter pylori*
Principal Investigator: Dr Ken Coleman
ARIETIS CORP
Agency: NIH 1R44AI102452-01
In this study, we will develop our lead series against *H. pylori*.

Program Director/Principal Investigator (Last, First, Middle): Lewis, Kim

NAME **Kim Lewis, Ph.D.**	POSITION TITLE University Distinguished Professor Director, Antimicrobial Discovery Center
eRA COMMONS USER NAME [redacted]	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Moscow University, Moscow, USSR	B.S.	1976	Biology
Moscow University, Moscow, USSR	Ph.D.	1980	Biochemistry

A. Personal Statement

The main focus of my research is on antimicrobial tolerance which limits the ability of antibiotics to sterilize an infection; and on antimicrobial drug discovery. Pathogen eradication is especially important for chronic infections such as those formed by biofilms, where the effectiveness of the immune system is limited. In the area of tolerance, my laboratory discovered persister cells in microbial biofilms and showed that this is the main culprit of recalcitrance. In recent years, we identified a number of genes involved in persister formation, and established detailed molecular mechanisms of dormancy in *E. coli* governed by the toxin components of toxin/antitoxin modules HipBA and TisAB.

My group has been involved in antimicrobial drug discovery for over a decade, and the program started with the discovery of synergistically-acting antimicrobials from medicinal plants. We found that many plant antimicrobials are effectively extruded by bacterial multidrug resistance pumps (MDRs). Plants solve the problem by simultaneously producing MDR inhibitors. In collaboration with Dr. Slava Epstein, we developed a method to grow uncultured bacteria, and identfified the first class of growth factors, siderophores, for these organisms. These approaches are being used by scientists from NovoBiotic to obtain novel secondary metabolites. My group together with Dr. Fred Ausubel developed an HTS approach using live infected animals, the worm *C. elegans* which is leading to identification of compounds that are missed by traditional in vitro screens.

The proposed project is a culmination of a decade-long effort by my group to identify a realistic approach to eradicate persisters. What we have learned over that decade is that persisters evolved to survive conventional antibiotics that hit specific targets. The targets in persisters are inactive, and the pathways of dormancy are redundant. It appears that acyldepsipeptides evolved to kill both growing and dormant bacteria by activating a protease, forcing cells to self-digest. We will use nature's elegant solution to the dormancy problem as a basis for developing a sterilizing therapeutic.

B. Positions and Honors:

From:	**To:**	**Title:**	**Institution:**
7/1/01	Present	Professor	Northeastern University
4/1/97	6/31/01	Research Assoc. Professor	Biotechnology Center, Tufts University
9/94	3/31/97	Associate Professor	Medical & Research Technology, UMAB
7/88	8/94	Assistant Professor	Department of Biology, MIT
1/88	1/88	Assistant Professor	Applied Biology, MIT (Department was disbanded)
7/87	1/88	Research Associate	Univ. of Wisconsin, Madison
4/84	7/87	Lost Academic position after applying to emigrate to the USA	
6/79	3/84	Senior Researcher	Moscow University
6/76	6/79	Researcher	Moscow University

Nobel Conference "Biofilm formation, its clinical impact and potential treatment", Karolinska Institutet, Stockholm, 2013

Leading Scientists Seminar Series, Imperial College London, 2013

Lester O. Krampitz Lecture, Case Western Reserve Univesity, 2013

Keynote Lecture, Lyme Disease Association annual meeting, Philadelphia, 2012

Fellow, American Academy of Microbiology, 2011

University Distinguished Professor, Northeastern University, 2011

Keynote lecture, Belgian Society for Microbiology, Brussels, 2011

Speaker and Panelist, The National Academies Forum on Microbial Threat, Washington, DC 2011

Speaker and Panelist, the NIAID/FDA/IDSA Public Workshop on Antibacterial Resistance and Diagnostic Device and Drug Development Research for Bacterial Diseases, Washington, DC 2010

Speaker and Panelist, The National Academies Forum on Antibiotic Resistance, Washington, DC 2010

NIH Director's Transformative Grant Award, 2009

Keynote Lecture, ASM Educational Meeting (ASMCUE), Fort Collins, CO 2008
Chair, NIAID-BARDA Application of Platform Technologies for the Development of Therapeutics for Biodefense study section, 2008
Chair, NIH Roadmap study section, High Throughput Screening in the Molecular Libraries Screening Centers Network, 2008
Convener, ICAAC symposium, Antimicrobial Tolerance: Persisters, Biofilms, And Infectious Disease, Washington, DC, 2008
Lecturer, Harvard University Microbial Science Initiative, 2006
Member, Faculty of 1000, Pharmacology & Drug Discovery section, 2006 -
Division A (Antimicrobial Chemotherapy) Lecture, ASM General Meeting, Atlanta, Georgia, 2005
NIH study section member, Drug Discovery and Mechanisms of Antimicrobial Resistance, 2004 –2006
Distinguished Research Fellow, Northeastern University, 2004
Convener, ASM General Meeting, New Orleans, 2004
Expert Lecture, V European Congress on Chemotherapy and Infections, Rhodes, Greece, 2003
Convener, ASM General Meeting, Washington, DC, 2003
Keynote lecture, Wind River Conference on Prokaryotic Biology, Estes Park, CO, 2001
Convener, Society for Industrial Microbiology Annual Meeting, Saint Louis, 2001
Convener, Society for Industrial Microbiology Annual Meeting, San Diego, 2000
Symposial Lecture at the Society for General Microbiology Meeting, Leeds, 1999
Chair, MDR Colloquium, 9th European Bioenergetics Meeting (EBEC), Louvain, Belgium, 1996
Convener, ASM General Meeting, Washington, DC, 1995
MIT C.E. Reed Faculty Initiative Award for an innovative research project. Competitively awarded to 1 Faculty member among the School of Science and the School of Engineering annually, 1992

INVENTIONS:

1. Solvent-resistant microorganisms. Klibanov, A. M., Lewis. K., Ferrante, A., Coyle, C. L., Zylstra, G., Logan, M. S. P., Grossman, M. J. US Patent #5,807,735. Awarded 9.15.98.
2. Culturing cells in presence of amphipathic weak bases and/or cations and multiple drug resistance inhibitor containing reserpine. Lewis, K., Hsieh, P-C. US Patent #6,410,041. Awarded 6.25.02.
3. Isolation and cultivation of microorganisms from natural environments and drug discovery based thereon. Lewis, K., Epstein, S.S., and Kaeberlein, T. US Patent #7,011,957. Awarded March 14, 2006. Licensed to NovoBiotic Pharmaceuticals, LLC.

4. Antimicrobial polymeric surfaces. Klibanov, A.M., Lewis, K., Tiller, J., Liao, C-J. US 10/123,860 (Patent pending). Licensed to Pulmatrix, Inc.

C. Selected publications (of 90 total).

Most relevant to the current application.

Schumacher, M.A., Piro, K.M., Xu, W., Hansen, S., Lewis, K., and Brennan, R.G. 2009. Molecular mechanisms of HipA mediated multidrug tolerance and its neutralization by HipB. Science 323:396-401.

Dörr, T., Vulić, M., and Lewis, K., 2010. Ciprofloxacin causes persister formation by inducing the TisB toxin in *Escherichia coli*. PLoS Biol 8(2): e1000317.

Mulcahy, L.R., Burns, J.L., Lory, S., and Lewis, K. 2010. Emergence of *Pseudomonas aeruginosa* strains producing high levels of persister cells in patients with cystic fibrosis. J. Bacteriol. 192: 6191–6199.

Keren, I., Wu, Y., Innocencio, J., Mulcahy, L., and Lewis, K. 2013. Killing by bactericidal antibiotics does not depend on reactive oxygen species. Science 339: 1213-1216.

Conlon, B.P., Nakayasu, E.S., Fleck, L.E., LaFleur, M.D., Isabella, V.M., Coleman, K., Leonard, S.N., Smith, R.D., Adkins, J.N. and Lewis, K. 2013. Protease activation kills persisters and eradicates a chronic biofilm infection. Nature 503: 365-370.

Additional recent publications of importance to the field.

Kaeberlein, T., Lewis*, K., and Epstein*, S.S. (*Equal contribution) 2002. Isolating "uncultivable" microorganisms in pure culture using a simulated natural environment. Science 296:1127-1129.

Keren, I., Shah, D., Spoering, A., Kaldalu, N., and Lewis, K. 2004. Specialized persister cells and the mechanism of multidrug tolerance in *Escherichia coli*. J. Bacteriol. 186:8172-8180.

Hansen, S., Lewis, K., and Vulic, M. 2008. Role of global regulators and nucleotide metabolism in antibiotic tolerance in *Escherichia coli*. Antimicrob. Agents Chemother. 52:2718-2726.

Correia ,F.F., D'Onofrio, A., Rejtar, T., Li, L., Karger, B.L., Makarova, K., Koonin, E.V., and Lewis, K. 2006. Kinase activity of overexpressed HipA is required for growth arrest and multidrug tolerance in *Escherichia coli*. J. Bacteriol. 188:8360-8367.

Moy, T.I., Ball, A., Anklesaria, Z., Casadei, G., Lewis, K., Ausubel, F.M. 2006. Identification of novel antimicrobials using a live-animal infection model. Proc. Natl. Acad. Sci. USA. 103:10414-10419.

D'Onofrio, A., Crawford, J.M., Stewart, E.J., Witt, K., Gavrish, E., Epstein, S., Clardy, J., and Lewis, K. 2010. Siderophores from neighboring organisms promote the growth of uncultured bacteria. Chem. & Biol. 17: 254–264.

Lafleur, M.D., Qi, Q., and Lewis, K. 2010. Patients with long-term oral carriage harbor high-persister mutants of *C. albicans*. Antimicrob. Agents Chemother. 54:39-44.

Keren, I., Minami, S., Rubin, E., and Lewis, K. 2011. Characterization and transcriptome analysis of *Mycobacterium tuberculosis* persisters. mBio 2: e00100-11.

Wu, Y., Vulić, M., Keren, I., and Lewis, K. 2012. Role of oxidative stress in persister tolerance. Antimicrob. Agents Chemother. 56:4922-4026.

LaFleur, M., Sun, L., Lister, I., Keating, J., Nantel, A., Long, L., Ghannoum, M., North, J., Lee, R., Coleman, K., Dahl, T., and Lewis, K. (2013) Potentiation of azole antifungals by 2-adamantanamine. Antimicrob. Agents Chemother. 57:3585-3592.

Lewis, K. (2013) Platforms for antibiotic discovery. Nat. Rev. Drug Disc. 12: 371–387.

D. Current Research Support/projects completed in the last 3 years:

Current:

Transformative (T-RO1) 1R01AI085585-01 (K. Lewis, P.I.) NIH 09/25/2009 – 08/31/2014
Super-persistent cells and the paradox of untreatable infections
The aim of the project is to test whether bacteria form super-persistent cells in vivo responsible for recalcitrance of disease.

Program Director/Principal Investigator (Last, First, Middle): Lewis, Kim

1 R01 AI076372-01A2 (K. Lewis, P.I.) NIH 2/2/2009- 2/1/2014
A synergy-based therapy against *C. difficile*
The aim of the project is to develop a therapeutic against *C. difficile* based on a combination antimicrobial.

Completed:

1R01AI085005-01A1 (K. Lewis, P.I.) NIH 07/01/2010 - 06/31/2013
A High-Throughput screen for specific anti-*M. tuberculosis* compounds
The aim of the project is to develop a screen for identifying natural product antimicrobials acting against *M. tuberculosis*.

1R01HG005824-01 (K. Lewis, P.I.) NIH 07/01/2010 - 06/31/2013
Culturing uncultivatable gut microorganisms
The aim of the project is to develop a high-throughput method to culture gut microorganisms.

3R01 GM061162 -05A1 (K. Lewis, P.I.) NIH 4/1/2008- 3/31/2012
A genomics approach to drug tolerance
The aim of the project is to identify the complement of persister genes in *E. coli*.

3R01GM061162-10S1 (K. Lewis, P.I.) NIH 9/30/2009 - 8/31/2011
A genomics approach to drug tolerance (ARRA competitive revision)
The aim of the project is to identify the mechanism of action of the TisB persister protein.

K. Lewis, P.I.; E. Rubin, Harvard Medical School, Co-PI. Bill & Melinda Gates Foundation 11/01/2007 – 10/31/2010
Isolation and properties of *M. tuberculosis* persisters.

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE
Leonard, Steven Nathaniel	Assistant Professor of Pharmacy Practice
eRA COMMONS USER NAME (credential, e.g., agency login) [redacted]	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Purdue University	B.S.	05/03	Pharmacy
Purdue University	Pharm.D.	12/04	Pharmacy
St. Vincent Hospital	Residency	06/06	Pharmacy Practice
Wayne State University	Fellowship	07/08	Infectious Disease

A. Personal Statement

The goal of this project is to develop the antibacterial ADEP for the treatment of *S. aureus* infections. During the first phase in vitro testing using an in vitro pharmacokinetic/pharmacodynamic (PK/PD) hollow fiber infection model will be employed to find the best companion antibiotic for ADEP. It is during this phase that my expertise will be utilized. I have extensive experience using a variety of PK/PD models, including the hollow fiber model, to test a variety of antibiotics and combinations of antibiotics. This experience includes preliminary work on ADEP with Arietis and theLewis lab.

B. Positions and Honors

Positions and Employment

2006-2008 Fellow, Anti-infective Research Laboratory, Wayne State University, Detroit, MI
2008-Present Assistant Professor, Department of Pharmacy Practice, Northeastern University, Boston, MA
2008-Present Infectious Diseases Pharmacist, Brigham and Women's Hospital, Boston, MA

Professional Memberships

2006-Present American Society for Microbiology
2006-Present Society of Infectious Diseases Pharmacists
2007-Present American College of Clinical Pharmacy
2008-Present American Association of Colleges of Pharmacy
2011-Present European Society of Clinical Microbiology and Infectious Diseases

Honors

2010 Faculty Inductee, Rho Chi Honor Society, Beta Tau Chapter, Northeastern University
2011 Northeastern University School of Pharmacy Teacher of the Year

C. Selected Peer-reviewed Publications (Selected from 23 total)

1. Rose WE, **Leonard SN**, Sakoulas G, Kaatz GW, Zervos MM, Sheth AA, Carpenter CF, Rybak MJ. Daptomycin Activity against *Staphylococcus aureus* following Vancomycin Exposure in an In Vitro Pharmacodynamic Model with Simulated Endocardial Vegetations. *Antimicrob Agents Chemother* 2008;52(3):831-6. PMID: 17999971.
2. LaPlante KL, **Leonard SN**, Andes DR, Craig WA, Rybak MJ. Activities of clindamycin, daptomycin, doxycycline, linezolid, trimethoprim-sulfamethoxazole, and vancomycin against community-associated methicillin-resistant *Staphylococcus aureus* with inducible clindamycin resistance in murine thigh infection and in vitro pharmacodynamic models. *Antimicrob Agents Chemother* 2008;52(6):2156-62. PMID: 18411321.
3. **Leonard SN**, Cheung CM, Rybak MJ. Activities of ceftobiprole, linezolid, vancomycin, and daptomycin against community-associated and hospital-associated methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2008;52(8):2974-6. PMID: 18519721.
4. Rose WE, **Leonard SN**, Rybak MJ. Evaluation of daptomycin pharmacodynamics and resistance at various dosage regimens against *Staphylococcus aureus* isolates with reduced susceptibilities to daptomycin in an in vitro pharmacodynamic model with simulated endocardial vegetations. *Antimicrob Agents Chemother* 2008;52(9):3061-7. PMID: 18591272.
5. Rybak MJ, **Leonard SN**, Rossi KL, Cheung CM, Sadar HS, Jones RN. Characterization of vancomycin-heteroresistant *Staphylococcus aureus* from the metropolitan area of Detroit, Michigan, over a 22-year period (1986 to 2007). *J Clin Microbiol* 2008;46(9):2950-4. PMID: 18632899.
6. **Leonard SN**, Kaatz GW, Rucker LR, Rybak MJ. Synergy between gemifloxacin and trimethoprim/sulfamethoxazole against community-associated methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother* 2008;62(6):1305-10. PMID: 18801920.
7. **Leonard SN**, Rybak MJ. Evaluation of vancomycin and daptomycin against methicillin-resistant *Staphylococcus aureus* and heterogeneously vancomycin-intermediate *S. aureus* in an in vitro pharmacokinetic/pharmacodynamic model with simulated endocardial vegetations. *J Antimicrob Chemother* 2009;63(1):155-160. PMID: 18984644.
8. Rose WE, **Leonard SN**, Rossi KL, Kaatz GW, Rybak MJ. Impact of inoculum and heterogeneous vancomycin intermediate *Staphylococcus aureus* (hVISA) on vancomycin activity and emergence of VISA in an *in vitro* pharmacodynamic model. *Antimicrob Agents Chemother* 2009;53(2):805-7. PMID: 19015334.
9. **Leonard SN**, Rossi KL, Newton KL, Rybak MJ. Evaluation of the Etest GRD for the detection of *Staphylococcus aureus* with reduced susceptibility to glycopeptides. *J Antimicrob Chemother* 2009;63(3):489-92. PMID: 19136530.
10. **Leonard SN**, Vidaillac C, Rybak MJ. Activity of Telavancin Against *Staphylococcus aureus* of Varying Vancomycin Susceptibilities in an In Vitro Pharmacokinetic/Pharmacodynamic Model with Simulated Endocardial Vegetations. *Antimicrob Agents Chemother* 2009;53(7):2928-2933. PMID: 19414568.
11. Vidaillac C, **Leonard SN,** Rybak MJ. In Vitro Activity of Ceftaroline Against Methicillin-Resistant *Staphylococcus aureus* and heterogeneous Vancomycin-Intermediate *S. aureus* Using a Hollow Fiber Model. *Antimicrob Agents Chemother* 2009;53(11):4712-7. PMID: 19738009.
12. **Leonard SN**, Szeto YG, Zolotarev M, Grigoryan IV. Comparative In Vitro Activity of Telavancin, Vancomycin, and Linezolid against Heterogeneously Vancomycin Intermediate *Staphylococcus aureus*. *Int J Antimicrob Agents* 2011;37(6):558-561. PMID: 21497067.
13. **Leonard SN**. Synergy between Vancomycin and Nafcillin against *Staphylococcus aureus* in an In Vitro Pharmacokinetic/Pharmacodynamic Model. *PLoS One* 2012;7(7):e42103. PMID: 22848719.
14. **Leonard SN**, Rolek KM. Evaluation of the Combination of Daptomycin and Nafcillin against Vancomycin-Intermediate *Staphylococcus aureus*. *J Antimicrob Chemother* 2013;68(3):644-7. PMID: 23152482.
15. **Leonard SN**, Supple ME, Gandhi RG, Patel MD. Comparative Activity of Telavancin Combined with Nafcillin, Imipenem, and Gentamicin against *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2013;57(6):2678-83. PMID: 23545527.

