AWARD NUMBER: W81XWH-18-1-0765

TITLE: Aerosol Delivery of CPZEN-45 for Treatment of Nontuberculous Mycobacterial (NTMs) Infections

PRINCIPAL INVESTIGATOR: Dr. Gail Cassell

CONTRACTING ORGANIZATION: Infectious Disease Research Institute SEATTLE, WA 98102

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6. AUTHOR(S) Gail Cassell, PhD; Diana Severynse-Stevens, PhD; Diane Ordway; Darrick Carter, PhD; E-Mail: Gail.Cassell@idri.org; Grant@IDRI.org		**5d. PROJECT NUMBER** **5e. TASK NUMBER** **5f. WORK UNIT NUMBER**
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Infectious Disease Research Institute 1616 Eastlake Ave E, Ste 400 Seattle WA 98102-3797		**8. PERFORMING ORGANIZATION REPORT**
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13. SUPPLEMENTARY NOTES		

14. ABSTRACT

This DOD Therapeutic Development Award is focused on a new antibiotic, CPZEN-45, discovered by our team for treatment of non-tuberculous mycobacterial infections (NTM) in patients with chronic obstructive pulmonary disease (COPD). We have two major objectives: **Objective 1**-To optimize fermentation and scale-up of manufacturing processes for high yield of CPZEN-45, including spray dried CPZEN-45. We have optimized fermentation and scale-up of CPZEN-45 and the processes have been successfully transferred to our manufacturing partner. **Objective 2**- To further define and characterize *in vitro* efficacy of CPZEN-45 against additional species of NTMs recently isolated from VA patients with COPD. We have obtained 26 recent NTM isolates from COPD patients (VA) Aurora, CO), phenotype and titers were determined, and DNA extracted for whole genome sequencing to evaluate phenotypic and genotypic correlation with resistance. Our objective to develop an efficacious regimen for COPD patients has started by screening multiple CPZEN-45 combinations with standard NTM compounds in human THP-1 cells using a checkerboard assay. **Synergy measurement by checkerboard analysis** will be used to determine the impact on potency of the combination of antibiotics in comparison to their individual activities. The optimized synergistic regimens will then be tested in COPD mouse and guinea pig efficacy models.

15. SUBJECT TERMS

Chronic Obstructive Pulmonary Disease, Veterans, CPZEN-45, Non-tuberculosis mycobacteria (NTM), NTM New Antibiotic Therapy, animal infection models, *M. avium*, *M. abscessus*.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRMC
a. REPORT U	**b. ABSTRACT** U	**c. THIS PAGE** U	UU		**19b. TELEPHONE NUMBER** *(include area code)*

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TABLE OF CONTENTS

1. INTRODUCTION:

Non-tuberculous mycobacteria (NTM) are environmental bacteria found commonly in soil, water, and biofilms. Chronic lung disease is the most frequent disorder caused by NTM; moreover, NTM lung infections not uncommonly complicate individuals with chronic obstructive pulmonary disease (COPD, aka emphysema). The incidence and prevalence of NTM lung disease (NTM-LD) in the U.S. is increasing yearly and now surpasses that of tuberculosis (TB). Veterans are three times more likely to develop COPD and NTM infection than the general population. NTM-LD is often treated for at least 18-24 months with at least three and sometimes a four or more-drug regimen. Despite this intense regimen – reflecting the high resistance of NTM to available antibiotics – the long-term cure rate is at best ~50% as the relapse rate is high. Thus, new antibiotics are urgently needed. Members of our research team have discovered a new chemical entity, CPZEN-45, which has been shown to have a novel mechanism of action. It is considered highly promising because it has been shown: (i) to directly kill many pathogenic species of NTM (both drug sensitive and drug resistant), (ii) to have efficacy in laboratory animals experimentally infected with NTM, (iii) to possess an acceptable toxicity profile, and (iv) to be able to be delivered directly to the lungs as a dry-powder. Before CPZEN-45 can be studied in patients with NTM-LD, we must do further pre-clinical work by making sure we can produce sufficient quantities of high quality CPZEN-45 as well as supply large amounts of the compound to do further testing in animals to further ensure efficacy and safety.