D. Research Support

Completed Research Support

Astellas Pharma and Theravance Inc. 8/2011-8/2012
Comparative Activity of Telavancin Combined with Nafcillin, Imipenem, and Gentamicin against *Staphylococcus aureus*
Role: PI

Cubist Pharmaceuticals 8/2011-6/2012
Daptomycin combined with nafcillin against vancomycin intermediate *Staphylococcus aureus*.
Role: PI

Astellas Pharma 4/2010-12/2010
Evaluation of Telavancin, Vancomycin, and Linezolid against Heterogeneously Glycopeptide Intermediate *Staphylococcus aureus* (hGISA).
Role: PI

Northeastern University Proposal Development Grant 6/2009-5/2010
Evaluation of the Combination of Nafcillin and Vancomycin Against Methicillin Resistant *Staphylococcus aureus* with Reduced Susceptibility to Glycopeptides.
Role: PI

OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Arietis Corporation

Delete Entry *** Start Date:** 07/01/2014 *** End Date:** 06/30/2015 **Budget Period** 1

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Kenneth		Coleman		PD/PI	[redacted]	1.20			[redacted]	[redacted]	[redacted]
2.													
3.													
4.													
5.													
6.													
7.													
8.													
9.	**Total Funds requested for all Senior Key Persons in the attached file**												
											Total Senior/Key Person		[redacted]

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

	* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
		Post Doctoral Associates						
		Graduate Students						
		Undergraduate Students						
		Secretarial/Clerical						
	1	Senior Scientist	3.00			[redacted]	[redacted]	[redacted]
	1	Research Associate	12.00			[redacted]	[redacted]	[redacted]
	1	Research Assistant	7.80			[redacted]	[redacted]	[redacted]
	3	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]

Total Salary, Wages and Fringe Benefits (A+B) [redacted]

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Arietis Corporation

Delete Entry *** Start Date:** 07/01/2014 *** End Date:** 06/30/2015 **Budget Period 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
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11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

D. Travel **Funds Requested ($)**

1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2.	Foreign Travel Costs	
	Total Travel Cost	

E. Participant/Trainee Support Costs **Funds Requested ($)**

1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 1

Next Period

* ORGANIZATIONAL DUNS: [redacted]

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Arietis Corporation

Delete Entry * Start Date: 07/01/2014 * End Date: 06/30/2015 Budget Period 1

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Hollow fiber	[redacted]
9.	
10.	
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	F&A	40.00	[redacted]	[redacted]
2.				
3.				
4.				
	Total Indirect Costs			[redacted]

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)
	[redacted]

K. * Budget Justification 1252-Budget.pdf Add Attachment Delete Attachment View Attachment

(Only attach one file.)

OMB Number: 4040-0001
Expiration Date: 06/30/2011

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Arietis Corporation

Delete Entry *** Start Date:** 07/01/2015 *** End Date:** 06/30/2016 **Budget Period** 2

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Kenneth		Coleman		PD/PI	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]
2.													
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8.													
9.	**Total Funds requested for all Senior Key Persons in the attached file**												
											Total Senior/Key Person		[redacted]

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
3	Senior Scientists	36.00			[redacted]	[redacted]	[redacted]
3	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]

Total Salary, Wages and Fringe Benefits (A+B) [redacted]

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Arietis Corporation

Delete Entry *** Start Date:** 07/01/2015 *** End Date:** 06/30/2016 **Budget Period 2**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
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11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel **Funds Requested ($)**

1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	[redacted]
2.	Foreign Travel Costs	
	Total Travel Cost	[redacted]

E. Participant/Trainee Support Costs **Funds Requested ($)**

1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 2

Next Period

* ORGANIZATIONAL DUNS: [redacted]

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Arietis Corporation

Delete Entry * Start Date: 07/01/2015 * End Date: 06/30/2016 Budget Period 2

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	[redacted]
2. Publication Costs	[redacted]
3. Consultant Services	[redacted]
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal costs	[redacted]
9. Compound resupply	[redacted]
10.	
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	F&A	40.00	[redacted]	[redacted]
2.				
3.				
4.				
	Total Indirect Costs			[redacted]

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)
	[redacted]

K. * Budget Justification 1252-Budget.pdf Add Attachment Delete Attachment View Attachment

(Only attach one file.)

OMB Number: 4040-0001
Expiration Date: 06/30/2011

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Arietis Corporation

Delete Entry *** Start Date:** 07/01/2016 *** End Date:** 06/30/2017 **Budget Period** 3

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Kenneth		Coleman		PD/PI	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]
2.													
3.													
4.													
5.													
6.													
7.													
8.													
9.	**Total Funds requested for all Senior Key Persons in the attached file**												
											Total Senior/Key Person		[redacted]

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
3	Senior Scientists	36.00			[redacted]	[redacted]	[redacted]
3	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]

Total Salary, Wages and Fringe Benefits (A+B) [redacted]

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Arietis Corporation

Delete Entry *** Start Date:** 07/01/2016 *** End Date:** 06/30/2017 **Budget Period 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
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11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel **Funds Requested ($)**

1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	[redacted]
2.	Foreign Travel Costs	
	Total Travel Cost	[redacted]

E. Participant/Trainee Support Costs **Funds Requested ($)**

1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 3

Next Period

* ORGANIZATIONAL DUNS: [redacted]

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Arietis Corporation

Delete Entry * Start Date: 07/01/2016 * End Date: 06/30/2017 Budget Period 3

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	[redacted]
2. Publication Costs	[redacted]
3. Consultant Services	[redacted]
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. animal supplies	[redacted]
9. compound resupply	[redacted]
10.	
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	F&A	40.00	[redacted]	[redacted]
2.				
3.				
4.				
	Total Indirect Costs			[redacted]

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)
	[redacted]

K. * Budget Justification 1252-Budget.pdf Add Attachment Delete Attachment View Attachment

(Only attach one file.)

OMB Number: 4040-0001
Expiration Date: 06/30/2011

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Arietis Corporation

Delete Entry *** Start Date:** 07/01/2017 *** End Date:** 06/30/2018 **Budget Period** 4

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Kenneth		Coleman		PD/PI	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]
2.													
3.													
4.													
5.													
6.													
7.													
8.													

9. **Total Funds requested for all Senior Key Persons in the attached file**

Total Senior/Key Person [redacted]

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	Senior Scientist	24.00			[redacted]	[redacted]	[redacted]
2	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]

Total Salary, Wages and Fringe Benefits (A+B) [redacted]

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Arietis Corporation

Delete Entry *** Start Date:** 07/01/2017 *** End Date:** 06/30/2018 **Budget Period 4**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel **Funds Requested ($)**

1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	[redacted]
2.	Foreign Travel Costs	
	Total Travel Cost	[redacted]

E. Participant/Trainee Support Costs **Funds Requested ($)**

1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 4

Next Period

* ORGANIZATIONAL DUNS: [redacted]

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: Arietis Corporation

Delete Entry * Start Date: 07/01/2017 * End Date: 06/30/2018 Budget Period 4

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	[redacted]
2. Publication Costs	[redacted]
3. Consultant Services	[redacted]
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal studies	[redacted]
9. Outsourced preclinical studies	[redacted]
10.	
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	F&A	40.00	[redacted]	[redacted]
2.				
3.				
4.				
	Total Indirect Costs			[redacted]

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)
	[redacted]

K. * Budget Justification 1252-Budget.pdf Add Attachment Delete Attachment View Attachment

(Only attach one file.)

Budget Justification

The project will include extensive *in vitro* and *in vivo* validation of ADEP4 in combination with a number of other antibiotics. A team of 6 Arietis scientists and two consultants will support the project.

Dr. Ken Coleman, PI, Arietis, (10% Y1, 20% Y2-4), is an expert in antimicrobial drug discovery and development with 35 years of experience in the Industry. He will be responsible for the overall supervision and administration of the project, coordinating interactions with the consultants, the CRO's and members of the team. He will be responsible for research summary reports, and will write the patent filings and papers that will result from this project.

Dr. Katya Gavrish, Senior Scientist, (25% Y1, 100% Y2-4), is a specialist in drug discovery and drug resistance. Dr. Gavrish contributed to generating the preliminary data that was included in the application. Dr. Gavrish will supervise the Research Associates on a day-to-day basis. She will assist as needed with individual experiments and data analysis. Dr. Gavrish will also assist Dr. Coleman in writing research reports, patent filings and papers that will results from this project.

Dr. Ida Lister, Senior Scientist, (100% Y2-4), is a microbiologist and biochemist. She specializes in mechanisms of bacterial drug resistance and will be responsible for performing the PK and efficacy studies. Dr. Lister received her PhD from Cambridge University, UK and did postdoctoral research fellowships at Harvard and Tufts University.

To be hired, Senior Scientist, (100% Y2-3), *an experienced PhD level pharmacologist with project management experience will be hired to support the project. This scientist will be primarily responsible for in vivo PK/PD data analysis and modelling. This scientist will also work with the business development team and PI at Arietis to integrate the data generated from the project into product development plans and target product profiles.*

Binu Shrestha, M.Sc. Research Associate, (100% Y1), will perform the hollow-fiber studies in year 1. Binu has a Master's Degree in Molecular Biology from Tufts University and she has worked with Dr. Coleman and Dr. Gavrish on the project since its inception.

David Charnuska, B.S., Research Associate, (65% Y1), will perform the in vitro susceptibility and biofilm assays in year 1. David will also support the hollow-fiber experiments.

Materials and Supplies
Funds of ~$20,000 for each full time equivalent each year are requested to the purchase of growth media, reagents, disposable plastics, and small equipment items such as automatic pipettes to support the work of the Arietis team.

Additional funds of $20,000 are requested in year 1, to support the hollow fiber studies.

Additional funds of $107,000 over years 2 and 3 is requested for synthesis of ADEP4 (100 grams), which will be sufficient to carry out the proposed studies. Synthesis will be performed [redacted] (or a comparable CRO), who has already optimized a scalable synthetic route and produced the ADEP4 that was used in preliminary studies (see quote).

Animal Studies
$44,000 per year (Y2-4) is requested for animal studies to be performed by Arietis at Boston University Medical Center. These costs include animal procurement, housing fees, and supplies associated with PK and efficacy studies. Additional costs for analytical blood quantification and histopathology to confirm efficacy results are included.

Additional costs of [redacted] are requested in year 4 to support the preclinical studies to be performed at [redacted]

Consultants:

Dr. Steven Leonard ([redacted], 40 hr/year, Y2-4), is a Clinical Pharmacist of Infectious Diseases at Brigham and Women's Hospital and an Assistant Professor of Pharmacy at Northeastern University. Dr. Leonard will advise us in all areas of research, especially hollow-fiber resistance studies, and in vivo PK, PD and efficacy.

Dr. Kim Lewis ([redacted], 40 hr/year, Y2-4), is a University Distinguished Professor and the Director of the Antimicrobial Discovery Center at Northeastern University. Dr. Lewis is an expert in drug discovery and antimicrobial tolerance. Dr. Lewis will advise us in all areas of research throughout this project, especially guiding the *in vitro* and *in vivo* biofilm studies.

Indirect Costs
Arietis is requesting F&A costs at 40% and fringe benefits at 35%.

Travel
$5000 per year (Y2-4) is requested to support travel of the Arietis team to present work at research conferences such as the ASM International Conference on Antimicrobial Agents and Chemotherapy.

Publication
$1000 per year (Y2-4) is requested for publication costs for journals such as Antimicrobial Agents and Chemotherapy.

Fee
Arietis request a fee of 7%.

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		[redacted]
Section B, Other Personnel		[redacted]
Total Number Other Personnel	11	
Total Salary, Wages and Fringe Benefits (A+B)		[redacted]
Section C, Equipment		
Section D, Travel		[redacted]
1. Domestic	[redacted]	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		[redacted]
1. Materials and Supplies	[redacted]	
2. Publication Costs	[redacted]	
3. Consultant Services	[redacted]	
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	[redacted]	
9. Other 2	[redacted]	
10. Other 3		
Section G, Direct Costs (A thru F)		[redacted]
Section H, Indirect Costs		[redacted]
Section I, Total Direct and Indirect Costs (G + H)		[redacted]
Section J, Fee		[redacted]

SBIR/STTR Information

OMB Number: 4040-0001
Expiration Date: 6/30/2016

*** 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)*

*** SBIR/STTR Type (select only one)**

☐ Phase I ☐ Phase II

☒ Fast-Track *(See agency-specific instructions to determine whether a particular agency participates in Fast-Track)*

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

☒ Yes ☐ No	* 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?
	* 1b. Anticipated Number of personnel to be employed at your organization at the time of award. 9
☐ Yes ☒ No	* 2. Does this application include subcontracts with Federal laboratories or any other Federal Government agencies? * If yes, insert the names of the Federal laboratories/agencies:
☐ Yes ☒ No	* 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? If no, provide an explanation in an attached file. * Explanation: Add Attachment / Delete Attachment / View Attachment
☐ Yes ☒ No	* 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? * If yes, insert the names of the other Federal agencies:
☒ Yes ☐ No	* 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 e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?
	* 7. Commercialization Plan: If you are submitting a Phase II or Phase I/Phase II Fast-Track Application, include a Commercialization Plan in accordance with the agency announcement and/or agency-specific instructions. * Attach File: 1250-CommercializationPlan.pdf Add Attachment / Delete Attachment / View Attachment

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.

☒ Yes ☐ No	* 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. * Attach File: 1251-Company Commercialization [Add Attachment] [Delete Attachment] [View Attachment]
☒ Yes ☐ No	* 9. Will the Project Director/Principal Investigator have his/her primary employment with the small business at the time of award?

STTR-Specific Questions:

Questions 10 and 11 apply only to STTR applications. If you are submitting ONLY an SBIR application, leave questions 10 and 11 blank.

☐ Yes ☐ No	* 10. Please indicate whether the answer to BOTH of the following questions is TRUE: (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?
☐ Yes ☐ No	* 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?

Commercialization Plan

Value of the SBIR Project, Expected Outcomes, and Impact.

The goal of this SBIR Fast-Track project is to develop a combination therapeutic consisting of ADEP4 and an approved antibiotic. This combination is for the treatment of refractory and recurrent *S. aureus* infections, including Methicillin Resistant *S. aureus* (MRSA). The FDA had published extensive Guidance for Industry on Developing Drugs for Treatment for Acute Bacterial Skin and Skin Structure Infections and we will use these guidelines throughout our development program. An important characteristic of our therapeutic is the ability to act against biofilms, stationary phase, persister cells and complicated forms of infection. The therapeutic should result in eradication rather than mere suppression of infection, which is critical for clearance of biofilm infected medical devices and in immunocompromised patients. This project constitutes a Phase 1 feasibility study, which will enable us to determine whether the resistance rate of the combined therapeutic is low enough to warrant further investigation. In Phase 2, the combination will be tested in animal models of MRSA infection, which are largely predictive of outcomes in humans. We have purposefully chosen models in which standard front-line therapies fail, allowing our therapeutic combination to demonstrate clear superiority. Phase 2 constitutes a series of standard preclinical development studies, which will enable a pre-IND meeting with the FDA upon this project's completion.

Staphyloccoccus aureus, was discovered in the 1880s and skin and soft tissue conditions such as boils were subsequently associated with the bacteria. *S. aureus* was also known to progress to bacterial pneumonia and septicemia, which were typically fatal prior to the age of antibiotics. Since the 1940's, antibiotic treatment of *S. aureus* infection was routine, however resistance to resistance to penicillin developed almost immediately. Similarly, the first case of methicillin resistance in a patient was documented in 1961 and the first case reported in the US was in 1968. MRSA refers to resistant to the entire class of beta-lactams, which includes the penicillins and cephalosporins. *S. aureus* continues to evolve resistance at a rapid pace. Vancomycin resistance has been documented since 2002 and it is not a matter of if resistance will develop, but when.

Thus, *S. aureus* has evolved from a controllable nuisance into a major problem, within a generation. *S. aureus* is ubiquitous - most people carry the bacteria on their skin and it does not typically cause an active infection. This is even true for carriers of MRSA. MRSA is often categorized according to where the infection was acquired: hospital-acquired MRSA (HA-MRSA) or community-associated MRSA (CA-MRSA). HA-MRSA has increased during the past decade and it is typically among the most frequent of all hospital acquired infections. CA-MRSA is caused by newly emerging strains and can infect healthy people with no warning. CA-MRSA may begin as skin or soft tissue infections, but can progress into a life-threatening infection. CA-MRSA is also occurring with increasing frequency and people who are in close physical contact, such as athletes and soldiers, are particularly susceptible. USA300 is a common strain of CA-MRSA that is responsible for rapidly progressive, fatal diseases including necrotizing pneumonia, severe sepsis and necrotizing fasciitis.

S. aureus is responsible for ~1,200,000 hospital infections each year and there are an estimated 94,000 life-threatening infections from MRSA. These represent 20% of all hospital infections. According to the Journal of the American Medical Association, MRSA is responsible for an estimated 18,650 deaths in the US. The CDC estimated 10,800 deaths occurred in the U.S. in 2005 and over half of these were linked to MRSA. The rate of MRSA infections recorded at U.S. academic hospitals doubled between 2003 and 2008 (David et al., 2012). Community acquired MRSA is estimated to have a $1.4-13.8 billion on society, while the US jail system and Army may be experiencing annual total costs of $7-11 million ($6-10 million direct medical costs) and $15-36 million ($14-32 million direct costs), respectively (Lee *et al.*, 2013). Surprisingly, for every death from MRSA, it is estimated we spend only $570 on research. This is a small amount compared to spending for AIDS, for which $69,000 is spent for every AIDS death, even though MRSA deaths far outnumber those from AIDS.

S. aureus biofilm infections such as endocarditis, osteomyelitis or those on implanted medical devices, are essential untreatable. For example, according to the Infectious Disease Society of America, a minimum 8-week course is recommended, although some experts suggest an additional 1-3 months and possibly longer for chronic infection or if debridement is not performed (Liu *et al.*, 2011). We found that a stationary population of *S. aureus* is completely refractory to killing by traditional antibiotics. This fact widely appreciated by researchers or clinicians. The refractory nature of stationary cells can be explained in the terms of target

availability - many of the cellular targets of traditional antibiotics are not active in stationary phase. Indeed, bactericidal antibiotics require an active target to be corrupted in order to produce killing. Thus, aminoglycosides cause mistranslation, which creates toxic misfolded proteins; fluoroquinolones convert DNA gyrase/topoisomerase into an endonuclease which cleaves DNA; and β-lactams activate autolysins that destroy the cell wall.

We reasoned that cells exhibiting drug tolerance could be killed by an antibiotic which activates and corrupts a cellular target. Remarkably, the antimicrobial acyldepsipeptide (ADEP), functions by precisely this mechanism. ADEP causes dysregulation of the ClpP protease, relieving it from regulatory control and the requirement to use ATP. ADEP activates proteolysis in stationary phase, causing even dormant cells to self-digest. Interestingly, proteolysis is responsible for the shutdown of many of the other cellular targets of antibiotics during dormancy and stationary phase. If these targets are not properly shut down, traditional antibiotics can be effective. This is a major conceptual advance that will allow us to treat incurable infection for the first time.

In support of our rational, we found that the dysregulation of proteolysis by ADEP caused exceptional killing of biofilms and dormant cells. ADEP also caused increased susceptibility to killing by other antibiotics, since the traditional antibacterial targets were not properly inactivated by proteolysis. Combining ADEP4 with antibiotics rifampicin or linezolid produced complete sterilization of stationary and biofilm *S. aureus* cultures. Importantly, ADEP4 in combination with rifampicin sterilized MRSA in a mouse model of deep-seated infection, where conventional antibiotics had very little effect.

ADEP does have a liability – ClpP is not essential, and a high-frequency of null mutations leads to resistance. However, knowing the potential of ADEP to target dormant cells and render cells to be more sensitive to killing by antibiotics, it makes sense to combine it with conventional antibiotics, which will also solve the resistance problem. In Phase I of this project, we will identify the best ADEP partnering antibiotic and we will evaluate the combinations for the potential to develop resistance in the hollow-fiber model. The frequency of resistance development will be the go-no-go decision point for this project. We will not enter into Phase 2 until we conclusively demonstrate that resistance development for the combined therapeutic will not be a concern. Typically, a resistance rate higher than 10^{-8} is considered too high to enter development for an antimicrobial. The approach of combining antibiotics to overcome resistance is not new. In *S. aureus*, spontaneous resistance mutants arise at a frequency of around 10^{-8} for selections with rifampicin or fusidic acids, but are undetectable (frequency $<10^{-11}$) for a combined treatment. Because an infection may contain more than 10^{8} cells, rifampicin is not used alone for the treatment of *S. aureus*, but only in combination with another agent. Since ADEP4 has a unique target and mechanism of action, and an amazing potential to target drug tolerant cells, it makes sense to combine it with another antimicrobial. In this project we will test combinations of ADEP4 and other antibiotics for activity against stationary phase, biofilms and difficult to treat infection models, to determine the best partnering antibiotic.

Because resistance to MRSA is a major problem, it continues to be the focus of new product launches, underscoring both an unmet medical need and a clear commercial potential. In particular, there are two lipoglycopeptides under development, dalbavancin and oritavancin, and one oxazolidinone, tedizolid. Based on their mechanisms, it is unlikely these drugs will have activity against biofilms or stationary cells, or would be effective in a model of deep-seated infection. However, these agents could make suitable partners for ADEP4, once they are available. Clearly the continued activity in this arena, and the commercial success of variants of existing antibiotics, highlights a market hungry for meaningful improvements over currently available drug products. Recurrence is most likely due to the survival of drug-tolerant persister cells that repopulate the infection once the drug concentration drops. The ability of our combination therapeutic to kill persisters, is likely to reduce recurrence and satisfy this unmet medical need.

All of the considerations outlined above also apply not only to skin and soft tissue infections, but to other infections caused by MRSA, such as osteomyelitis, endocarditis, and biofilm infected joints and catheters. The current project is expected to result in multiple new drug products such as topical agent to eradicate nasal carriage, creams or ointments for topical use, combinations with systemic agents for systemic use, catheter lock solutions and medical device coatings. A product for treatment of skin and soft tissue infections is the primary objective of this development plan. Other commercial product paths are envisioned as future market expansions.

Key technology objectives.

The ultimate goal of the project will be preparation of a development candidate suitable for entry into focused preclinical drug development with input from the FDA in order to support Investigational New Drug (IND)-enabling studies. A summary of key technology objectives relating to the milestones follows:

Aim 1. Identify the best sterilizing combination of ADEP4 and an approved antibiotic. ADEP4 will be paired with known antibiotics such as daptomycin, linezolid, ciprofloxacin, gentamicin, and oxacillin, and examined for its ability to sterilize stationary and biofilm populations of S. aureus. ADEP/antibiotic combinations will also be tested for maximum killing and low resistance development using a hollow-fiber model of in vitro infection. Milestone: Advance 2 ADEP4/antibiotic combinations to in vivo studies. Combinations which effectively sterilize biofilms and stationary populations of *S. aureus*, and have a rate of resistance development $<10^{-9}$ in the hollow fiber model will be advanced.

Aim 2. *In vivo* testing of sterilizing combinations. PK parameters and initial efficacy of two ADEP4/antibiotic combinations will be performed in mice. The PK/PD driver of ADEP efficacy will be determined using the mouse model of deep-seated MRSA infection. Efficacy of each combination will be tested in an implanted tissue cage biofilm model. Milestone: Determine the PK of the combined agents, the pharmacodynamic driver of efficacy and treat a previously incurable model of *in vivo* biofilm MRSA infection.

Aim 3. Preclinical development. Acute, dose-range finding, and 14 day repeated dose toxicity studies will be performed in rats. PK studies in rats [redacted] will provide the basis for selecting doses to be tested in humans. Ames and chromosomal aberration genotoxicity studies will also be performed.

Taken together, these studies will form the basis for a risk-benefit assessment prior to the pre-IND meeting with the FDA and entry into human clinical trials. We will work with the FDA to define the remaining preclinical steps if not already covered.

- In house studies in the mouse model to determine the PK/PD driver and magnitude of each drug, will help with human dose prediction.
- Preliminary toxicology and local irritation assessments in preparation for selection of the IND combination and dose selection to proceed into IND-enabling studies.
- Prior to the final design of the definitive GLP toxicology and safety program, a Medical Advisory Board will be convened to aid in the design of the clinical program to ensure that the preclinical program submitted with the IND properly supports the intended development path.
- Dose range-finding studies along with metabolite/CYP profiling and plasma protein-biding assays will be performed in house.
- GLP and preclinical studies, which will be outsourced to [redacted] will include genotoxicity, bioanalytical method development and validation in 2 species, definitive Toxicology studies in 2 species, and single-dose PK/ADME studies in 2 species.
- Safety pharmacology studies, to include CNS and respiratory in the rat and [redacted], will also be outsourced [redacted]
- Formulation development and validation, GMP production, stability of both drug substance and drug product will be outsourced to [redacted] specialists in GMP production of clinical trial materials.
- Oversight of all outsourced GLP and GMP work will be provided by Ken Coleman who has 35 years of experience designing development programs and providing these capabilities in both large pharmaceutical and small biotech companies.

Success in these milestones will enable Arietis to enter Phase III of the project, focused drug development, ultimately leading to commercial products – a novel skin and soft tissue treatment capable of overcoming complicated MRSA infection. The ultimate product profile will be determined by data obtained during research and development efforts, in conjunction with the clinical input of a Medical Advisory Board (MAB) composed of key opinion leaders in the field of infectious disease. Specific clinical input will be gathered on the best ways to use the drug combination based on its inherent pharmacological characteristics, and how best it can be used as an addition to the existing armamentarium.

The FDA has provided an excellent guidance to inform our development path in its "Guidance for Industry on Developing Drugs for Treatment for Acute Bacterial Skin and Skin Structure Infections" and "Guidance for Industry and FDA Staff: Early Development Considerations for Innovative Combination Products". *The FDA has also recently released "Draft Guidance for Industry on Antibacterial Therapies for Patients With Unmet Medical Need for the Treatment of Serious Bacterial Diseases." The guidance presents streamlined approaches to evaluating new antibacterials for patients with unmet medical needs, and for pathogen-focused antibacterials*. *We expect our combination therapeutic to fall under Tier C, which could significantly streamline both the cost of clinical trials and the time to market. Pathogen-focused MRSA treatments addressing unmet medical need would fall in between, in tiers B and C. Approval would rely heavily on human pharmacokinetic (PK) data combined with preclinical data in animals. Tier B drugs would include broad-spectrum antimicrobials that also act against a multidrug-resistant pathogen, such as multidrug-resistant Enterobacteriaceae. Tier C would include antimicrobials with very narrow multidrug-resistant pathogen coverage, such as an antibiotic that is effective only against Acinetobacter or one that targets only Pseudomonas aeruginosa. Deciding whether a drug should be assigned to tier B versus tier C would depend on the feasibility of studies, the degree of unmet medical need, and the strength of preclinical data, based on conversations with the FDA.* Coupled with the clinical guidance of the MAB this input will be used in outlining our clinical program design, which will in turn guide the detailed definition of the preclinical program needed to support it. Arietis management has extensive experience in establishing and coordinating the input from MABs, Regulatory Affairs, and designing clinical and preclinical programs, as well as clinical study design, implementation, and reporting.

In addition to our in house capabilities, several contract research organizations (CROs) are available with the expertise to conduct preclinical studies compliant with current good laboratory practices (GLPs) for inclusion in an IND. Arietis management has extensive experience in choosing and managing high quality CROs for this purpose. Successful completion of Phase II is therefore an integral part of our research and development plan, aimed at commercializing a new, more effective MRSA therapy.

Scientific benefits and the non-commercial impacts of the project. In the course of Phase I and transition to Phase II work, Arietis has identified scientific questions relating to the benefit of an antimicrobial, which targets drug tolerance and functions through an activation mechanism. Conceptually, one can image numerous added benefits conferred by eradicating an infection as opposed to suppressing it. The clinical benefits of eradicating an infection may especially pertain to deep-seated and complicated infections which are refractory to current treatments. Now, for the first time we have the tools to answer some fundamental questions: For example, will targeting drug tolerance cell populations lead to shorter durations of therapy and less frequent emergence of resistance? Is recurrent infection caused by pathogen re-colonization or drug tolerance? Indeed, we are excited about the possibility of uncovering a link between difficult to treat infection, drug tolerance, classical resistance and biofilms. New findings in this area may help shift the paradigm of how we think about drug tolerance and drug resistance.

Company, Production and Marketing Plan, Finance Plan, and Revenue Stream

Arietis Corporation was founded by Dr. Kim Lewis to commercialize his method for eliminating persisters, and thereby identify or create agents that sterilize, rather than simply suppress, infections. In May, 2008 the Company was awarded an SBIR Phase I grant, which was successful in establishing compound library screens and identifying validated hits *in vitro* for further evaluation *in vivo* as potential drug development candidates. In November, 2008 the company moved into its present location at 650 Albany Street in Boston, Massachusetts, a mecca of innovation in the biotech and pharma industries. The Company currently occupies 1400 sq. ft. of laboratory and office space, with additional access to animal facilities at Boston University School of Medicine. The laboratory space is allocated to biology. The current space will be sufficient for us to

perform the planned work, supplemented as necessary by collaborations with outside labs for the conduct of some specialized animal studies and synthetic chemistry scale up.

Dr. Kenneth Coleman serves as PI. Dr. Coleman has a long history of successful antimicrobial drug discovery and development, both in major global pharma companies and small start-up environments. Daily scientific activities are overseen by Michael LaFleur, Chief Operating Officer, one of the inventors of the company's core technologies. His team includes both PhD-level scientists and a veterinarian. *We anticipate hiring one additional employee, to serve as senior scientist and project manager, analyzing and model PK/PD data generated during the project.* Thus a team of 6 people will be working on the project, along with extramural expertise in the areas of pharmacology, chemistry and pre-clinical animal studies. Dr. Coleman will also coordinate the input of Drs. Leonard, Lewis and LaFleur as well as members of the SAB. Dr. Coleman will manage outsourcing of work conducted off-site under this program.

The core competence of our team is in anti-infective drug discovery and preclinical and clinical drug development, with emphasis on antiinfectives and especially activity against persister cells. Our expertise is supplemented by a Scientific Advisory Board made up of outstanding scientists: [redacted] In addition to these key experts, the Company benefits from the advice of its founder, Dr. Kim Lewis, Professor of Biology at Northeastern University.

As we move on to Phase III and beyond, the company will undergo substantial expansion as required to transition from a research to a drug development focus. It should be noted that creation of drug development capabilities is something that Coleman has accomplished for many of his previous employers.

The Team

● Dr. Kenneth Coleman, PI, has a number of successful drugs in his portfolio, including clavulanic acid and gemifloxacin. Dr. Coleman has more than 35 years of experience in antimicrobial drug discovery and development in both large pharma and small biotech companies.

● Dr. Michael LaFleur, Chief Operating Officer, was responsible for coordinating the work described in Preliminary Studies. He serves as the project manager and grant administrator.

● Dr. Kim Lewis, Consultant, is Director, Antimicrobial Discovery Center of NEU, and Founder of Arietis. Dr. Lewis is an expert in antimicrobial drug resistance, drug tolerance, and anti-infective drug discovery.

● Dr. Steven Leonard, Consultant, is a Clinical Pharmacist of Infectious Diseases at Brigham and Women's Hospital and an Assistant Professor of Pharmacy at Northeastern University. Dr. Leonard will contribute all areas of research, especially hollow-fiber resistance studies, and in vivo PK, PD and efficacy.

● Dr. Ida Lister, Senior Scientist is a microbiologist and biochemist. She specializes in mechanisms of bacterial drug resistance. Dr. Lister received her PhD from Cambridge University, UK and did postdoctoral research fellowships at Harvard and Tufts University.

● Dr. Ekaterina Gavrish, Senior Scientist, is a microbiologist who worked with Dr. Lewis for many years. She is a specialist in drug discovery and drug resistance.

● Ms. Binu Shrestha, is a veterinarian who also holds a Master's Degree in Molecular Biology from Tufts University.

Several outstanding scientists are members of the Scientific Advisory Board of Arietis. In addition to Drs. Lewis:

● [redacted]

[redacted]

- [redacted]
- [redacted].
- [redacted].

Funding history. The company has been supported by SBIR funds in the past.

NIH/NIAID 5R43AI098327-02 [redacted]
Compounds to treat Helicobacter pylori infection.
06/15/2012-05/31/2014.
Establish proof of concept for developing novel small molecules capable of eradicating *H. pylori* infection

NIH/NIAID 1R44AI102452-01 [redacted]
Narrow-spectrum Agents Acting against *Helicobacter pylori*
07/01/2012-06/30/2013
This successful Phase I project was continued in a Phase II grant

NIH/NIAID 4R44AI102452 - 02 [redacted]
Narrow-spectrum Agents Acting against *Helicobacter pylori*
07/01/2013-06/30/2016
Iterative medicinal chemistry optimization will be combined with detailed validation of each series in Phase II.

NIH/NIAID 1R43AI074258-01A1 [redacted]
Therapy against Recalcitrant *C. albicans* Infection
05/15/2008 – 05/14/2010.
This successful Phase I project was continued in a Phase II grant:

NIH/NIAID 5R44AI074258-04. [redacted]
Therapy against Recalcitrant *C. albicans* Infection
07/01/2010 – 12/30/2013
This project seeks to develop topical therapy for recurrent vulvovaginal candidiasis.

NIH/NIDCR 5R43DE020880-02 [redacted]
2-adamantanamine based therapeutic for recurrent oropharyngeal *Candidiasis*.
05/01/2010 – 04/30/2012. This project seeks to develop therapy for oral thrush.

Corporate vision – from small R&D business to a commercial entity.

Arietis was founded on a basic scientific breakthrough: a means to eliminate the persister cells that are responsible for recurrent and recalcitrant fungal infections. The Company has successfully leveraged this invention into a drug discovery engine, with tangible results warranting further exploration and development into safe and effective therapies made available to patients through commercialization.

As drug development proceeds through its lifecycle, development costs increase along with probabilities of success. The key to success in very early stage endeavors is to very carefully match funds available with strategically chosen experimentation that maximizes the chances of proceeding to the next stage of development.

Next steps in commercialization include identifying ways to defray or defer costs while simultaneously reducing program risk. This can best be done by visualizing what the end commercial product will look like and who will be using it (both the patient and prescriber). The result is a product profile that can be used to drive the roadmap of further development. We plan to take advantage of the NIH's Niche Assessment program during our Phase I progress, with the result that we have had access to Key Opinion Leaders and selected end user (prescriber) advisory input in defining our product profile, as well as initial contacts with targeted pharma companies expressing an interest in learning more about this project and our lead candidate as accumulate additional data through the conduct of this Phase II program.

Focusing on the primary (initial) commercial profile – in this case skin and soft tissue infections caused by MRSA -- we can identify the most likely regulatory requirements for marketing approval by the FDA, and design an appropriate and efficient clinical trial program strategy for each commercial application. For example, the most likely initial approval will be for second line treatment after failure with a current standard treatment, linezolid. With an emphasis on effecting a microbial cure in addition to a clinical cure and decreasing frequency of recurrence, this product also has the potential to move into a first line position in the future for immunocompromised patient populations most affected by issues of recurrence.

With a clinical trial program outlined we will then design an appropriate and efficient preclinical program to support entry into clinical trials. Outlining a preclinical program allows us to identify an efficient late research plan to position us for the timeliest and most cost-effective entry into focused drug development with the most worthwhile drug candidate or candidates.

Once entering focused development, the costs and risks are well understood from a historical perspective. At this time, during initial preclinical development, we anticipate having a sufficiently robust dataset and understanding of likely development pathways and regulatory requirements to begin entering into discussions with potential industry partners and investors, to further the goals of the company. While we will continue to be open to both types of investment, as well as continued research support through programs such as SBIR, it is most likely that we would seek an industry partner for our first and most advanced commercial product. The reasons for this are 2-fold: from an industry perspective, a proven program from a small R&D entity can compete favorably with unproven internal research programs, providing faster and more cost-effective ways to bring a product to market; and from Arietis' perspective, the existence of a mature, established marketing and distribution capability within the corporate partner provides the fastest and most efficient means to get safe and effective therapies into the hands of the physicians who will prescribe them and the patients whose lives will benefit from them. Through the NIH's Niche Assessment process we will become familiar with the potential partners most interested in licensing this product, and have initiated discussions anticipated to progress as more data become available through the studies supported by this application. *We have already entered into discussions with several large pharmaceutical companies based on our preliminary data and the Nature publication. There is a high level of interest however, based on our considerable experience with large Pharma and Biotech, it may be unrealistic to obtain a meaningful commitment from a partner at this early stage of development. We plan to partner after we have generated preclinical data in Phase II.*

The revenue stream to be generated through successful partnership and commercialization of this first product then make possible both the continued development of other therapies, and the establishment of additional capabilities such as manufacturing, marketing and distribution necessary to transform a research and development undertaking into a fully integrated pharmaceutical company, with a robust pipeline of drug candidates to treat an array of diseases across the anti-infective spectrum.

Initially and in the absence of a full integration of pharmaceutical company capabilities we will outsource required work, especially in order to comply with government regulations (e.g., concerning good manufacturing practices, or GMP, for the production of drug substance and drug product; good laboratory practices or GLPs for the studies to be included in the IND, and eventually good clinical practices, or GCPs, for the conduct of

clinical trials to support the marketing application). Work such as basic research, mechanism of action, resistance testing, etc., that is not subject to specific regulation we will perform in-house, supplemented as necessary or desirable with academic expertise (e.g., novel assays or animal models) through collaboration with leaders in the relevant areas.

Decisions on whether to seek additional private investment, additional partnerships, or access public funds via an IPO will be data-driven in the future, based on factors such as the number and stage of additional drug candidates, the costs and risks of carrying them forward, and any available revenue streams such as royalties or commercial sales. In general, it is our bias to hold both individual programs and company stock as long as possible in order to reduce risk and increase value, while still providing the resources necessary to advance drug candidates efficiently toward the marketplace.

Market, Customer, and Competition.

Market and Customer.

Despite a global market size of $42 billion for anti-infectives, there is a substantial unmet need for novel antimicrobial compounds (Hamad, 2010). Very few novel antimicrobials have reached market in the last 10 years. However, there are no sterilizing antimicrobials which are capable of eradicating, rather than suppressing, an infection. Reflecting the paucity in the discovery of new drugs, even narrow-spectrum antibiotics which are novel and not subject to pathogen resistance do very well in the marketplace. The total US market for antibiotics is approximately $25 billion, with several narrow-spectrum compounds each earning more than $1 billion annually.

Glycopeptides, such as vancomycin, are the treatment of choice against MRSA. In 2006, sales of glycopeptides totaled $680 million. Vancomycin-resistant strains have begun to appear and in such cases linezolid and daptomycin are typically used. Worldwide sales of linezolid (Zyvox, Pfizer) exceeded $1B per year for the period 2007-2010 and daptomycin (Cubicin, Cubist) generated revenue $600M in the US. The market for these agents is expected to grow.

It is important to note that a combined agent, such as Arietis is developing, does not need to replace current therapies in order to gain market share, because it is intended to be added to -- thereby increasing the effectiveness of -- existing products. Most importantly, the customer for these new agents will benefits from successful treatment without the pitfalls of inadequate infection control and persistent recurrence.

Skin and soft-tissue infections can become complicated, (cSSTI), defined as patients with deeper soft-tissue infections, surgical/traumatic wound infection, major abscesses, cellulitis, and infected ulcers and burns and these infections are often difficult to treat. Bactericidal agents are preferred for these deep-seated infections, although they are still difficult to treat, often requiring longer courses of therapy and surgical debridement. Recurrent SSTI is not considered complicated, but is still a problem and nasal decolonization is recommended if optimized wound care and hygiene were followed. Oral therapy is not recommended decolonization and should be reserved for active infection unless initial decolonization attempts fail. An oral agent in combination with rifampin, may be considered for decolonization, provided the strain is susceptible. No clinical trials have evaluated the role of oral antimicrobials for treatment of recurrent CA-MRSA SSTI and even the definition of recurrence is not standardized.