2. KEYWORDS:

Chronic Obstructive Pulmonary Disease, Veterans, CPZEN-45, Non-tuberculosis mycobacteria (NTM), animal infection models, NTM New Therapy, *M. avium, M. abscessus.*

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Goal 1: Optimize fermentation and scale-up of manufacturing processes for CPZEN-45, including spray dried CPZEN-45.

Goal 2: Define and characterize in vitro and in vivo efficacy of CPZEN-45 against NTM recently isolated from VA patients with COPD using our well characterized COPD mouse models and to evaluate CPZEN-45 inhaled therapy using a chronic NTM model in guinea pigs.

What was accomplished under these goals?

SUMMARY OF ACHIEVEMENTS RELATIVE TO SOW [See Appendix for supporting data (Figures 1-10 and Tables 1-2) and the SOW]:

Specific Aim 1: Optimize fermentation and scale-up of manufacturing processes. PROJECT LEADERS and SITES [PROJECT LEADERS – G. Cassell and D. Carter, Site 1- Infectious Disease Research Institute; T. Zhu, Site 2- Hisun Pharmaceuticals; M. Shibasaki, Y. Ishizaki, and K. Yamazaki, Site 2A, Institute for Microbial Chemistry (IMC); D. Stevens and A. Hickey, Site 4 - Research Triangle Institute (RTI)]

Major Task 1 – Improve drug substance yield to reduce cost. Our major accomplishment in Year 01 has been to improve the yield of CPZEN-45 and to successfully transfer manufacturing processes for CPZEN-45 from IMC to Hisun Pharmaceuticals, our manufacturing partner. IMC performed the production of 4 (500g total) of CPZEN-45 fromCaprazene, starting material. Caprazene was supplied from the mixture of caprazamycins A-G (CPZs) obtained by fermentation by Hisun. The general and synthesis of caprazene from CPZs yielded 500 g of highly pure CPZEN-45 **(Appendix, Figures 1-8)** with demonstrated activity against well characterized, standard strains of NTM **(Appendix, Table 1)**. Furthermore, Hisun independently manufactured 100g of highly pure and efficacious CPZEN-45 including large scale manufacturing of the intermediates and final synthesis of CPZEN-45. The MIC values for two different species of NTM and the reference Mycobacterium tuberculosis strain H37RV were identical for each of the different batches. Two CPZEN-45 HCl samples [(HISUN, S181121) and (IMC standard, Lot16-2)] were recently analyzed by IMC and compared using two HPLC analysis methods (IMC method and HISUN method). No significant difference was found in the contents of the main component and the impurity between the two samples. Based upon the HPLC and MIC analyzes, we can state unequivocally that the transfer of technology for manufacturing of CPZEN-45 were successfully transferred from IMC to HISUN. During the past 6 months, in order to continue to improve the productivity of caprazamycins which are parent compounds of CPZEN-45, IMC applied the genetical technique called ZouA method to a caprazamycins producing strain Streptomyces sp. MK730-62F2. This method enables one to drastically increase the copy number of a biosynthetic gene cluster of certain antibiotics. By using this technique, IMC succeeded to enhance the copy number of caprazamycin biosynthetic gene cluster to 30 copies/genome and the the resulting strain produced 5 times higher concentrations of caprazamycins than the parent strain. IMC is now trying to construct some other strains which can keep the copy number of the cluster more stable and which show even higher yields of caprazamycins. Milestones Achieved- Drug Product Manufacturing improvements 60% completed and technology successfully transferred to Hisun.