In addition to SSTI, MRSA bacteremia and infective endocarditis are serious diseases associated with high morbidity, and mortality rates are 30%– 37% for MRSA endocarditis. Vancomycin is still the mainstay of therapy for MRSA bacteremia and endocarditis, but obviously it is not effective in all cases. Daptomycin is an alternative to vancomycin for the treatment of MRSA bacteremia or endocarditis. Emergence of reduced susceptibility to daptomycin was observed in several daptomycin-treated patients who experienced failure of therapy.

Similarly, high failure rates have been observed in the treatment of MRSA pneumonia, particularly in ventilator-associated pneumonia (VAP). Failure of vancomycin may be related to poor penetration into the lung epithelial. Linezolid is an alternative, while daptomycin bind to lung surfactant, rendering it ineffective.

Osteomyelitis is also very problematic. Surgical debridement of necrotic bone, drainage of abscesses and antimicrobial therapy is required. Vancomycin remains the primary treatment for MRSA osteomyelitis and failure rates from 35%–46% have been reported. There are no controlled trials of MRSA osteomyelitis, but small trials of MSSA osteomyelitis suggested higher cure rates were associated with combination rifampicin therapy. The optimal duration of therapy for osteomyelitis is unknown.

Since we expect our combinational therapeutic to be more effective compared to current agents, our therapeutic has the potential to improve cure rates and prevent relapse. The main driver for the current project is that a therapeutic that specifically targets drug tolerant cells and is highly cidal to bacteria will improve the effectiveness of chemotherapy and decrease relapse. An antibiotic with these performance characteristic is therefore anticipated to have a market advantage.

Expert opinion, collected through consultants and clinicians, noted an urgent need to find novel technologies and new classes of antimicrobials within this arena. They also noted that the severe debilitation is frequently observed in these patients with osteomyelitis, endocarditis and VAP. Current antibiotic therapies' inability to completely eliminate infection may be a major cause of clinical failure. Based on this input, end-users expect that this type of therapy would be used initially as a second line agent for uncomplicated infections with the possibility for first line agent using for the complicated infections which we target.

In another regard, and consistent with our business plan, the customer is one or more pharmaceutical companies with currently marketed antibiotic agents that can improve their product offerings, both in terms of therapeutic effectiveness and consequent increased market share. We have identified and initiated conversations with potential partners, and will continue these discussions as more data on the performance of our product candidates become available through the performance of the studies proposed in the current application.

Competition.

Vancomycin has long been the standard of care for MRSA treatment, but its market share is increasingly being replaced with newer agents, such as dapotmycin or ceftobiprole. These and other products currently available for treatment of *S. aureus* are summarized in the Competition Assessment Table below. None of these agents has activity against stationary and persister cell populations of *S. aureus.* There are currently no antibiotics capable of sterilizing infection, and therefore all products are subject to failure, are ineffective or require long-term regiments. We have not found a strongly competitive pipeline of new classes in development vying for FDA approval at this point. A majority of pharmaceutical companies have suspended new research in the gram positive area or are developing molecules based on iterative improvements of known agents. In this regard, there is no direct competitor for Arietis' approach, as it represents a first in class novel therapeutic. In addition, approved agents that demonstrate activity complementary to ADEP4 will benefit from combination therapy, meaning that markets for both are expected to expand, rather than compete with each other. In one sense, the market for Arietis' will not be limited by competition but by the range of currently available agents that provide enhanced activity in combination. Determining the scope of this is an active area of investigation. At the same time, however, an effective combination can be expected to out-compete any single agent in the treatment of recurrent infections, as no single agent can achieve the sterilization necessary to prevent recurrence.

Competition Assessment Table

Product Name	***Manufacturer***	***Relevance***	***Activity against Persisters***
B-lactams	multiple	newer agents like Ceftobiprole, may be effective against MRSA	No
Ceftaroline	Forest/AstraZeneca/Dainippon Sumitomo	IV only, cephalosporin, approved for complicated skin/soft tissue infections and community-acquired pneumonia	No
Clindamycin	multiple	bacteriostatic	No
Daptomycin	Cubist	IV only, cidal, membrane acting	No

Linezolid	Pfizer	bacteriostatic new drug of last resort	No
Quinupristin-Dalfopristin	King Pharmaceuticals	IV only, off label MRSA use, bactericidal	No
Rifampicin	Sanofi Aventis	cidal, used in combination with other antibiotics due to high resistance	No
Telavancin	Astellas	not currently available due to manufacturing issues	No
Tetracyclines	multiple	bacteriostatic, resistance a concern	No
TMP-SMX	Roche, GSK	bacteriostatic, off label use for MRSA	No
Vancomycin	ViroPharma	mainstay of MRSA therapy, slow bacteriocidal activity and resistance are concerns	No
Dalbavancin	Durata Therapeutics	IV, lipoglycopeptide under development	No
Oritavancin	The Medicine Co.	IV, lipoglycopeptide under development	No
Tedizolid	Trius/Cubist	oxazolidinone under development	No

The trend for the basis of competition in this arena will continue to be best performance as there are numerous products available as well as generics. In this case, performance will be measured by ability to treat complicated SSTI, osteomyelitis, VAP, bacteremia and endocarditis, where there is clearly a need for improvement.

Intellectual Property (IP) Protection.

[redacted]

David MZ, Medvedev S, Hohmann SF, Ewigman B, Daum RS, (2012) Increasing burden of methicillin-resistant *Staphylococcus aureus* hospitalizations at US academic medical centers, 2003-2008. Infect Control Hosp Epidemiol.

Hamad, B., (2010) The antibiotics market. Nat. Rev. Drug Discov. 9, 675-676.

Lee, B. Y., A. Singh, M. Z. David, S. M. Bartsch, R. B. Slayton, S. S. Huang, S. M. Zimmer, M. A. Potter, C. M. Macal, D. S. Lauderdale, L. G. Miller & R. S. Daum, (2013) The economic burden of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA). *Clin Microbiol Infect* **19**: 528-536.

Liu, C., A. Bayer, S. E. Cosgrove, R. S. Daum, S. K. Fridkin, R. J. Gorwitz, S. L. Kaplan, A. W. Karchmer, D. P. Levine, B. E. Murray, J. R. M, D. A. Talan & H. F. Chambers, (2011) Clinical practice guidelines by the infectious diseases society of america for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children: executive summary. *Clin Infect Dis* **52**: 285-292.

Information also collected from the NIH CDC websites.

Company Commercialization History

Arietis Corporation has not received more than 15 SBIR Phase II awards from the Federal Government during the preceding 5 fiscal years.

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix: Dr.
* First Name: Kenneth
Middle Name:
* Last Name: Coleman
Suffix:

2. Human Subjects

Clinical Trial? ☐ No ☐ Yes

* Agency-Defined Phase III Clinical Trial? ☐ No ☐ Yes

3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix: Dr.
* First Name: Michael
Middle Name:
* Last Name: LaFleur
Suffix:

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

* Title: Chief Operating Officer

* Street1: 650 Albany ST
Street2: [redacted]
* City: Boston
County/Parish:
* State: MA: Massachusetts
Province:
* Country: USA: UNITED STATES
* Zip / Postal Code: 02118-2518

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4. Human Embryonic Stem Cells

* Does the proposed project involve human embryonic stem cells? ☒ No ☐ Yes

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://stemcells.nih.gov/research/registry/. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

OMB Number: 0925-0001

PHS 398 Research Plan

1. Application Type:

From SF 424 (R&R) Cover Page. The response provided on that page, regarding the type of application being submitted, is repeated for your reference, as you attach the appropriate sections of the Research Plan.

*Type of Application:

☐ New ☒ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

2. Research Plan Attachments:

Please attach applicable sections of the research plan, below.

1. Introduction to Application (for RESUBMISSION or REVISION only)	1243-Introduction_.pdf	Add Attachment	Delete Attachment	View Attachment
2. Specific Aims	1244-Specific_Aims.pdf	Add Attachment	Delete Attachment	View Attachment
3. *Research Strategy	1245-Research_Strategy_.pdf	Add Attachment	Delete Attachment	View Attachment
4. Inclusion Enrollment Report		Add Attachment	Delete Attachment	View Attachment
5. Progress Report Publication List		Add Attachment	Delete Attachment	View Attachment

Human Subjects Sections

6. Protection of Human Subjects		Add Attachment	Delete Attachment	View Attachment
7. Inclusion of Women and Minorities		Add Attachment	Delete Attachment	View Attachment
8. Targeted/Planned Enrollment Table		Add Attachment	Delete Attachment	View Attachment
9. Inclusion of Children		Add Attachment	Delete Attachment	View Attachment

Other Research Plan Sections

10. Vertebrate Animals	1246-Vertebrate_Animals_F.p	Add Attachment	Delete Attachment	View Attachment
11. Select Agent Research		Add Attachment	Delete Attachment	View Attachment
12. Multiple PD/PI Leadership Plan		Add Attachment	Delete Attachment	View Attachment
13. Consortium/Contractual Arrangements		Add Attachment	Delete Attachment	View Attachment
14. Letters of Support	1247-LettersOfSupport.pdf	Add Attachment	Delete Attachment	View Attachment
15. Resource Sharing Plan(s)	1248-ResourceSharing.pdf	Add Attachment	Delete Attachment	View Attachment

16. Appendix Add Attachments | Remove Attachments | View Attachments

We are encouraged by the generally positive evaluation of our proposal: “Significance resides in addressing an area of unmet medical need for novel therapeutics that overcome issues with recalcitrant infections and are effective against dormant persisters and biofilm growth…” “The outstanding investigator is highly qualified to lead a very strong team that is highly experienced in drug development and discovery…” “Strengths of this application are the clever, innovative mechanism of action of ADEP4 to activate dormant cells to increase antibiotic sensitivity and the preliminary results that most reviewers regarded as strong and promising as they reveal combinations of ADEP4/antibiotic are effective against biofilms and stationary bacteria.” ” Overall, there is very strong confidence in the likelihood of this project’s success and that the proposed work will lead to development ADEP-antibiotic combinations as effective candidate therapeutics for recalcitrant S. aureus infections.” – Summary Statement.

We are grateful to the Reviewers for their constructive comments that allowed us to prepare a stronger proposal. Our replies to critiques follow. Changes in the proposal have been italicized.

Reviewer I. 1. The background on combination therapeutics for treating *S. aureus* infections is too narrative. – We include specific data on combinations in the revision.
2. Development of combination therapeutics for drug-resistant pathogens is not new. Using a ClpP activator, ADEP4 a derivative of ADEP is not new. – In the revised application, we note that existing combinations do not eradicate biofilm infections. Regarding ADEP4, several studies suggested that ADEP4/ClpP only attacks targets in growing cells. We show that ADEP4 activates proteolysis in non-growing cells, and detail this in the revision.
3. It is unclear whether the combination treatment eradicated *S. aureus* from infected thighs or still had 3 log CFU (Fig. 4B). – There were no remaining CFUs suggesting sterilization and we clarify this in the revision.
4. Lack of rationale to repeat to combine ADEP4 with rifampicin and linezolid in Aim 1. – Agreed, redundant repetition eliminated, section rewritten.
5. Lack of detailed information of how many *S. aureus* isolates will be utilized for the validation studies. – Information provided.
6. Lack of detailed critical information regarding in vitro ADME/DMPK/toxicity studies in Aim 1. – Information provided.
7. The Reviewer notes that “This phase I application has clear, appropriate, measurable goals (milestones) that should be achieved prior to initiating Phase II.”, but also “However, it is unclear when the applicant is able to obtain letters of interest, additional funding commitments, and/or resources from the private sector or non-SBIR/STTR funding sources that would enhance the likelihood for commercialization.”, and concludes that fast track is therefore unacceptable. Our plan is to enter into partnership once strong data from Phase II are available. Based on our considerable experience with Pharma and Biotech, it is unrealistic to obtain a meaningful commitment from a partner at this early stage of development.

Reviewer II. 1. ADEP compounds were previously published by other companies many years ago. – Please see reply 2, Reviewer I.
2. It is unclear if ADEP has the potential problems common to peptide antibiotics, such as solubility, in vivo stability and other undesirable pharmacokinetics. Another unclear is if the uncontrolled proteolysis due to an ADEP-activated ClpP causes any undesirable effect, in addition to the desirable activation of the dormant cells biofilms and dormant cells. – Preliminary pharmacokinetics show that the drug achieves a reasonable level, and this is confirmed by the complete sterilization of a biofilm infection within 24 hours. This is also in agreement with sterilizing results from a hollow fiber model. Rapid sterilization resulting in a very short duration of treatment is likely to diminish any potential side-effects.
3. The project relies on outsourced synthesis of compounds and pharmacology, toxicology, and other tests. -The total amount of outsourced work is well below the 50% SBIR limit. It represents only ~13% of the budget.
4. VAS does not address the use of rats [redacted] use of mice is appropriately described. – VAS modified accordingly.
5. Biohazards: Protections are not adequately described. – Additional biohazard and PPE details provided.
6. Recommended reductions in 3 Research Associates (Year 2 and Year 3) and 1 Research Associate (Year 4) as these are not well justified. - These reductions were made as recommended.

Reviewer III.
1. VAS on use of [redacted] rats is not provided while use of mice is well-described. – Please see reply 4, Reviewer II.
2. Recommended reductions in 3 Research Associates (Year 2 and Year 3) and 1 Research Associate (Year 4) as these positions are not well justified. – Please see reply 6, Reviewer II.

The goal of this project is to develop the antibacterial ADEP to treat recalcitrant *S. aureus* infections. This molecule is uniquely capable of sterilizing biofilm-related recalcitrant infections, such as abscesses, osteomyelitis and endocarditis. These infections are essentially untreatable and lead to substantial morbidity and mortality. In many cases, recalcitrance is not caused by drug resistance, but by the inability of current antibiotics to target the cells within a biofilm. Indeed, biofilms contain heterogeneous populations of stationary, slowly growing, non-growing, and dormant persister cells, which are highly tolerant to killing by traditional antibiotics (Lewis, 2010). When the antibiotic concentration drops, these cells are able to regrow and repopulate the biofilm. In *S. aureus,* a stationary population is completely refractory to killing by traditional antibiotics. Bactericidal antibiotics require an active target to be corrupted in order to kill. For example, the fluoroquinolones turn DNA gyrase into an endonuclease and the aminoglycosides cause mistranslation. This approach does not work on cells within a biofilm, which contain a high proportion of non-growing cells. Biofilms are protected from the immune system, compounding the problem. We reasoned that dormant cells could be targeted by an antibiotic which causes killing through the *activation* of a target. Acyldepsipeptide (ADEP) was a possible candidate for such a compound. ADEP causes dysregulation of the ClpP protease, relieving it from regulatory control and the requirement to use ATP. *However, ADEP/ClpP was reported to only attack targets in growing cells. We reexamined this and find that ADEP induces massive proteolysis in stationary cells of S. aureus, forcing them to self-digest (Conlon et al., 2013). We showed that ADEP has a remarkable ability to kill persisters and stationary cells.*

ADEP has been previously examined and was effective in mouse models of uncomplicated bacteremia, however conventional antibiotics are highly effective in these models as well. ADEP has a liability – ClpP is not essential, and a high-frequency of null mutations leads to resistance. For this reason, development of ADEP4 was terminated. However, knowing the potential of ADEP to target dormant cells, it makes sense to combine it with conventional antibiotics, which will solve the resistance problem. Indeed, combining ADEP4 with rifampicin or linezolid produced complete sterilization of stationary and biofilm *S. aureus* cultures. Importantly, ADEP4 in combination with rifampicin sterilized MRSA in a mouse model of deep-seated infection, where conventional antibiotics had very little effect. In Phase I of this project, we will identify the best ADEP partnering antibiotic and we will evaluate the combinations for the potential to develop resistance in the hollow-fiber model. Phase II will focus on *in vivo* PK, efficacy and pre-IND enabling studies.

This project is a result of a collaboration between the PI, Dr. Ken Coleman, an expert in drug discovery and development (Arietis), Kim Lewis, an expert in antimicrobial resistance and tolerance (NU) and Steven Leonard, an expert in the hollow fiber model and pharmacology (NU).

Phase 1 Segment- ***Aim 1. Identify the best sterilizing combination of ADEP4 and an approved antibiotic.*** ADEP4 will be paired with known antibiotics such as daptomycin, gentamicin, and oxacillin, and examined for its ability to sterilize stationary and biofilm populations of *S. aureus.* ADEP/antibiotic combinations will also be tested for maximum killing and low resistance development using a hollow-fiber model of *in vitro* infection. Milestone: Advance 2 ADEP4/antibiotic combinations to *in vivo* studies. Combinations which effectively sterilize biofilms and stationary populations of *S. aureus,* and have a rate of resistance development $<10^{-9}$ in the hollow fiber model will be advanced.

Phase 2 Segment- ***Aim 2. In vivo testing of sterilizing combinations.*** PK parameters and initial efficacy of two ADEP4/antibiotic combinations will be determined in mice. The PK/PD driver of ADEP efficacy will be determined using the mouse model of deep-seated MRSA infection. Efficacy of each combination will be tested in an implanted tissue cage biofilm model. Milestone: Determine the PK of the combined agents, the pharmacodynamic driver of efficacy, and treat a previously incurable biofilm MRSA infection.

Aim 3. Preclinical development. Acute and dose-range finding toxicity studies will be performed in rats [redacted]. A 14 day repeated dose toxicity study will be performed in rats. PK studies in rats [redacted] will provide the basis for selecting doses to be tested in humans. Ames and chromosomal aberration genotoxicity studies will also be performed. Taken together, these studies will form the basis for a risk-benefit assessment prior to the pre-IND meeting with the FDA and entry into clinical trials.

Research Strategy

(a) *Significance*.

The goal of this project is to develop a therapeutic capable of treating chronic drug-tolerant infections. While drug resistance is a formidable challenge, there is a considerable arsenal of antibiotics that are effective against most disseminated infections, and new compounds are slowly moving through the drug discovery pipelines. The situation with tolerance is very different – there has never been a therapeutic capable of sterilizing a chronic infection. Antibiotics are effective because of cooperation with the immune system. An antimicrobial kills or slows the growth of the pathogen, and the immune system cleans up the rest. But this does not work when an exopolymer matrix restricts access of the immune system to the pathogen within a biofilm. The result is a chronic infection, requiring treatment with multiple antibiotics over the course of months to years, accompanied by significant morbidity and mortality. These include abscesses, deep-seated infections, endocarditis, osteomyelitis, cystic fibrosis, infections of indwelling devices, and dental diseases. Only recently did we gain insight into the cause for the limited efficacy of antibiotics against chronic infections. All communities of cells produce persisters, dormant variants of the wild type which are tolerant to antibiotics (Lewis, 2010). In order to kill, an antibiotic requires an active target. Aminoglycosides cause mistranslation, which creates toxic misfolded proteins; fluoroquinolones convert DNA gyrase/topoisomerase into an endonuclease, which cleaves DNA; and β-lactams activate autolysins that destroy the cell wall. Drug targets are largely inactive when a cell is dormant, and antibiotics fail (Keren *et al.*, 2004b). Once the concentration of an antibiotic drops, persisters repopulate the biofilm, causing a relapsing chronic infection.

What is known about persisters can be summarized as follows: persisters are not resistant mutants but phenotypic variants of regular cells produced stochastically in the population (Keren *et al.*, 2004a); all pathogens form persisters (Lewis, 2010); the relative abundance of *E. coli* persisters is higher in late-exponential and biofilms, reaching 1% (Keren et al., 2004a); toxin-antitoxin modules (TA) contribute to persister formation (Keren *et al.,* 2004b, Schumacher *et al.*, 2009, Dorr *et al.*, 2010); persisters are non-growing (Balaban *et al.*, 2004), dormant (Shah *et al.*, 2006) cells, which explains their tolerance to bactericidal antibiotics and their role in recalcitrance of disease (LaFleur *et al.*, 2010, Mulcahy *et al.*, 2010); pathways of persister formation are highly redundant (Hansen *et al.*, 2008, Maisonneuve *et al.*, 2011). This presents an obvious problem – persisters cannot be eliminated with traditional antibiotics, or by the immune system if the cells are located within the biofilm.

It was recently suggested that adding sugars such as glucose or mannitol to a stationary culture will resuscitate persisters which can then be killed by aminoglycosides (Allison *et al.*, 2011). Given that the bloodstream has an abundant supply of glucose, the practical implications of this result are unclear. A recent report described a compound resulting from an HTS that potentiated norfloxacin, completely killing an exponentially-growing culture of *E. coli* (Kim *et al.*, 2011). This is an interesting observation, but conventional antibiotics do slowly kill *E. coli* persisters formed in an exponential culture as well. The challenge is to sterilize a stationary or biofilm culture. Influx tried finding potentiators of bactericidal antibiotics that kill stationary cells by screening a synthetic compound library but failed (Penny Markham, personal communication). There was also a claim that all bactericidal antibiotics kill by forming reactive oxygen species, and activating ROS formation or suppressing protective mechanisms will eliminate tolerant cells (Kohanski *et al.*, 2007, Dwyer *et al.*, 2009, Nguyen *et al.*, 2011). These finding were recently refuted (Liu & Imlay, 2013) and our careful side-by-side examination showed virtually no difference in MBC under aerobic vs. anaerobic growth (Keren *et al.*, 2013). The problem of drug-tolerant cells is highly significant to human health, and the challenge of eliminating persisters is formidable.

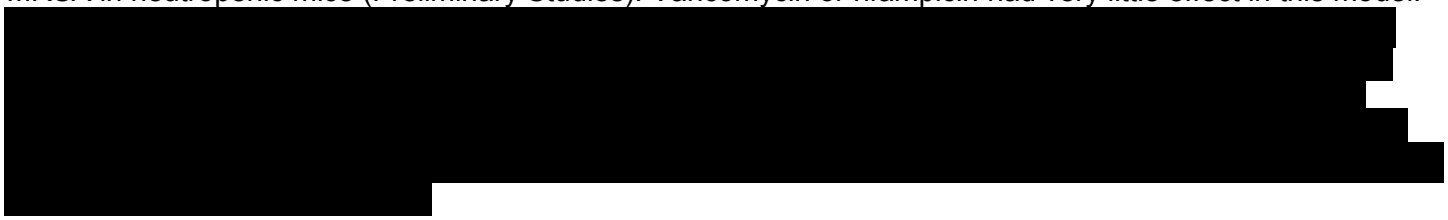
(b) Innovation.

We reasoned that activation of a target, rather than inhibition, could lead to the killing of dormant cells. Indeed, there is an antimicrobial compound, acyldpepsipeptide (ADEP), which activates the ClpP protease, relieving it both from regulatory control and from the requirement to use ATP (Brotz-Oesterhelt *et al.*, 2005). The ClpP protease is fairly selective, clearing misfolded proteins that are fed into the enzyme by accessory ClpA, ClpC and ClpX subunits, which use ATP to recognize, unfold and translocate substrates into the ClpP proteolytic chamber for degradation. ADEP binds to the entry pocket of ClpP, keeping the proteolytic chamber open (Lee *et al.*, 2010). The result is a non-specific protease that does not require ATP. *However, ADEP/ClpP was reported to only attack targets in growing cells. Nascent polypeptides emerging from the ribosome, rather than mature folded proteins, were reported to be targets of ADEP4/ClpP (Kirstein et al., 2009). This would indicate that ADEP4 targets growing cells with active protein synthesis. A particular mature protein, FtsZ, has been reported to be a major target of ADEP4/ClpP* (Sass *et al.*, 2011)*. FtsZ forms the cell division ring, suggesting*

activity of ADEP4 against growing cells as well. We noted that the studies were performed with very short time exposures, and decided to re-examine the action of ADEP. We found that ADEP induces massive proteolysis in stationary cells of S. aureus, forcing them to self-digest (Conlon et al., 2013). Importantly, ADEP was able to effectively sterilize a culture of stationary phase *S. aureus* (Preliminary Studies). The only other antibiotic that has activity against stationary *S. aureus* is the membrane-acting agent daptomycin. At high concentrations daptomycin is active against stationary *S. aureus in vitro* (Murillo *et al.*, 2009), but it does not sterilize at clinically achievable concentrations. Daptomycin is the best available bactericidal compound, but treatment failures do occur and they may be caused by its limited activity against persisters at therapeutic concentrations (Lamp *et al.*, 2007). The exceptional killing ability of ADEP opens the possibility to develop the first therapeutic which specifically targets recalcitrant chronic infections.

Acyldepsipeptide is produced by a soil streptomycetes and was discovered 26 years ago by scientists from Eli Lilly (Michel & Kastner, 1985). They evaluated the compound, found that it had activity against gram-positive but not gram-negative bacteria, and dropped it. Twenty years later, there was a serious need to treat infections caused by drug resistant gram-positive pathogens. By that time Eli Lilly was no longer in the business of antiinfectives, and Bayer picked up acyldepsipeptide for evaluation. They produced a more active derivative, ADEP4, and determined its mechanism of action (Brotz-Oesterhelt *et al.,* 2005). Importantly, ADEP4 was found to be safe and effective in animal models of uncomplicated infection caused by *S. aureus, S. pneumoniae* and *E. faecalis*. However, resistance development to ADEP4 was high due to null mutations in ClpP, and the compound was dropped again. Bayer has since closed its antiinfective research division and its patent for ADEP4 has lapsed. Knowing that ADEP has the potential to sterilize infections, we will combine it with conventional antibiotics to solve the resistance problem.

Combining ADEP with antibiotics such as rifampicin or linezolid produced complete sterilization of stationary *S. aureus* cultures. Given that a stationary population contained 10^9 cells, and *clpP* mutants arise with a frequency of 10^{-6}, we expected to see 10^3 surviving cells in this experiment. Unexpectedly, we found that ClpP mutants become highly susceptible to killing by most antibiotics, explaining the sterilizing effect of the combination seen *in vitro*. ADEP + rifampicin also completely sterilized a deep-seated biofilm infection of MRSA in neutropenic mice (Preliminary Studies). Vancomycin or rifampicin had very little effect in this model.



(c) Approach.

<u>Preliminary Studies</u>. *Previous studies showing that ADEP targets nascent peptides and FtsZ in particular were performed with short exposure times and with rapidly growing cells, and we considered the possibility that longer incubation with ADEP may result in nonspecific degradation of proteins in non-growing cells. A stationary phase population of S. aureus was chosen to test this, as cells are not dividing and synthesis of nascent polypeptides is strongly down regulated (Michalik et al., 2009). Stationary cells of methicillin-resistant S. aureus (MRSA) were exposed to ADEP4 for 24 h and the resulting proteome was compared with that of an untreated control (Fig. 1).*

Proteomic analysis of untreated stationary cells led to the detection of 1,712 proteins representing 65% of the predicted open reading frames. Treatment with ADEP4 resulted in decreased abundance of 243 proteins ($P \leq 0.05$ and twofold decrease) (Fig. 1A). Essential ribosomal proteins were among the most strongly diminished by ADEP4/ClpP, with proteins S21, L9, S1 and ribosomal recycling factor all showing between 17- and 64-fold reduction in the ADEP4 treated sample. Elongation factor Tu, pyruvate kinase and fructose bi-phosphate aldolase were among the proteins with the largest increase in non-trypsin cleavage sites. FtsZ was also one of the many strongly degraded proteins perhaps because of its disordered carboxy terminus (Buske & Levin, 2013). Other than the ribosome, degraded proteins belonged to various functional types, including purine metabolism, glycolysis and aminoacyl-tRNA biosynthesis, among others (Fig. 1B). Taken together, the proteomic data indicated that ADEP4 forced the cell to self-digest, suggesting that it could be effective in killing dormant cells.

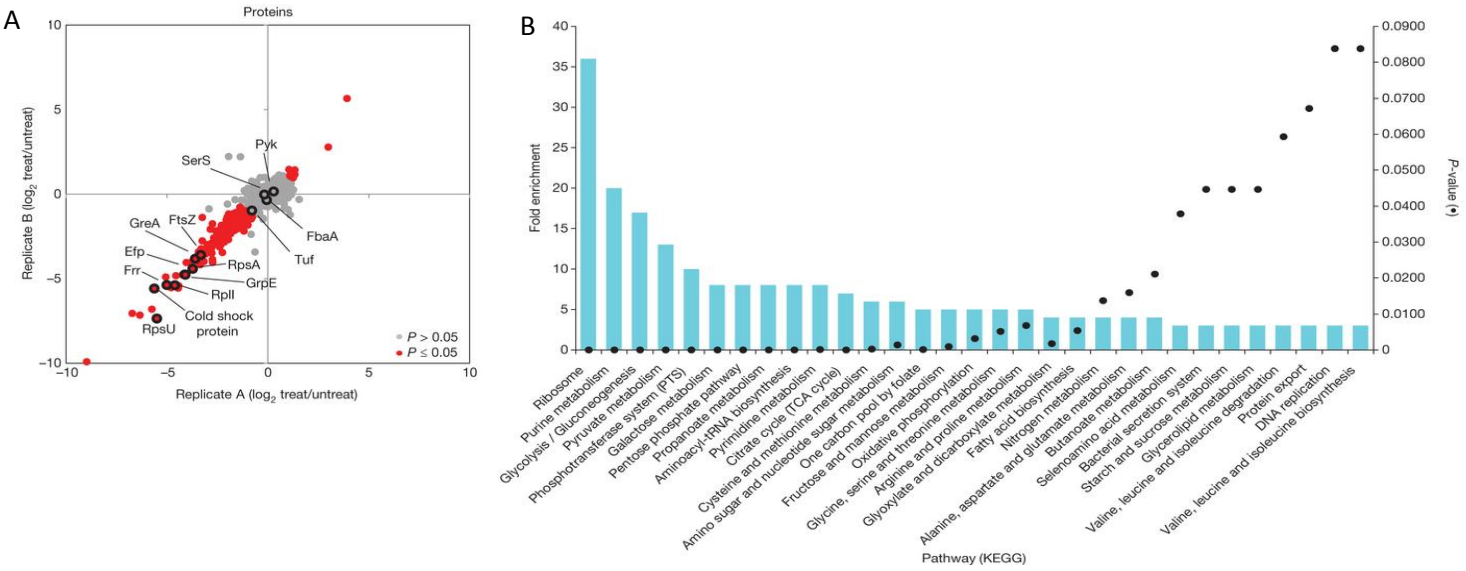


Figure 1. Proteomic analysis of *S. aureus* cells treated with ADEP4 reveals extensive protein degradation. A) The dispersion graphs show the relative abundances (treated/untreated) of total proteins in different biological replicates (*n* = 2). The significant changes in abundances (*P* ≤ 0.05 and >twofold) are represented in red circles. B) Function–enrichment analysis of proteins degraded by ADEP4. Functions overrepresented among proteins degraded by ADEP4 were annotated using Database for Annotation, Visualization and Integrated Discovery (DAVID) and the overrepresented pathways compared to the genome background are shown as columns, whereas their *P*-values are represented by the black dots. Bayesian moderated *t*-test was used to provide *P*-values that were further corrected by the data set size.

The natural product acyldepsipeptides are not particularly potent, but a medicinal chemistry program conducted by Bayer resulted in ADEP4, with an IC_{50} of 0.05 µg/ml against *S. aureus*. The killing ability of ADEP4 had not been examined. Another analog, ADEP-10c was subsequently described by a different group, with a reported MIC of 0.6 µg/ml (Socha *et al.*, 2010). We decided to test the killing properties of both compounds. In our hands, ADEP 10c had a *S. aureus* MIC of 5 µg/ml, and ADEP4 had an MIC of 0.75 µg/ml. Since the Bayer group only reported IC50 rather than MIC, our results are probably in accordance with their findings. ADEP 10c is considerably less active; apart from the compounds synthesized for this project, we also tested a sample from the authors (Socha *et al.*, 2010), and got the same MIC, 5 µg/ml. We therefore decided to focus on ADEP4. ADEP4 had a MBC against *S. aureus* of 0.75 µg/ml, which is identical to the MIC and a good indication of its excellent killing ability.

As mentioned previously, ADEP4 uncouples ClpP from the requirement to use ATP, which would help kill persisters with low energy levels. In a control experiment, ciprofloxacin was added to an exponentially growing culture of S. aureus, which produced a typical biphasic killing pattern with surviving persisters (Fig. 2A).

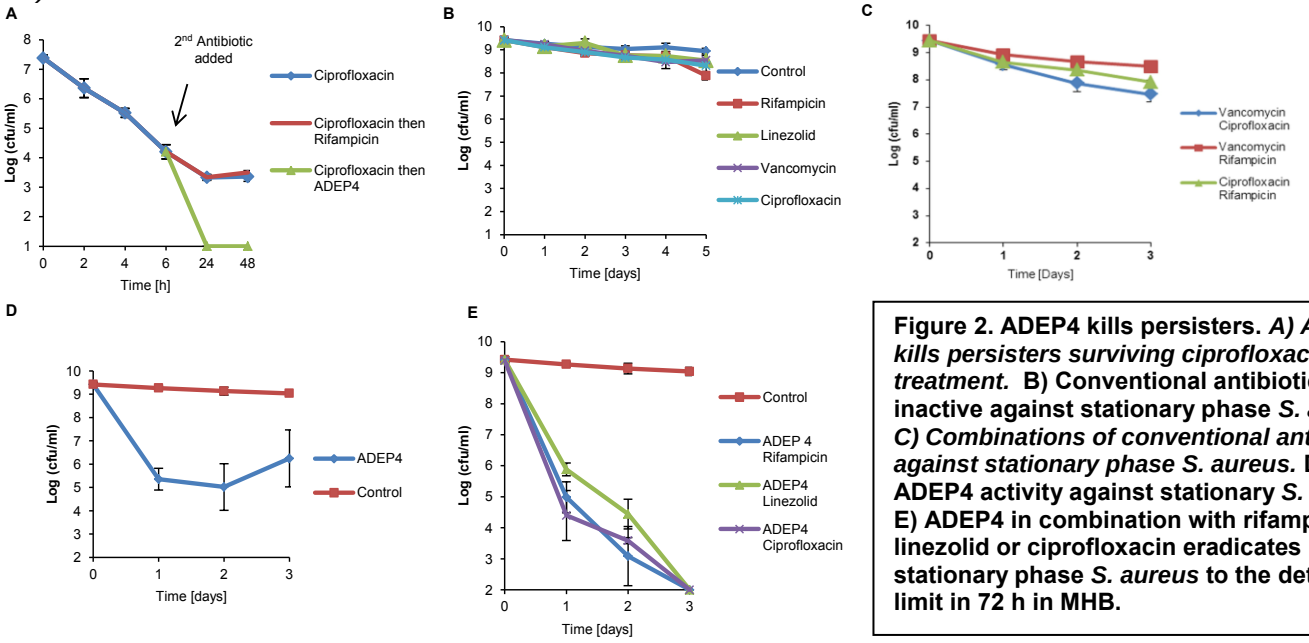


Figure 2. ADEP4 kills persisters. *A) ADEP4 kills persisters surviving ciprofloxacin treatment.* B) Conventional antibiotics are inactive against stationary phase *S. aureus*. *C) Combinations of conventional antibiotics against stationary phase S. aureus.* D) ADEP4 activity against stationary *S. aureus*. E) ADEP4 in combination with rifampicin, linezolid or ciprofloxacin eradicates stationary phase *S. aureus* to the detection limit in 72 h in MHB.

Addition of rifampicin to surviving persisters had no effect on their viability, in agreement with previous observations on the multidrug tolerant nature of these cells. By contrast, addition of ADEP4 led to eradication of persisters (Fig. 2A). In agreement with our previous observations (Keren *et al.,* 2004a), high concentrations (10x MIC) of bactericidal antibiotics vancomycin, ciprofloxacin, rifampicin or linezolid had little effect on a stationary population of *S. aureus* cells even after a 5 day incubation (Fig. 2B). *Furthermore, combinations of vancomycin, rifampicin and ciprofloxacin had very limited activity (Fig. 2A, 2C, 6B, and 7B).* ADEP4 at 1x MIC produced much more killing after a single day of incubation, decreasing cell numbers of a stationary population by 4 logs (Fig. 2D). After that, the culture partially rebounded, due to the appearance and growth of *clpP* mutants, which were validated by PCR and sequencing. However, combining ADEP with rifampicin, linezolid *or ciprofloxacin* produced complete sterilization by day 3 (Fig. 2E).

Complete sterilization in these experiments was unexpected—the frequency of *clpP* mutants is 10^{-6}, and in a population of 10^9 cells, there should have been 10^3 survivors. To investigate this, a *ΔclpP* mutant was examined for its susceptibility to linezolid, rifampicin and ciprofloxacin (Fig. 3). The *ΔclpP* strain had the same minimum inhibitory concentration (MIC) as the wild type, but stationary phase counts were reduced 10- to 100-fold upon exposure to linezolid, rifampicin or ciprofloxacin. A mutation in *clpP* apparently diminishes the fitness of cells and makes them vulnerable to certain antibiotics. In agreement with this, a *clpP* mutant was reported to be avirulent in a murine skin abscess model of infection (Frees *et al.*, 2003).

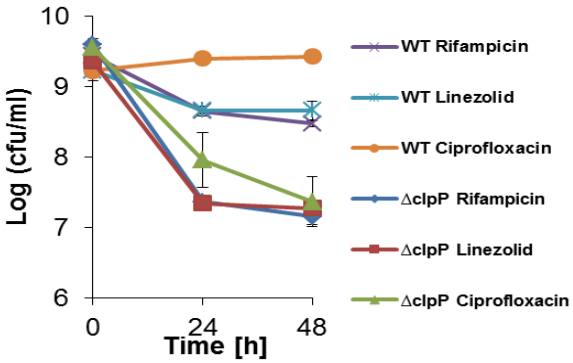


Figure 3. *clpP* mutants are susceptible to killing by antibiotics. Stationary cultures of *S. aureus* were exposed to 10x the MIC of each antibiotic for 2 days.

We then tested the eradicating potential of the ADEP4 and rifampicin combination against a variety of S. aureus strains. These included the laboratory strain SA113, as well as clinical isolates USA300, UAMS-1 and strain 37. USA300 is a community acquired MRSA and is the most common cause of staphylococcal skin and soft tissue infections in the United States(Stryjewski & Chambers, 2008). UAMS-1 is a highly virulent clinical isolate associated with chronic osteomyelitis (Gillaspy et al., 1995). Strain 37 was isolated from a patient undergoing vancomycin therapy who succumbed to infection (Miyazaki et al., 2011). No colonies were detected in any of these strains after 72 h of incubating stationary cultures with the combination of ADEP4 and rifampicin (Fig. 4).