Major Task 2- Transfer spray-drying method to Contract Manufacturing Organization, produce drug product for animal studies and develop drug product processes for GMP scale-up: No progress to report. Studies will not begin until Year 2.

Specific Aim 2 - Define and characterize in vitro and in vivo efficacy of CPZEN-45 against clinical NTM isolates PROJECT LEADERS and SITES [G. Cassell and D. Carter, Site 1-IDRI; D. Ordway, Site 3- Colorado State University (CSU); E. Chan, Site 3A-Denver Veterans Administration and National Jewish Hospital; D. Stevens and A. Hickey, Site 4 -RTI; M. Braunstein, Site 5- University of North Carolina (UNC)].

Major Task 3 - Quantify CPZEN-45 activity against a representative panel of recent clinical isolates: Collect and speciate NTM clinical isolates from VA COPD patients - 100% completed; Characterize phenotypically and genotypically clinical isolates – all clinical isolates phenotypically characterized and whole genome sequencing completed on 30% of clinical isolates; Determine i*n vitro* susceptibility of clinical isolates to CPZEN-45 – 100% completed; Establish optimal combination for treatment regimen by checkerboard titration – 100% completed once. Repetition for confirmation is ongoing. Milestone Achieved: Recent clinical isolates from COPD patients collected and characterized phenotypically and over half of isolates characterized genotypically. Repetition for confirmation is ongoing. Milestone Achieved: Recent clinical isolates from COPD patients collected and characterized phenotypically and over half of isolates characterized genotypically. Determination of optimal combination for in vivo treatment regimen by checkerboard titration 50% completed. Specifically, Dr. E. Chan at the Denver VA, has obtained 30 isolates from VA/UCH (Colorado) and ten isolates were obtained from VA (NYU) for the CPZEN-45 study. No patient information is associated with the isolates. MALDI-TOF MS was used to identify isolates. NTM strains were from COPD patients infected with *M. abscessus* (mixed rough/smooth), *M. massiliense* (mixed rough/smooth) and *M. bolletii*. Dr.Ordway has grown the NTM strains to high titers, bottled, frozen and quantified the bacterial colony forming units (CFU). She has completed THP-1 cell checkerboard MICs against *M. abscessus* 103 showing CPZEN-45 was synergistic with improved MICs when combined with Clofazimine, Amakacin, Vancomycin, Cefepime, and Rifampicin **(Appendix, Figure 9; Table 2).** Dr. M. Strong of NJH has established the phylogenetic tree of clinical isolates of NTM, using results of whole genome sequencing of the first10 isolates from VA patients. Samples were sequenced on the MiSeq (2x300bp), data processed by genomic and phylogenomic analysis. The read coverage was adequate for all isolates (35x to 116x). All 5 MAB isolates are confirmed as *M. abscessus* subspecies *abscessus* based on this analysis. The isolates are phylogenetically diverse membersof *M. abscessus* subspecies *abscessus* (indicated in re in the phylogenetic tree generated and shown in **Appendix, Figure 10**). All 5 MAC isolates are confirmed as *M. avium* based on this analysis, and are phylogenetically diverse. Targeted analysis of specific gene variation also, pertinent to drug activity in Year 02.

Major Task 4 - Evaluate efficacy in animal models: ACURO review of mouse and guinea pig protocols completed and aproved. Evaluation of CPZEN-45 in COPD NTM murine models, and aerosol efficacy in guinea pig model will begin Year 02.

What opportunities for training and professional development has the project provided?

Nothing to report

How were the results disseminated to communities of interest?

A meeting of the teams involved in the DOD grant occurred on 09/24-09/26, 2019 at National Jewish Heath, Denver, Colorado to review progress for Year 01 and to discuss future studies to accomplish the grant objectives. A major emphasis was evaluation of drug susceptibility data on recent clinical isolates of NTM from VA patients with COPD and selection of strains to use for animal infectivity and efficacy testing. In addition to project directors from CSU, NJH and Denver VA, IDRI, 3 international NTM expert consultants (S. Hoffner, Karolinska Inst., M. Salfinger, Univ.So.FL., and P. Brennan, CSU) attended and others from IDRI and RTI participated by Zoom. A meeting with the experts and physicians from NJH and the VA was organized to discuss the importance of NTM infections in veterans. In addition, S. Hoffner presented a seminar ("Epidemiological Trends of NTM in Europe and a Second Look at the Role of in vitro Drug Susceptibility Testing and How to Do IT") to over 50 physician specialists, residents and fellows, graduate students, and basic scientists attended.

What do you plan to do during the next reporting period to accomplish the goals?

Specific Aim 1

There are two goals for IMC in Year 02. One is for IMC to continue to elucidate the mechanism of action of CPZEN-45 on NTM. This includes the elucidation of drug resistance mechanisms. IMC will also conduct a comparative analysis of the titers, metabolites, and impurities between the production strain improved by Hisun and the original production strain of IMC, and if possible, genetic analysis of the strains. IMC will perform genome sequencing of MK730-62F2 strain and construct plasmids for induction of zouA-RsA and RsB with certain drug resistant genes.
Also, as set out in the original research plan, in the following 6 months we will begin spray drying to generate material for the small animal studies in Aim 2. Material will be analyzed for particle size and uniformity as well as chemical composition and concentration. On completion of these studies, material for the mouse and guinea pig challenges will be available and we will be positioned to begin scale-up activities with Recipharm. Specifically, IDRI and Research Triangle Institute International (RTI) will:

a. spray dry CPZEN-45 for efficacy evaluation in the guinea pig inhalation study.
b. develop processes suitable for scaling to spray drying kilogram quantities of CPZEN-45 to form 1-5μm aerodynamic diameter particles suitable for delivery by inhalation to be used in future cGMP inhalation toxicology studies using NIH RO1 funds.
c. Batch fed spray drying will be used to produce gram scale lots that will be combined after suitable characterization to ensure each lot is comparable. Characterization will include particle size and drug content as key quality parameters.

Lots produced will be placed on a stability program to provide data for longer term storage and ensure materials are stable for the duration of in-life studies

c. Methods for production, HPLC analysis of drug content, and particle size determination will be transferred to Recipharm, a contract manufacturing company that can prepare drug for nonclinical studies and cGMP drug product for clinical studies.
d. Recipharm will optimize and qualify the transferred analytical methods including HPLC analysis, particle size, moisture by Karl Fisher, and foreign particulate matter analysis.
e. Packaging will be optimized and stress tested.

a. Once method transfers are complete they will initiate a scaled spray-drying run of ~50 grams of API to demonstrate scalability of the process and produce materials for further guinea pig studies.
a. The produced material will be tested for aerosol performance using the cyclohaler and materials packaged in capsules.
b. Final, released product will be placed on a formal stability program.

Specific Aim 2
CSU will test the VA Chronic COPD NTM strains using *in vitro* MIC/MBC checkerboard macrophage assays to evaluate the single and combination treatments with CPZEN-45, Amikacin, Rifampin, Vancomycin, Ethambutol, Cefepime, and Clofazamine. This approach will allow us to understand not only the optimal MIC/MBC but also compound synergies and antagonisms to help us develop a new effective regimen to use in our *in vivo* acute and chronic mouse efficacy studies. Miriam Braunstein of the University of North Carlolina will collaborate with RTI and CSU to establish the chronic guinea pig model using a recent NTM clinical isolate from a COPD patient from Denver VA. This model will be utilized to evaluate the efficacy of CPZEN-45 delivered by aerosol.

4. **IMPACT:**
What was the impact on the development of the principal discipline(s) of the project?

Nothing to report

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS: Changes in approach and reasons for change

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

There was a substantial delay in submitting and receiving approval of animal protocols by CSU and UNC (subcontractor to RTI) as well as getting legal documents processed for establishing the Subaward Agreements.