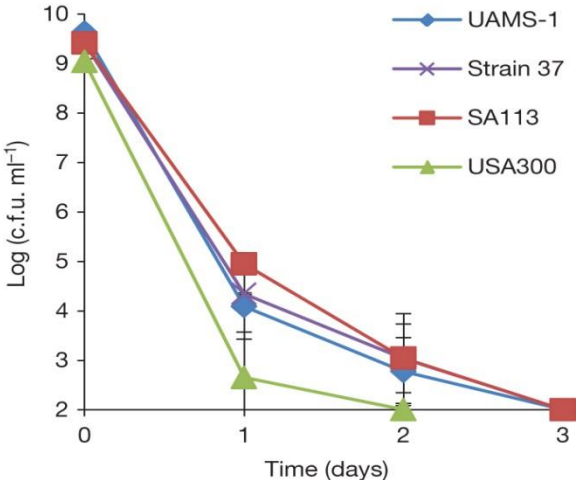


Figure 4. S. aureus was grown in MHB for 16 h and challenged with 10× MIC of ADEP4 and rifampicin. Colony counts were performed every 24 h. The x axis is the limit of detection. Data are representative of 3 independent experiments. Error bars represent standard deviation.

Based on these encouraging results, we decided to test the antibiotics against biofilms. Biofilms of *S. aureus* UAMS-1 were grown overnight in 96-well plates in BHI broth. Spent media was replaced with fresh BHI containing 10x MIC of various antibiotics and incubated for 1 or 3 days. Wells were washed with PBS and the biofilms were disrupted with sonication and plated on BHI agar for colony counts. As expected, conventional antibiotics had little effect on the biofilm (Fig. 5A). A combination of ADEP4 with rifampicin resulted in eradication of cells in the biofilm to the limit of detection (Fig. 5A). The replacement of antibiotics with fresh medium did not result in re-growth after 3 days of ADEP/rifampicin treatment, confirming the complete eradication of living cells. Confocal microscopy also showed that the combination of ADEP + rifampicin eradicated the biofilm (Fig. 5B). An elimination of a biofilm is unprecedented for such low, clinically achievable concentrations of compounds.

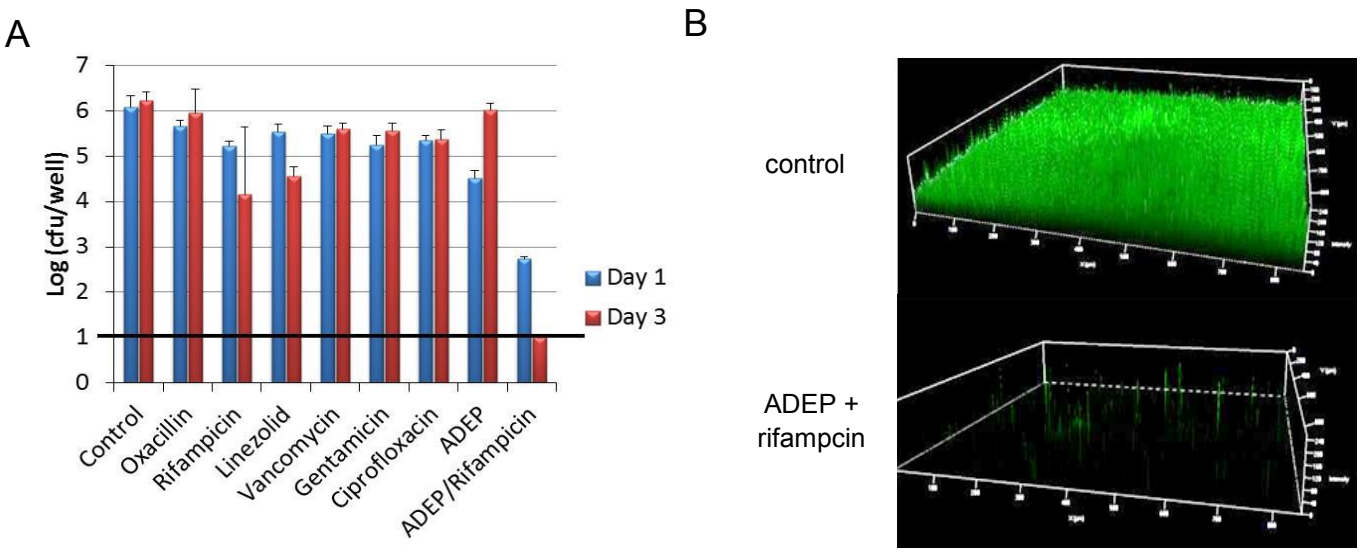


Figure 5. The effect of antibiotics on the survival of *S. aureus* biofilms. A) Biofilms were grown in 96 well plates and exposed to antibiotics at concentrations of 10x the MIC for 24 and 72 hours. B) Confocal microscopy live cell imaging of a control biofilms or one treated with the combination of ADEP and rifampicin at 1 and 10 x the MIC for 72 hours. *S. aureus* strain UAMS-1 was transformed with a GFP plasmid under the control of xylose inducible promoter. Biofilms were seeded and grown in an imaging flow cell for 24 hours. After drug exposure, the biofilms were washed with fresh medium containing xylose to induce GFP expression in live cells.

Eradication of stationary and biofilm populations was an encouraging sign that ADEP4 could be a very useful antibiotic against untreatable chronic infections. To test this, we used a deep-seated mouse thigh infection model. In a standard thigh model, a mouse is infected with a low dose of pathogen and antibiotic therapy begins within a few hours of infection. Under these conditions, conventional antibiotics are very effective. In the deep-seated model, the mouse is made neutropenic by treatment with cyclophosphamide, a large dose of pathogen is delivered and the infection is allowed to develop for 24 h before therapy, leading to a severe, recalcitrant, deep-seated infection. This model emulates a difficult to treat human deep-seated chronic infection in immunocompromised patients. We performed histopathology of the infected thigh, and detected massive aggregates of *S. aureus* cells with Gram staining (Fig. 6A).

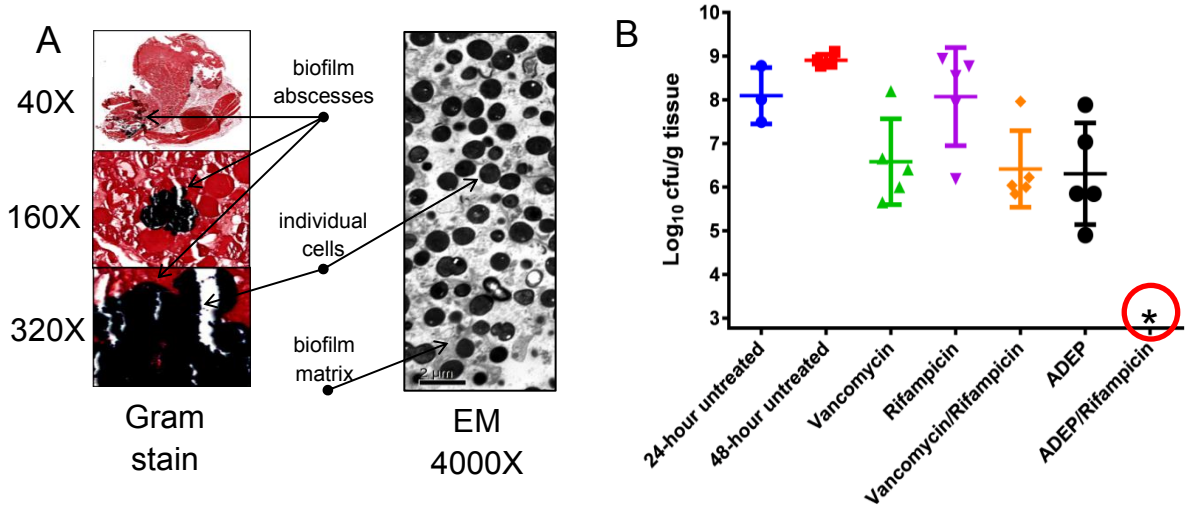


Figure 6. *In vivo* model of deep seated *S. aureus* infection. A) Gram staining and electron microscopy histopathology cross-sections of infected thigh tissue harvested 24h post infection. Tissues were fixed in 10% formalin or EM fixative (paraformaldehyde/glutathione) prior to processing and imaging. B) *In vivo* activity of antibiotics in a deep seated neutropenic mouse thigh lesion model. Groups of 5 mice were infected with 1x 10^6 cells of *S. aureus* MRSA ATCC 33591 and infection was allowed to develop for 24 hours. Vancomycin was administered at 110 mg/kg twice a day. Rifampicin and ADEP were administered once at 30 mg/kg and 50 mg/kg respectively alone or in combination. Mice were euthanized and the thigh muscle was removed aseptically and homogenized in PBS. Serial dilutions were spotted on MHA and incubated overnight at 37ºC. **No cell counts were present in the ADEP + rifampicin group, even when the entire thigh homogenate from each animal was plated.*

A

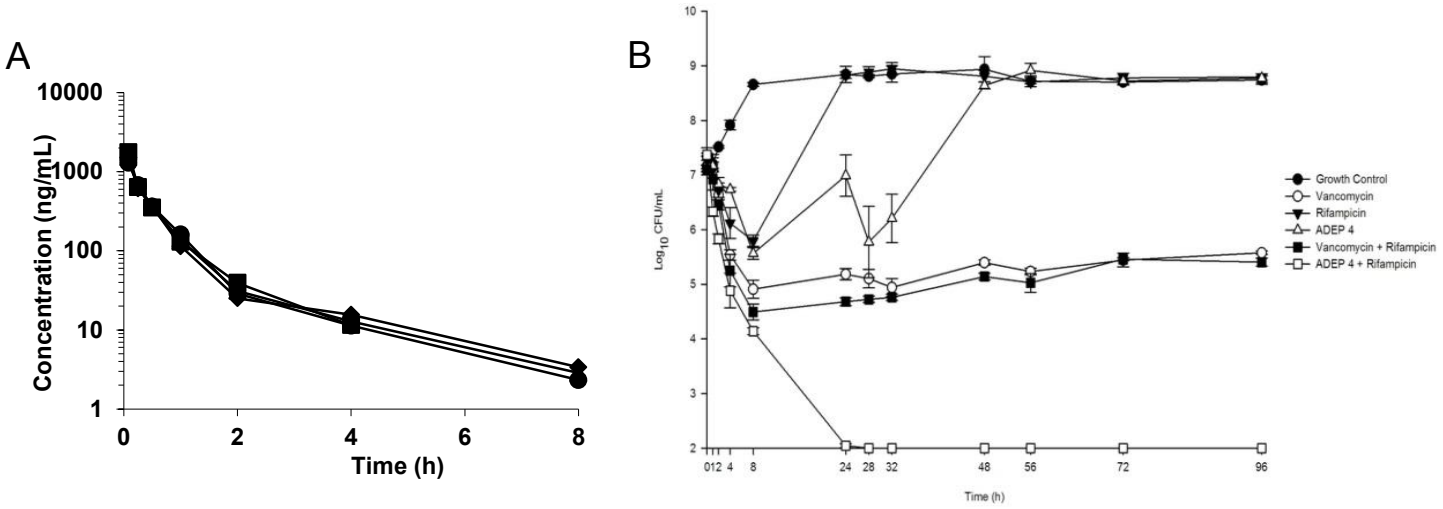


Figure 7. A) ADEP concentrations in the hollow fiber experiment were based on the AUC calculated from free drug plasma concentrations in mice. B) Survival of *S. aureus* exposed to antibiotics for 4 days in a hollow-fiber infection model. Antibiotic concentrations were based on physiologically relevant concentrations achieved in humans and were adjusted for protein binding. No cell counts were present after 24 hours.

Electron microscopy of cross-sections of the infected tissue revealed *S. aureus* growing in biofilms adhered to muscle cells (Fig. 6A). Administration of vancomycin, rifampicin or a combination of both decreased the viable counts, but did not clear the infection (Fig. 6B). Furthermore, no notable difference was observed between mice treated for 24 h or 48 h with vancomycin in this model, indicating the presence of a persister subpopulation surviving the antibiotic treatment (Conlon et al., 2013). *Remarkably, an ADEP4 and rifampicin combination led to sterilization of the infected tissue within 24 h (Fig. 6B). No colonies were present even when the entire thigh homogenate from each animal was plated. Based on this efficacious dose and our mouse pharmacokinetics data (Fig. 7A), we performed a hollow-fiber experiment and found that the combination of ADEP and rifampicin also resulted in complete eradication of the pathogen to the limit of detection in these experiments (Fig. 7B).* Complete sterilization of a chronic infection with a single dose of an antimicrobial combination bodes well for development of an effective therapeutic to target difficult to treat chronic infections.

Aim 1. Identify approved antibiotics to combine with ADEP4 and validate the combination treatment. *So far, we have tested a combination of ADEP4 with rifampicin; linezolid; and ciprofloxacin. For complete characterization, we will also examine the remaining main classes of antibiotics, a β-lactam (oxacillin), an aminoglycoside (gentamicin), and a lipopeptide (daptomycin). Again we will test osteomyelitis isolate UAMS-1, MRSA strains ATCC33591 and community acquired USA300, and a clinical isolate which was responsible for death of a patient undergoing vancomycin therapy (Miyazaki et al., 2011).* Combinations will be tested at clinically achievable concentrations against stationary cultures of these pathogens. Complete sterilization will indicate a good combination. We will then examine combinations that pass this test against biofilms. For testing biofilms, we will use two different models. One is a Calgary™ device with prongs which is placed in a suspension of bacteria in nutrient medium for the biofilms to form. Another method is based on forming a biofilm in a flow cell, which probably better resembles an environment *in vivo* due to shear flow. The *osteomyelitis isolate UAMS-1 will be used for biofilm testing.* As a final validation, ADEP/antibiotic combinations will be tested for maximum killing and low resistance development using an *in vitro* hollow-fiber infection model. The *MRSA strains ATCC33591 and community acquired USA300 strains will be used for the hollow-fiber model. Since we are registered with the Network on Antimicrobial Resistance in Staphylococcus Aureus (NARSA), and have access to their strain collection, newly emergent isolates which are deposited in their repository will also be available for testing.*

The reason for using combinations is to minimize the probability of resistance. As mentioned, null *clpP* mutants resistant to ADEP4 arise with a probability of 10^{-6}. As we report, these mutants are highly attenuated; they lose virulence and become susceptible to killing by other antibiotics. The frequency of rifampicin resistance is around 10^{-8}, which suggests that mutants resistant to the combination will arise with a frequency of 10^{-14}. Resistance development for other antibiotics is $<10^{-9}$, suggesting that the combination will have an even greater safety margin. So far, we have not seen spontaneous resistant mutants develop for the combination of ADEP/rifampicin tested at 10X the MIC on agar plates. However, resistance may occur as a result of changing drug concentrations and exposures. Therefore we will determine the relationship between

ADEP4/antibiotic exposure and drug resistance in the hollow-fiber infection model. *ADEP4 will be paired with known antibiotics such as daptomycin, linezolid, ciprofloxacin, gentamicin, and oxacillin, and examined for the ability to sterilize and prevent resistant population take-over in the hollow fiber infection model*. The hollow-fiber model is a pharmacodynamic system that allows a pathogen to grow in a chamber within a hollow-fiber cartridge. The bacterial growth chamber is separated from the central compartment by a semi-permeable membrane, which allows nutrients and drugs to pass through, while retaining the cells. Fresh media and drugs are pumped into the central compartment allowing different half-lives and exposure conditions to be simulated, without diluting the pathogen. Sample ports allow drug concentrations and culture densities to be monitored.

We have performed a preliminary experiment using this model in order to validate this approach (Fig. 7B). Antibiotic concentrations will be calculated and delivered based on known standard dose regimens in humans, adjusted for each antibiotic half-live and the extent of protein binding. ADEP concentrations were calculated in a similar fashion based on a preliminary determination of PK in mice. The ADEP drug concentration vs time area under the curve (AUC) was determined based on a 5 mg/kg dose delivered by IV tail vein injection (Fig. 7A). The AUC was multiplied by 10 to estimate the 50 mg/kg dose which was effective *in vivo* (Fig. 6B), assuming a linear correlation, and adjusted for plasma protein binding. These experiments mimicked the *in vivo* results with the neutropenic murine thigh model of deep seated MRSA infection. The combination of ADEP and rifampicin caused complete sterilization after 24 hours and no resistance occurred over 4 days (Fig. 7B). Resistance to rifampicin occurred within 24 hours, while dosing with vancomycin or vacomycin + rifampicin achieved a steady state, 2 log reduction in colony counts.

In Aim 1, a more detailed analysis of resistance will be performed. The experiments will be expanded to 10 days in duration and the frequency of resistance development for 3 different ADEP4/partner antibiotic ratios will be determined. The relationship between drug exposure and resistance is an inverted-U. Resistance is less for low and high drug exposures compared to intermediate levels. Therefore, several doses of ADEP will be evaluated. The published maximum tolerated dose of ADEP4 is 100 mg/kg (Brotz-Oesterhelt et al., 2005) and 50 mg/kg sterilized in the mouse model. Rifampicin was dosed at 30 mg/kg once per day for the *S. aureus* infection model, which mimics the 600 mg daily dose in humans. Therefore, ADEP will be added at 1:1, 2:1 and 3:1 dosing ratios (30, 60 and 90 mg/kg). Samples will be taken from the bacterial chamber, washed, diluted, and plated to determine the total live bacterial population for each time point. A portion of each sample will also be plated on agar containing 3 x MIC of each drug, in order to determine whether a resistant subpopulation exists and at what level. Similar experiments will be repeated for each antibiotic - linezolid, ciprofloxacin, gentamicin, daptomycin and oxacillin. Dosing for each known antibiotic will mimic the standard human dose and three different concentrations of ADEP4 will be tested for each antibiotic. Combinations with a resistance rate less than 10^{-9} will be considered acceptable for further advancement. Ultimately the ADEP4/antibiotic combinations with the best killing potential and lowest frequency of resistance will emerge.

<u>In vitro ADME/DMPK/Toxicity.</u> The potential for secondary drug interactions will be examined by testing ADEP4 in combination with each partner antibiotic against a safety panel of human enzymes and receptors using the Eurofins lead profiling + CYP screening. This screen detects potential adverse activity against 68 primary molecular targets and the five major CYP450s. The full list of the enzymes to be tested is available from Eurofins and includes human adenosine, adrenergic, bradykinin, calcium channel, dopamine, GABA, glutamate, histamine, muscarinic, opiate, potassium channel, purinergic and serotonin receptors. [redacted] *This data supports other published findings regarding the excellent safety of ADEP4 (Brotz-Oesterhelt et al., 2005). A combination of ADEP4 and each antibiotic will be tested in duplicate at 10 µM and screened for inhibition >50% compared to negative controls. Any hit will be confirmed in a dose response follow-up experiments comparing the activity of each component to the combination. Any combinations showing significantly higher adverse activitiy compared to each agent alone will be deprioritized.*

Recent reports have shown that ADEPs are able to activate human mitochondrial hClpP in vitro (Lowth et al., 2012). However, toxicity associated with unregulated protein degradation in mitochondria has not been observed either in cultured eukaryotic cells or in animal studies (Brotz-Oesterhelt et al., 2005). Preliminary pharmacokinetics and efficacy in mice shows that ADEP achieves a reasonable level, confirmed by the complete sterilization of a biofilm infection within 24 hours. This is also in agreement with sterilizing results from a hollow fiber model. Rapid sterilization resulting in a very short duration of treatment is likely to diminish any potential side-effects. If we observe that a given combination of ADEP4 + antibiotic shows toxicity, this combination will be deprioritized.

Pitfalls and Alternatives: We do not anticipate any problems with these studies as we have already validated these models. Additional approved antibiotics and higher or more frequent dosing regimens may be explored.