Changes that had a significant impact on expenditures

The delay in submitting and review of animal use protocols plus delay in getting legal documents processed for establishing Subaward Agreements resulted in delayed start of work and therefore lower expenditures in Year 01 than planned. Some of these unexpended funds will be reallocated in Year 02 to purchase larger quantities of CPZEN-45 for use in establishing spray dried scale up and establishment of standards plus spray drying of more compound to perform additional mouse and guinea pig studies. The additional animal studies will result more thorough characterization of the animal models using previously untested clinical isolates from COPD patients.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to report.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Dr. Gail Cassell
Project Role: Principal Investigator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 4.2
Contribution to Project: Dr. Cassell has coordinated all work with participating organizations, participated in experimental design, and reviewed the results. She organized and chaired the annual meeting of all Project Directors and most other key personnel.
Funding Support: N/A

Name: Dr. Darrick Carter
Project Role: Scientist
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1
Contribution to Project: Steering the project, attending regular meetings, and guiding the contractor on the scale up of the spray drying.
Funding Support: N/A

Name: Anthony Hickey
Project Role: Researcher
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 0.07
Contribution to Project: Dr. Hickey is providing scientific advisory support for the spray drying activities under Specific Aim 1

Name: Diana Severynse-Stevens
Project Role: Principal Investigator of the Subcontract
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 0.37
Contribution to Project: Dr. Severynse-Stevens is performing coordination of CPZEN-45 materials and managing the relationship with UNC in support of Specific Aim 2

Name: Mr. Grayson Stowell
Project Role: Project Manager
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 0.19
Contribution to Project: Mr. Stowell is performing coordination of CPZEN-45 materials and providing project management support.

Name: Ian Stewart
Project Role: Researcher
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 1.53
Contribution to Project: Dr. Stewart is performing spray drying activities in support of Specific Aim 1.

Name: Cathy Simpson
Project Role: Project Manager
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 0.15
Contribution to Project: Ms. Simpson is providing project management support for the project.

Name: Dr. Diane Ordway
Project Role: Associate Professor
Researcher Identifier (e.g. ORCID ID): http://orcid.org/0000-0003-0003-326X
Nearest person month worked: 1.2
Contribution to Project: Dr. Ordway is responsible overall direction of then mouse model studies, for monthly conference calls, report preparation and directing staff experiments.
Funding Support: Dr. Ordway is also supported by multiple contracts and Cystic Fibrosis, NIAID R21 grants.

Name: Mr. Drew Wilson
Project Role: Student work study
Researcher Identifier (e.g. ORCID ID): None
Nearest person month worked: 1.02
Contribution to Project: Student work study preparing agar plates and broth media.
Funding Support: This DOD proposal

Name: Miss Gina Im
Project Role: Student work study
Researcher Identifier (e.g. ORCID ID): None
Nearest person month worked: 2.47
Contribution to Project: Student work study preparing agar plates and broth media.
Funding Support: This DOD proposal

Name: Mr. Jacob Gadwa
Project Role: Research Associate II
Researcher Identifier (e.g. ORCID ID): None
Nearest person month worked: 2.04
Contribution to Project: Mr. Gadwa performed THP-1 checkerboard assays.
Funding Support: Mr. Gadwa is also supported by multiple contracts and Cystic Fibrosis.

Name: Kazushige Sasaki
Project Role: Reseacher
Researcher Identifier: N/A
Nearest person month worked: 3.25 Calendar Months
Contribution to Project: Dr. Sasaki worked on the synthesis of 500 g of highly pure CPZEN-45.

Name: Katsuhisa Yamazaki
Project Role: Reseacher
Researcher Identifier: N/A
Nearest person month worked: 3.25 Calendar Months
Contribution to Project: Dr. Yamazaki worked on the synthesis of 500 g of highly pure CPZEN-45.

Name: Yashuhiro Takehana
Project Role: Reseacher
Researcher Identifier: N/A
Nearest person month worked: 3.25 Calendar Months
Contribution to Project: Dr. Takehana worked on the synthesis of 500 g of highly pure CPZEN-45.

Name: Masaki Hatano
Project Role: Reseacher
Researcher Identifier: N/A
Nearest person month worked: 5.4 Calendar Months
Contribution to Project: Dr. Hatano worked on the synthesis of 500 g of highly pure CPZEN-45.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Anthony Hickey, RTI – Key Investigator on this DOD grant has been awarded the following NIH/NIAID grant as the PI: "Development of Inhaled CPZEN-45; 03/01/2019-02/28/2023; $3,981,680. This grant is to develop CPZEN-45 for tuberculosis, specifically to fund cGMP tox studies and document preparation to support pre-IND meeting with FDA. There is no duplication with the goals of the current DOD grant. Dr. Gail Cassell and Dr. Darrick Carter are both listed as Key Personnel on a subaward from RTI to IDRI for this project with an annual effort of 1.32 Person Months and 0.6 Person Months, respectively.

What other organizations were involved as partners?

Colorado State University, Mycobacteria Research Laboratory
Department of Microbiology, Immunology and Pathology
1682 Campus Delivery
200 West Lake Street
Fort Collins, CO 80523-1682
Phone (970)-491-7840
Fax (970)-491-1815

- Financial support of staff at CSU to conduction project aims of obtaining COPD patient strains and growth of strains.
- Purchasing mouse model to conduct experiments using CPZEN-45 in THP-1 checkerboard assays and mouse infection models.

8. SPECIAL REPORTING REQUIREMENTS

QUAD CHARTS:

Aerosol Delivery of CPZEN-45 for Treatment of Non-Tuberculous Mycobacterial (NTMs) Infections

Log Number: PR171209

Award Number: W81XWH1810765

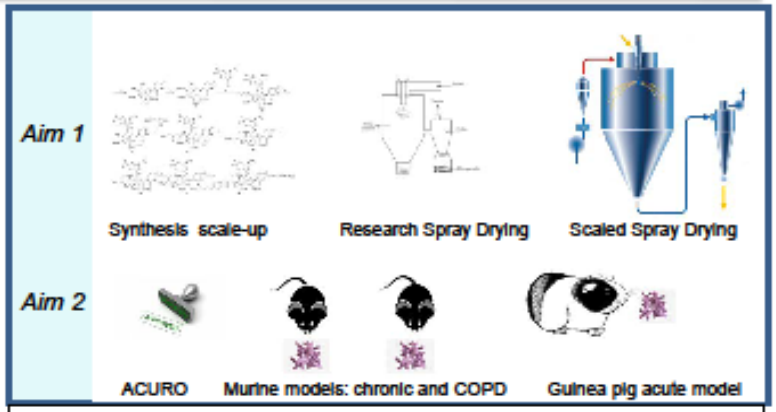
PI: Dr. Gail Cassell **Org:** Infectious Disease Research Institute **Award Amount:** $ 3,273,025

Study/Product Aim(s)

- Study Aim 1: Optimize fermentation and scale-up of manufacturing processes for CPZEN-45, including spray dried CPZEN-45.
- Study Aim 2: Define and characterize in vitro efficacy of CPZEN-45 against additional species of NTMs recently isolated from VA patients with COPD and to evaluate efficacy in our well characterized acute and chronic COPD mouse models and to evaluate CPZEN-45 Inhaled Therapy Using a Chronic NTM Model in guinea pigs