Criteria for Phase II advancement and Fast-track justification. ADEP4 has a unique mechanism of action and an exceptional ability to kill stationary populations and biofilms. These properties suggest there is an untapped potential for ADEP based therapeutics to cure recalcitrant infection. In phase I, we will determine the best sterilizing combinations of ADEP/antibiotic. ClpP mutants are avirulent and highly susceptible to killing by traditional antibiotics, suggesting it is unlikely for resistance to the ADEP/antibiotic combination to occur. However, a more detailed study of resistance is warranted. We will therefore identify combinations that result in resistance frequency below 10^{-9}. **Milestone: Identify ADEP4/antibiotic combinations which sterilize MRSA in stationary, biofilm and hollow fiber models, and have a frequency of resistance development <10^{-9}.** ADEP4 is already known to be safe and effective in multiple animal models of uncomplicated infections. Our preliminary data show that ADEP and rifampicin cure a deep seated biofilm infection, while other antibiotics are ineffective. These findings suggest a realistic opportunity to develop an ADEP/antibiotic combination and justify our fast-track application. For purposes of clarity, a simplified project overview is given below.

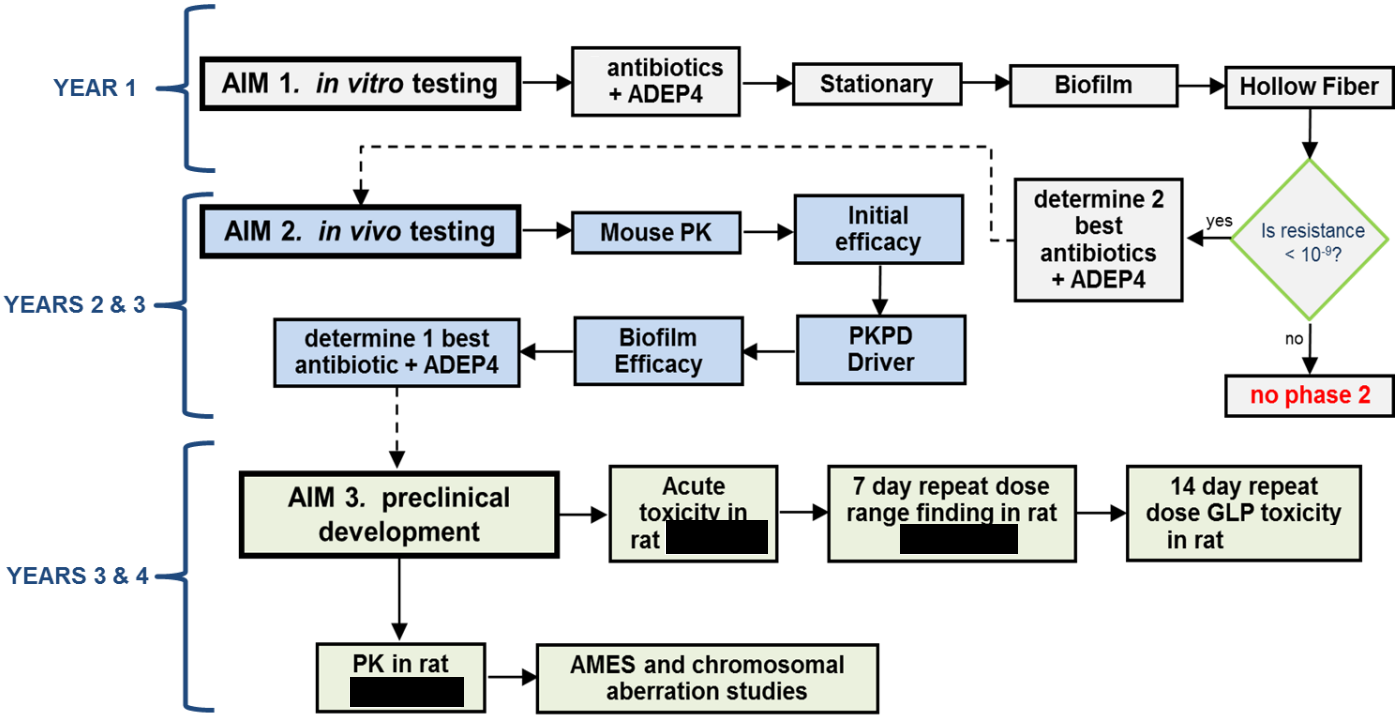


Figure 8. Overview and timeframe for the entire project. Phase 1 will be completed in 1 year, when the feasibility of developing ADEP4/antibiotic combination will be evaluated. Phase 2 will continue for 3 subsequent years and these studies will allow us to focus on a single antibiotic partner and enable a pre-IND meeting with the FDA.

Phase 2- Aim 2. *In vivo* evaluation of sterilizing combinations. Evaluating multiple combinations will improve the chance for success. We expect most of the combinations to work well *in vitro*, and since the initial *in vivo* efficacy model is straightforward, 2 of the best combinations will be advanced. The goal of this aim is to determine, validate, and describe the parameters responsible for the *in vivo* efficacy of the combination therapeutic. In order to optimize dosing, it is necessary to determine the amount of each component to administer, and the frequency of administration. A determination of the pharmacokinetics (PK) and pharmacodynamics (PD) of the two agents is essential to enable this optimization. PK describes the changes in the plasma concentration of the drug over time, while PD describes the effect of the drug on the infecting organism. Although PD data can be collected *in vitro*, this may correlate poorly with *in vivo* PD data. For virtually all antiinfectives studied, the best predictor of efficacy, referred to as the PKPD driver, is one of the following parameters: 1) The area under the plasma concentration profile of unbound (free) drug (fAUC) compared to MIC (fAUC/MIC); 2) The duration within a 24h period for which the unbound (free) drug concentration exceeds the MIC (fT>MIC); or 3) The peak plasma concentration of unbound drug compared to MIC (fC_{Max}/MIC). For example, if the PKPD driver of an agent is found to be a fAUC/MIC ratio of 100, and the infecting organism has an MIC of 4 µg/mL, the minimum efficacious dose is one which delivers a total 24h fAUC of 400 µg.h/mL.

The PKPD driver for ADEP4 will be determined in combination with a fixed concentration of antibiotic using the methods described below. Since our primary aim for these combination agents is to eradicate an infection, these PKPD studies are critical for determining optimal dosing and efficacy. For example, if the PKPD driver proves to be fT>MIC, then drug dosing is optimized by prolonging the duration of exposure of the organism to the drug, either by administering more frequently at lower doses or by continuous infusion. If fAUC/MIC is the PKPD driver, then antimicrobial efficacy is optimized by the infrequent administration of large doses. The PKPD of the approved antibiotics are well defined and, by determining the PKPD driver for ADEP4 in the presence of the antibiotic, it should be possible to optimize the ratio of the two drugs in combination, and determine the total daily dose of the combination and the dosing frequency. We will start by determining the PK of ADEP4 in combination with each antibiotic, and will then determine the PKPD driver of efficacy in the thigh model of deep seated MRSA infection.

Pharmacokinetics. The PK of ADEP4 with antibiotic 1 or antibiotic 2 will be determined in mice (the PK of single agents is already known). PK will be determined for ADEP4 following IV and IP doing at 25, 50 and 100 mg/kg, all in combination with a standard dose of antibiotic 1 and antibiotic 2, using LC-MS/MS (Table 1). In the unlikely event of non-linear PK being detected, intermediate doses will also be studied. The oral bioavailability, elimination half-life, peak plasma concentration (C_{Max}), area under the curve (AUC) and time above MIC (T>MIC) will be determined for each drug in the combination. These experiments will follow a typical 8-time point, single dose set-up. Serial micro- sampling from submandibular bleeds will be used to minimize mice and plasma will be collected at predetermined intervals of 15, 30, 45, 60, 90, 270, 480 and 1440 minutes. The PK data will be used to adjust the dosing strategy for the initial efficacy studies, if necessary.

Treatment	# Samples	Mice	Route I	Route II	Total
ADEP4 (25) + Antibiotic 1	8	3	IV	IP	6
ADEP4 (50) + Antibiotic 1	8	3	IV	IP	6
ADEP4 (100)+Antibiotic 1	8	3	IV	IP	6
ADEP4 (25) + Antibiotic 2	8	3	IV	IP	6
ADEP4 (50) + Antibiotic 2	8	3	IV	IP	6
ADEP4 (100)+Antibiotic 2	8	3	IV	IP	6
10% excess					4
Total number of mice					**40**

Table 1. Mouse PK study design and animal numbers.

Initial efficacy. We have previously used the neutropenic murine deep-seated S. aureus thigh infection as a model of recalcitrant infection. The murine staphylococcal thigh lesion model has been a standard *in vivo* test for antimicrobials for fifty or more years. In this model, an appropriate inoculum is introduced into the thigh muscle of the mouse to form an abscess. The infection remains localized for 4 or more days, causing enlargement of the thigh but no systemic infection. In most reported studies, chemotherapy commences within 2h of infection, as delaying therapy beyond this point usually results in failure to cure. In our hands, using immunocompromised mice and delaying therapy until 24h post-infection results in a highly recalcitrant biofilm infection, where even high doses of vancomycin or rifampicin dosed twice/day for 2 days gave less than a 2-log reduction in viable count relative to an untreated control. A single dose combination of rifampicin with ADEP4 cleared the infection. We will continue to use this recalcitrant abscess model to evaluate 2 ADEP4/antibiotic combinations. Initial studies will deliver 50 mg/kg of ADEP4 (½ of MTD) and the standard dose of the partner antibiotic, each adjusted based on the combined PK. Later studies will focus on optimizing the ratio of ADEP and finding the best dosing regimen for cure by determining the pharmacodynamic driver for efficacy. Each study will contain the following test groups: vehicle control, antibiotic alone, ADEP4 alone, antibiotic + ADEP4, and the positive control, ADEP4 + rifampicin (Table 2).

Treatment	Mice
Vehicle	5
Antibiotic 1 control	5
Antibiotic 2 control	5
Antibiotic 1 + ADEP4	5
Antibiotic 2 + ADEP4	5
ADEP4 alone	5
rifampicin + ADEP4	5
10% excess	4
Total	**39**

Table 2. Initial efficacy study design and animal numbers.

PKPD driver of ADEP4/antibiotic efficacy. Once an initial efficacious dose of ADEP4 in combination with each antibiotic is validated, it will be possible to determine the PD driver for ADEP4 using the methods described by Craig (Craig, 2007). Using the neutropenic mouse model described above, groups of 5 mice will be treated for 1 day with a range of 4 ADEP4 administrations based around the PK and the effective dose from the initial efficacy experiment (Table 2). 50 mg/kg ADEP4 sterilized in combination with rifampicin so doses above and

below this dose were selected as an example. ADEP4 doses above the MTD will not be tested, e.g. 4x q24h (Table 3). Groups of 5 mice will also be required for untreated, ADEP4 alone and antibiotic alone groups. Doses will be fractionated to allow administration of each daily dose q24h, q12h, q8h and q6h to give a broad range of AUC, T>MIC and C_{Max} values. The known antibiotic will be administered to test groups using standard dosing adjusted for the combined PK similar to the initial efficacy study above. One day after the final dose, thigh burden will be determined as previously described. Plotting pathogen count at each dose against AUC, T>MIC and C_{Max} will reveal the PK parameter which most closely correlates with efficacy – the PKPD driver. Following this design, a total of 105 mice will be required (Table 3).

Treatment	**Antibiotic** (standard dose)	**Total**
Vehicle	N	5
Antibiotic control	Y	5
ADEP4 alone (~50 mg/kg)	N	5
ADEP4 (**0.5x** mg/kg q24h)	Y	5
ADEP4 (**0.5x** mg/kg q12h)	Y	5
ADEP4 (**0.5x** mg/kg q8h)	Y	5
ADEP4 (**0.5x** mg/kg q6h)	Y	5
ADEP4 (**1x** mg/kg q24h, q12h, q8h, q6h)	Y	20
ADEP4 (**2x** mg/kg q24h, q12h, q8h, q6h)	Y	20
ADEP4 (**4x** mg/kg q12h, q8h, q6h)	Y	15
Uninfected	N	5
10% excess	N	10
Total number of mice		**105**

Table 3. Dose fractionation study design and animal numbers.

Efficacy in implanted biofilms. Combinations which display efficacy in the thigh model will progress to an *in vivo* biofilm model (Kristian *et al.*, 2003). In the mouse tissue cage (TC) model, a *S. aureus* biofilm was not cleared with three broad-spectrum antibiotics dosed either alone or in combination (Lucet *et al.*, 1990), but we anticipate that our combinations will clear the biofilm. Briefly, a sterile TC containing sintered glass beads is implanted subcutaneously in the back of the anaesthetized mouse. Two weeks after surgery, the TC is verified to be sterile, the mice are then immunocompromized, 200 µL of a *S. aureus* culture is introduced into the TC and the animals are left for 14 days to allow the infection to stabilize. Mice are then dosed for 7 days and euthanized to allow removal of the TC. Bacterial counts will then be performed on the TC fluid and on the glass beads (following washing and sonication). Lucet *et al.* used a fixed dose of each agent for their studies and our initial efficacy study will directly compare their combination of rifampicin (25 mg/kg) + vancomycin (50 mg/kg) with our chosen test combinations. A vehicle control group will also be studied. 10 mice per group and 20% excess will be required due to the variability of this model and the possibility that once implanted, the tissue cages will not remain sterile (Table 4).

Treatment	**Mice**
Vehicle	10
Antibiotic 1 control	10
Antibiotic 2 control	10
Antibiotic 1 + ADEP4	10
Antibiotic 2 + ADEP4	10
ADEP4 alone	10
rifampicin + vancomycin	10
20% excess	14
Total	**84**

Table 4. Biofilm study design and animal numbers.

Aim 3. Preclinical development. These studies are generally required by the FDA prior to pre-IND discussions. They also provide the basis for a risk-benefit assessment to be completed prior to entry into human clinical trials. They will also provide the basis for selecting doses to be tested in humans. Only a single partner antibiotic will enter preclinical development. Studies will include: (1) acute and repeated dose toxicity studies in 2 species with local irritation assessments; (2) single dose PK studies in 2 species; (3) genotoxicity including Ames and chromosomal aberration studies. Preclinical development studies will be out-sourced to a contract research organization, which is a standard practice in the Industry. We have chosen [redacted] (see accompanying letter), which will provide cost effective support for the project.

Single dose MTD determination will be performed in 24 Sprague Dawley rats divided into four groups (3/sex/group). ADEP4 will be delivered by IV injection in combination with the standard dose of the partner antibiotic in an up/down procedure with 2 days between doses. Clinical observations are performed twice daily with body weights determined pre-dose and on day 7. Terminal blood draws are performed for clinical chemistry and hematology, along with gross necropsy. Organ weights are determined by standard by ICH/OECD guidelines and histopathology is performed on lesions as applicable.

Next, a 7-day repeat dose range-finding study in Sprague Dawley rats will be performed. This study will identify acceptable dosing for the 14 day repeat dose GLP toxicity study. The 7-day repeat dose range-finding study will be similar to the MTS determination except body weights will be measured daily. Provided reports include all observations and dose administration tables for in-life phase, as well as clinical pathology, gross observations, and pathology results, as applicable.

A 14 day repeat dose GLP toxicity study will also be performed using Sprague Dawley rats. Low, middle and high doses of ADEP4 will be given along with standard fixed dosing of the partner antibiotic, daily for 14 days by IV injection. Local irritation assessments will be performed at the site of injection. Each group will contain 10 male and 10 female rats and the study will also include vehicle control groups, bringing the total number animals to 80 for this study. Blood sampling will be performed on the first and last day of the dosings for analytical dose verifications. Food consumption and body weight will be measured weekly. Opthamalic examination will be performed prior to the first dose and during week 2. Clinical pathology will be performed at sacrifice and include standard panels include for clinical chemistry, hematology, coagulation, and urinalysis. Necropsy will also be performed and tissues will be stored in formalin prior to histopathology.

[redacted]

Single dose PK studies will also be performed in rats [redacted] For Sprague Dawley rats, 6 animals will be required per group. Doses of ADEP4 will be delivered by IP and IV injection in combination with a standard single dosing regimen of the partner antibiotic. ADEP doing will be selected based single dose MTD experiments that were previously performed using this species. High and low ADEP doses will be selected for each route of administration and 24 animals will be required. Blood plasma will be sampled at 15, 30, 60, 120, 240, 360, 720 and 1440 minutes. Drug levels will be determined using LC-MS/MS and standard PK parameters for each antibiotic will be reported including oral bioavailability, elimination half-life, peak plasma concentration (C_{Max}), area under the curve (AUC) and time above MIC (T>MIC). [redacted]

Genotoxicity will be tested using the Ames reverse mutation assay in *S. typhimurium* and *E. coli*. The tests are conducted using strains of *Salmonella* containing a mutation in the histidine gene and a strain of *E.coli* containing a mutation in the tryptophan gene. In this assay, the auxotrophic strains are exposed to the chemical of interest. The cells are plated on auxotrophic minimal media, such as His-, and the number of revertants are counted and compared to untreated and mutagenic chemical controls. The number of mutants represent the genotoxic potential of the test compound. 6 concentrations of ADEP4 in combination with the partner antibiotic will be tested. Chromosomal aberration assays will also be performed with the Chinese hamster ovary (CHO) cell line. The cells will be exposed to the ADEP4/antibiotic combination at three concentrations. Metaphase cells in the test groups and negative controls will be examined for structural chromosomal aberrations using karyotyping. A confirmatory assay will be performed with an extended period of exposure in the event of negative results. A similar study will be performed using human lymphocytes.

Pitfalls and alternatives. Antiinfective drug discovery and development are inherently risky. We have attempted to mitigate some of these risks by discovering unique properties of a known agent. These properties, namely the ability to sterilize biofilm and stationary populations of cells, have the potential to solve a large unmet medical need. ADEP4 was previously optimized, but its development was discontinued. It is effective in multiple animal models and its target is known and unique. By combining ADEP4 with other antibiotics we expect the potential for resistance development to be low. [redacted]

Project management. Dr. Ken Coleman, PI, will be responsible for the overall oversight of the project, including communications between Arietis; our consultants, Steven Leonard and Kim Lewis of Northeastern University; [redacted] and the NIH. The team will hold monthly conference calls, and yearly meetings in Boston to discuss problems and progress of the project. Dr. Coleman has many years of experience leading antibiotic drug discovery and development programs at large and small pharmaceutical companies. The end result of this project will be a validated lead combination that will enable us to enter into a partnership with a Pharmaceutical Company for additional preclinical development studies leading towards an IND, clinical trials, marketing and sales of a new therapeutic which targets chronic infections.

Vertebrate Animal Section

These studies will be conducted at Boston University Medical Center by Arietis staff under animal assurance number [redacted] following protocols [redacted], which were approved by Boston University Institutional Animal Use and Care Committee on May 29 , 2013 and January 29th, 2013 respectively. Additional studies described in the proposal will be outsourced [redacted]

1) Provide a detailed description of the use of animals in the work previously outlined in the experimental design and methods section. Identify species, strains, ages, sex, and numbers of animals to be used.

The aim of the animal studies is to determine pharmacokinetics and efficacy of ADEP4/antibiotic combinations following *in vitro* evaluation.