Approach

New antibiotics for Non-Tuberculosis Mycobacterial Lung Disease (NTM-LD) are urgently needed. We have discovered a new chemical entity, CPZEN-45, which has is highly promising since it: (i) directly kills many pathogenic species of NTM (both drug sensitive and drug resistant), (ii) has *in vivo* efficacy, and (iii) possess an acceptable toxicity profile, and (iv) can be delivered directly to the lungs as a dry-powder. Before CPZEN-45 can be studied in patients with NTM-LD, we must do further pre-clinical work - making sure we can produce sufficient quantities of high quality CPZEN-45 as well as supply large amounts of the compound to do further testing in animals to further ensure efficacy and safety



Accomplishments: Aim 1: CPZEN-45 drug synthesis scaled to 500 g;
Aim 2: ACURO approvals for both models received

Timeline and Cost

Activities / CY	18	19	20
Aim1: Scale up / Task 1: Improve drug substance yield			
Aim1: Scale up / Task 2: Transfer spray drying			
Aim2: Define and characterize efficacy of CPZEN-45 / Task 1: ACURO review			
Aim2: Define and characterize efficacy of CPZEN-45 / Task 2: In vivo Models			
Estimated Budget ($K)	**$1,203k**	**$1,270k**	**$798k**

Updated: October 23rd, 2019

Goals/Milestones

CY18 Goals – Synthesis Scale-up and Animal Modeling
- ☑ CPZEN-45 scaled to 500 g
- ☑ ACURO approval received

CY19 Goal – Spray Drying Scale-up and Murine Testing
- ☐ Research spray dried lots for guinea pig and mouse models
- ☑ Begin transfer to spray-dry manufacturer
- ☐ Begin murine testing

CY20 Goal – Manufacturing and Animal Modeling
- ☐ Complete scale-up manufacturing
- ☐ Complete murine testing
- ☐ Complete guinea pig testing

Comments/Challenges/Issues/Concerns
- N/A

Budget Expenditure to Date

Projected Expenditure: $ 1,203,977

Actual Expenditure: $ 406,900

9. **APPENDICES:**

AWARD CHART:

PR171209: Aerosol Delivery of CPZEN-45 for Treatment of Nontuberculous Mycobacterial (NTMs) Infections

PI: DR. GAIL CASSELL, INFECTIOUS DISEASE RESEARCH INSTITUTE

Budget: $3,273,025.00 **Topic Area:** Antimicrobial Resistance **Mechanism:** W81XWH-17-PRMRP-TTDA

Research Area: Chemotherapy/Pharmacotherapy (803), Drug Resistance / Multidrug Resistance (804) **Award Status:** 9/30/18 – 9/29/21

Study Goals:

To improve the clinical outcome for patients with nontuberculous mycobacterial (NTM) lung disease by further development of a new antibiotic, CPZEN-45.

Specific Aims:

1) Optimization of fermentation and scaling up manufacturing to support IND enabling activities;
2) Evaluation of efficacy in a more relevant animal model of chronic obstructive pulmonary disease with a chronic NTM lung infection,
3) To identify the most optimal combination of drugs for eradicating NTM in this model, and to prove efficacy by administration of CPZEN- 45 by aerosol.

Key Accomplishments:

Publications: None
Patents: None
Funding Obtained: None

Figures 1-10 and Tables 1-2 in Support of Accomplishments in Year 01

Figure 1: Karl Fischer titrations to determine water content of new lots.