We will begin by performing a detailed PK study of ADEP4 in combination with 2 known antibiotics, which will inform the dosing for efficacy. For the vast majority of antibacterials, the salient pharmacokinetic driver for efficacy is the *Area Under the Free Plasma Concentration Time Curve* (fAUC) and the usual pharmacodynamic driver is *Minimum Inhibitory Concentration* (MIC), with an fAUC/MIC value of 125 usually sufficient for effective therapy with suppression of resistant isolates. The plasma pharmacokinetics of ADEP in combination with 2 antibiotic partners will be determined following dosing by the IV and IP routes. Blood will be withdrawn by submandibular bleeds at 15, 30, 45, 60, 90, 270 and 480 minutes post-dose using microsampling and not to exceed IACUC blood volume guidelines. At 1440 minutes post-dose, the mice will be euthanized and bled out by cardiac puncture. ADEP4 will be dosed at 25 mg/kg, 50 mg/kg and 100 mg/kg to determine whether PK is linear. The chosen antibiotic partners will be dosed using the mouse equivalent of the standard human dose. Plasma concentrations will be quantified using LC-MS/MS. The PK will inform dosing strategy for subsequent efficacy studies. Following this design, 40 mice will be required for the PK study. 6-8 week old female C57BL/6 mice will be used for the PK study.

Treatment	# Samples	Mice	Route I	Route II	Total
ADEP4 (25) + Antibiotic 1	8	3	IV	IP	6
ADEP4 (50) + Antibiotic 1	8	3	IV	IP	6
ADEP4 (100)+Antibiotic 1	8	3	IV	IP	6
ADEP4 (25) + Antibiotic 2	8	3	IV	IP	6
ADEP4 (50) + Antibiotic 2	8	3	IV	IP	6
ADEP4 (100)+Antibiotic 2	8	3	IV	IP	6
10% excess					4
				Total	**40**

Efficacy. We have used a murine abscess model as a model of recalcitrant infection. The murine staphylococcal thigh lesion has been a standard *in vivo* test for antimicrobials for fifty years or more. In this model, an appropriate inoculum is introduced into the thigh muscle of the mouse to form an abscess. The infection remains localized for 4 or more days, causing enlargement of the thigh but no systemic infection. In most reported studies, chemotherapy commences within 2h of infection, as delaying therapy beyond this point usually results in failure to cure. In our hands, using immunocompromised mice and delaying therapy until 24h post-infection results in a highly recalcitrant infection where high dose vancomycin or rifampicin dosed twice/day IP for 2 days gives less than a 2-log reduction in viable count relative to an untreated control, while a single dose combination of rifampicin with ADEP4 cleared the infection. We will continue to use this recalcitrant abscess model to evaluate two ADEP4/antibiotic combinations. Initial studies will use the level of ADEP4 that was effective in the preliminary work (50 µg/ml; ½ of MTD), and the standard mouse adapted human equivalent of the partner antibiotic to demonstrate efficacy, and later studies on successful combinations will focus on optimizing the ratio of the partners and the best dosing regimen for cure. Each

Treatment	Mice
Vehicle	5
Antibiotic 1 control	5
Antibiotic 2 control	5
Antibiotic 1 + ADEP4	5
Antibiotic 2 + ADEP4	5
ADEP4 alone	5
rifampicin + ADEP4	5
10% excess	4
Total	**39**

study will contain the following test groups: vehicle control, antibiotic alone, ADEP4 alone, antibiotic + ADEP4, ADEP4 + rifampicin (positive control). Following this design 39 animals will be required for the initial efficacy study. 6-8 week old female ICR/Swiss mice will be used for efficacy studies.

Next the combinations will progress to an *in vivo* biofilm model [1]. In a mouse TC model, a staphylococcal biofilm is not cleared with three broad-spectrum antibiotics dosed either alone or in combination [2] but we anticipate that the lead combination from our treatment optimization studies will clear the biofilm in this model. Briefly, a sterile TC containing sintered glass beads is implanted subcutaneously in the back of the anaesthetized mouse. Two weeks after surgery, the TC is verified to be sterile, the mice are then immunocompromised with cyclophosphamide (150 mg/kg), and 200 µL of a *S. aureus* culture is introduced into the TC and the animals are left for 14 days to allow the infection to stabilize. Mice are then dosed for 7 days and euthanized to allow removal of the TC. Bacterial counts will then be performed on the TC fluid and on the glass beads (following washing and sonication). Lucet *et al.* used a fixed dose of each agent for their studies and our initial efficacy study will directly compare their combination of rifampicin (25 mg/kg) + vancomycin (50 mg/kg) with our chosen test combination. An infected untreated control group will also be studied. Following this design 84 animals will be required for the tissue cage study. 6-8 week old female ICR/Swiss mice will be used for these studies.

Treatment	Mice
Vehicle	10
Antibiotic 1 control	10
Antibiotic 2 control	10
Antibiotic 1 + ADEP4	10
Antibiotic 2 + ADEP4	10
ADEP4 alone	10
rifampicin + vancomycin	10
20% excess	14
Total	**84**

Once an initial efficacious dose of ADEP4 in combination with each antibiotic is validated, it will be possible to determine the PD driver for ADEP4 using the methods described by Craig [3]. Using the neutropenic mouse model described above, groups of 5 mice will be treated for 1 day with a range of 4 ADEP4 administrations based around the PK and the effective dose from the initial efficacy experiment. 50 mg/kg ADEP4 sterilized in combination with rifampicin so doses above and below this dose were selected as an example. ADEP4 doses above the MTD will not be tested, e.g. 4x q24h. Groups of 5 mice will also be required for untreated, ADEP4 alone and antibiotic alone groups. Doses will be fractionated to allow administration of each daily dose q24h, q12h, q8h and q6h to give a broad range of AUC, T>MIC and C_{Max} values. The known antibiotic will be administered to test groups using standard dosing adjusted for the combined PK similar to the initial efficacy study above. One day after the final dose, thigh burden will be determined as previously described. Plotting pathogen count at each dose against AUC, T>MIC and C_{Max} will reveal the PK parameter which most closely correlates with efficacy – the PKPD driver. Following this design, a total of 105 mice will be required. 6-8 week old female ICR/Swiss mice will be used for these efficacy studies.

Treatment	Antibiotic (standard dose)	Total
Vehicle	N	5
Antibiotic control	Y	5
ADEP4 alone (~50 mg/kg)	N	5
ADEP4 (**0.5x** mg/kg q24h)	Y	5
ADEP4 (**0.5x** mg/kg q12h)	Y	5
ADEP4 (**0.5x** mg/kg q8h)	Y	5
ADEP4 (**0.5x** mg/kg q6h)	Y	5
ADEP4 (**1x** mg/kg q24h, q12h, q8h, q6h)	Y	20
ADEP4 (**2x** mg/kg q24h, q12h, q8h, q6h)	Y	20
ADEP4 (**4x** mg/kg q12h, q8h, q6h)	Y	15
Uninfected	N	5
10% excess	N	10
	Total	**105**

Single dose maximum tolerated dose determination will be performed in 24 Sprague Dawley rats divided into four groups (3/sex/group). The rats will be prepubertal to young adult, approximately 7-9 weeks of age and approximately 150-400 g at the time of randomization. ADEP4 will be delivered by IV injection in combination with the standard dose of the partner antibiotic in an up/down procedure with 2 days between doses. Clinical observations are performed twice daily with body weights determined pre-dose and on day 7. Terminal blood draws are performed for clinical chemistry and hematology, along with gross necropsy. Organ weights are determined by standard by ICH/OECD guidelines and histopathology is performed on lesions as applicable.

Single dose MTD (acute) in Rats		
Sprague Dawley Rats	*Males*	*Female*
dose level 1	*3*	*3*
dose level 2	*3*	*3*
dose level 3	*3*	*3*
dose level 4	*3*	*3*
		total N = 24

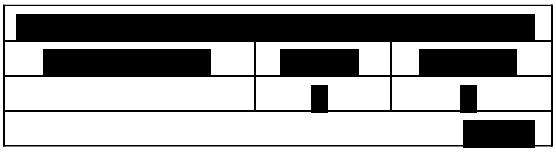
A 7-day repeat dose range-finding study in Sprague Dawley rats will be performed. The rats will be prepubertal to young adult, approximately 7-9 weeks of age and approximately 150-400 g at the time of randomization. This study will identify acceptable dosing for the 14 day repeat dose GLP toxicity study. The 7-day repeat dose range-finding study will be similar to the MTD determination except body weights will be measured daily. Provided reports include all observations and dose administration tables for in-life phase, as well as clinical pathology, gross observations, and pathology results, as applicable.

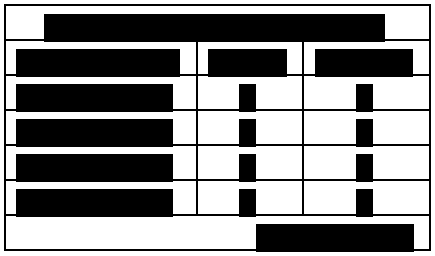
Repeat dose MTD in Rats		
Sprague Dawley Rats	*Males*	*Female*
dose level 1	*3*	*3*
dose level 2	*3*	*3*
dose level 3	*3*	*3*
dose level 4	*3*	*3*
		total N = 24

A 14 day repeat dose GLP toxicity study will also be performed using Sprague Dawley rats. Low, middle and high doses of ADEP4 will be given along with standard fixed dosing of the partner antibiotic, daily for 14 days by IV injection. The rats will be prepubertal to young adult, approximately 7-9 weeks of age and approximately 150-400 g at the time of randomization. Local irritation assessments will be performed at the site of injection. Each group will contain 10 male and 10 female rats and the study will also include vehicle control groups, bringing the total number animals to 80 for this study. Blood sampling will be performed on the first and last day of the dosings for analytical dose verifications. Food consumption and body weight will be measured weekly. Opthamalic examination will be performed prior to the first dose and during week 2. Clinical pathology will be performed at sacrifice and include standard panels include for clinical chemistry, hematology, coagulation, and urinalysis. Necropsy will also be performed and tissues will be stored in formalin prior to histopathology.

Sprague Dawley Rats	*Males*	*Females*
Control	*10*	*10*
Low	*10*	*10*
Mid	*10*	*10*
High	*10*	*10*
		total N = 80

[redacted]





Single dose PK studies will also be performed in rats [redacted]. For Sprague Dawley rats, 6 animals will be required per group. Doses of ADEP4 will be delivered by IP and IV injection in combination with a standard single dosing regimen of the partner antibiotic. ADEP doing will be selected based single dose MTD experiments that were previously performed using this species. High and low ADEP doses will be selected for each route of administration and 24 animals will be required. Blood plasma will be sampled at 15, 30, 60, 120, 240, 360, 720 and 1440 minutes. Drug levels will be determined using LC-MS/MS and standard PK parameters for each antibiotic will be reported including oral bioavailability, elimination half-life, peak plasma concentration (C_{Max}), area under the curve (AUC) and time above MIC (T>MIC). [redacted]

Single Dose PK Study in rats					
Species	*Dose Route*	*Animals per group*	*Doses per Group*	*Collection Time points*	*Sample Collection*
Sprague Dawley Rats	*IV*	*6*	*4*	*8 time points*	*plasma*
					N = 24

[redacted]					
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
					[redacted]

2) Justification for the use of animals.

The use of animals in a drug discovery pipeline is essential for prioritization of lead compounds into development candidate drugs for humans. Additionally, animal safety and efficacy data are a regulatory requirement in the majority of countries. Many potential drugs fail for PK reasons. Understanding the factors that lead to good PK properties is an important part of the design and selection of drug candidates.

Justification for animal numbers.

For PK studies, typically an 8 time-point pharmacokinetic profile is generated using 3-6 animals per group. We will use 3 mice per group in order to minimize the number of animals used based on similar studies using the same model to study drug efficacy and PK testing of approved antibiotics [3, 4]. For example, using three animals per group, there was 80% power to detect a difference of 25 ml/min/kg in the clearance of midazolam, at a significance level of 0.05 [5]. Three animals per group was also used to calculate the PK profile for a pre-clinical anti-*H. pylori* evaluation of the antibiotic finafloxacin [6].

[redacted]

[redacted]

For efficacy studies, we will need 5 mice per treatment group in order to produce statistically relevant results. The number of mice per group is based on similar studies using the same model to measure antibiotic efficacy, which used 5-10 mice per group [7]. We will use the lowest number of mice which has conferred statistical significance in this model. Based on variability of this model described in the literature, we expect to detect at least a 90% decrease in virulence with a probability of 80% at a two sided 5% significance level using 5 animals per group. Since we aim to eradicate infection, we expect our combination therapies to produce more than 90% decreased virulence compared to negative controls and individual antibiotics, serving as an excellent indicator of efficacy at this stage of development. The Mann-Whitney rank sum test will be used to determine differences in the median values of the bacterial count per thigh between antibiotic treated mice and controls.

For tissue cage studies, we will require 10 mice per group in order to produce statistically relevant results. The larger number of mice per group compared to the thigh model is a result of the higher variability of this model. Based on variability of this model described in the literature, we expect to detect at least a 99% decrease in virulence with a probability of 80% at a two sided 5% significance level using 10 animals per group. As most antibiotics are not effective in this model, and detection of a 2-log reduction in CFU with a probability of 80% will be sufficient to justify further evaluations for our combined therapeutic. The Mann-Whitney rank sum test will be used to determine differences in the median values of the bacterial count per thigh between antibiotic treated mice and controls.

These animal studies will be performed at Boston University Medical Center which is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and operates in full compliance with all federal and state regulatory agencies.

3) Veterinary care of the animals involved.

The animals studies performed by Arietis will be housed in the AAALAC accredited facilities of the Boston University Medical Campus (BUMC). The animal facilities [redacted] comprise approximately 45,780 sq feet of animal housing and support space. The Laboratory Animal Science Center is a team of administrative, managerial, technical and professional staff committed to the advancement of science in collaboration with the research community by promoting the humane care and use of animals used in biomedical research and teaching. The Laboratory Animal Science Center (LASC) oversees veterinary medical care, animal care services, and actively participates in all facility design. Day-to-day care of animals and technical assistance are provided by approximately thirty animal caretakers. The care staff is overseen by Operations Managers and Animal Care Supervisors. In addition, three veterinary technicians are supervised by a Veterinary Services Manager, under the direction of the Attending Veterinarian. Administrative, business and purchasing functions are supported by a Business Manager and three support staff. Use of these animal facilities is included in the BioSquare lease that Arietis has negotiated with BUMC. Standard Operating Procedures and reference materials are available from the IACUC Office for animal use. The animal health program for all Boston University owned laboratory animals is directed by the attending veterinarian, [redacted], and provided by two full-time veterinarians. All studies involving animals will be performed in the Laboratory Animal Sciences Center (LASC). Animals in each LASC room are observed daily for signs of illness by the animal technician responsible for providing husbandry. Medical records and documentation of experimental use are maintained individually for non-rodents and individually or by cage group for rodents. Veterinary technicians under the direction of the attending veterinarian provide routine veterinary medical care to all animals. Animal care and use is additionally monitored for training and compliance issues by the Training and Compliance Manager. [redacted] Staff from Northeastern University and Arietis have previous experience with the murine thigh abscess model and Arietis has a qualified veterinary surgeon on staff.

[redacted]

[redacted]

4) Describe procedures for ensuring that discomfort, distress, pain, and injury will be limited to that which is unavoidable to conduct scientifically sound research. Describe the use of analgesic, anesthetic, and tranquilizing drugs and comfortable restraining devices, where appropriate, to minimize discomfort, distress, pain, and injury.

For studies performed by Arietis, all IACUC policies on humane endpoints will be followed. Animals will be monitored at least every 24 hours for signs of distress or pain. If pain or distress is detected, the choice of appropriate analgesic will be made upon consultation with the attending veterinarian. For example, pharmaceutical grade carprofen may be given orally at 5 mg/kg every 12-24 h as needed. Sedation is required for the manipulation of each mouse prior to thigh injections. Isoflurane will be administered at 1-3% inhalant to effect (up to 5% for induction). The animals will not be allowed to reach a moribund state. However if for some reason the animals reach a moribund state, they will be immediately euthanized. Animals observed experiencing the following humane endpoints will be euthanized: hunched posture,sunken eyes, with or without discharge; respiration that has increased, decreased, or appears labored; cyanosis (blue color to skin or mucous membranes); hypothermia or hyperthermia; ruffled hair coat, erection of hair or fur, lack of grooming behavior; diarrhea or constipation; prolonged bleeding from any orifice; self-mutilation; no response to external stimuli; vomiting; unsteady gait or lameness not induced by experimental manipulation; ulcerated tumors; severe or ulcerative dermatitis; inability to reach food and/or water; inability to remain upright; refusal of food and water monitored through disappearing from bottle and feed container; clinical dehydration and/or prolonged decreased food intake (more than 48 hours); muscle atrophy and signs of lethargy and lack of physical activity; Decreased body condition score(2.5 or less on a scale of 1-5; mouse in not well conditioned, vertebral column may be prominent). The animals will be observed at least once daily during the workdays, and at least once daily on holidays and weekends. An animal entering, but not yet in, the moribund state will be euthanized if the next observation is going to be after a period longer than 12 hours.

[redacted]

5) Describe any euthanasia method to be used and the reasons for its selection. State whether this method is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association.

For studies performed by Arietis, every animal will be euthanized by CO_2 inhalation, with secondary euthanasia by cervical dislocation. Methods for euthanasia are consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association and the Boston University animal welfare regulations and policies.

[redacted]

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Northeastern University
Bouvé College of Health Sciences

August 1, 2013

Dr. Ken Coleman
Arietis Corporation
650 Albany St, [redacted]
Boston, MA 02118

Dear Ken,

School of Pharmacy

Department of Pharmacy Practice

360 Huntington Ave., R218TF
Boston, MA 02115

[redacted]

I am happy to consult on your project, "Antibiotics for Recalcitrant Infection." As you know, I specialize in pharmacology and specifically using the hollow-fiber model to test the effectiveness of antibiotics against *S. aureus*. It is clear from the hollow-fiber study that ADEP4 has exceptional killing properties and I was not surprised that these results translated nicely into animal efficacy. In my opinion, the resistance studies that you propose are well designed and will also be predictive whether resistance development for your combined therapeutic will be a concern. Of course, these experiments will also be useful in prioritizing which antibiotic partner to choose. In addition to the hollow-fiber model, I can assist on many other aspects of the proposal including PK/PD and dosing for *in vivo* efficacy studies. I wish you luck with this important application and I look forward to continuing to work on this exciting project together.

Sincerely,

[redacted]

Steven N. Leonard, Pharm.D.
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Northeastern University School of Pharmacy
Clinical Pharmacist, Infectious Diseases
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Kenneth Coleman, Ph.D. 8/1/2013
Arietis Corporation
650 Albany St., [redacted]
Boston, MA 02118

Dear Ken,

I will be happy to continue our collaboration on developing a sterilizing therapeutic based on ADEP, and act as a consultant for your SBIR project. As you know, the focus of my lab is on mechanisms of antimicrobial drug tolerance and persister cells. What we learned over the years is that the mechanisms that form drug-tolerant persisters are redundant, and no realistic drug target emerged from our studies. The only thing that would sterilize a stationary or biofilm population of cells in our hands was peracetic acid. The finding that the ClpP activator, ADEP forces cells to self-digest and sterilizes a mouse deep-seated infection in combination with rifampicin is truly exciting. I think we finally have a good candidate to address untreatable chronic infections.

Best wishes,

[redacted]

We will disseminate the results of this study by publishing it in professional Journals such as Antimicrobial Agents and Chemotherapy, and by presenting at professional meetings (ICAAC). [redacted]

PHS 398 Checklist

OMB Number: 0925-0001

1. Application Type:

From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

☐ New ☒ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

Federal Identifier: [redacted]

2. Change of Investigator / Change of Institution Questions

☐ Change of principal investigator / program director

Name of former principal investigator / program director:

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

☐ Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For renewal applications only)

* Inventions and Patents: Yes ☐ No ☐

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

* Previously Reported: Yes ☐ No ☐

4. * Program Income

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)

5. * 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 e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

☒ Yes ☐ No