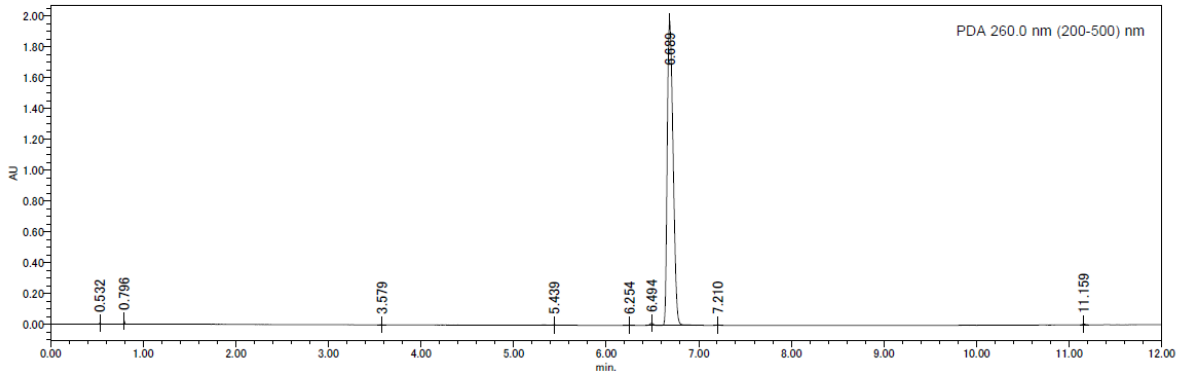
Summary of Karl Fischer titration

- CPZEN-45 HCl (Lot.S180301)
  - Immediately after drying: 24382.8 ppm (water content: 2.44%)
- CPZEN-45 HCl (Lot.S180401)
  - Immediately after drying: 24198.6 ppm (water content: 2.42%)
- CPZEN-45 HCl (Lot.S180501-1)
  - Immediately after drying: 24315.8 ppm (water content: 2.43%)
- CPZEN-45 HCl (Lot.S180501-2)
  - Immediately after drying: 24202.5 ppm (water content: 2.42%)

- CPZEN-45 HCl $1H_2O$: water content: 2.42%
  $2H_2O$: water content: 4.73%
  $3H_2O$: water content: 6.94%

Figure 2: HPLC purity analysis of CPZEN-45 Lot S180301

Sample name: CPZEN-45 HCl Lot.S180301



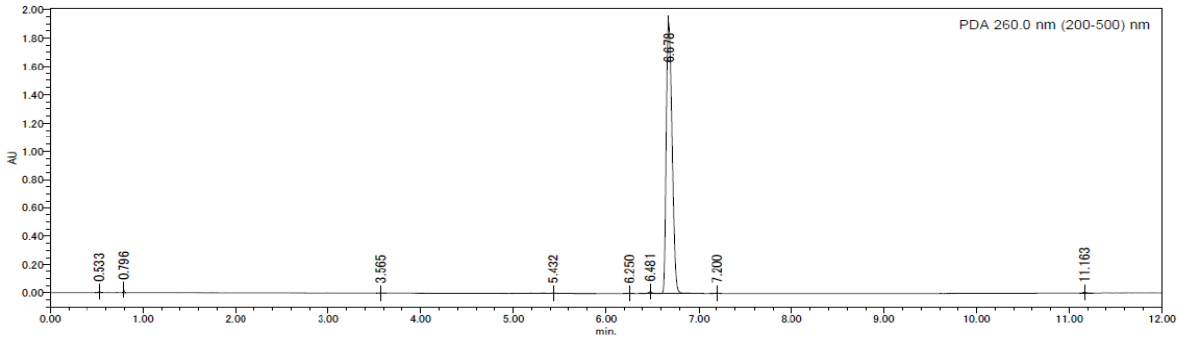
Table

Retension Time (min)	Area	Height	% (area)
0.532	8019	6835	0.09
0.796	14505	23499	0.17
3.579	2742	1122	0.03
5.439	16828	2320	0.19
6.254	4639	1616	0.05
6.494	35754	14032	0.41
6.689	8636569	1973759	98.86
7.210	6543	2761	0.07
11.159	10562	4362	0.12

Waters Acquity UPLC H Class System with PDA
Sample diluent: 1000µg/mL, 50%MeCN
Injection: 3µL
Column: Waters, Symmetry C18 3.5µm 2.1 mm x 150 mm
Flow rate: 520µL/min
Temperature: 30°C
Gradient: A : H_2O + TFA 0.01%
B : MeCN + TFA 0.01 %
B = 5% (0min) - 50% (12min)

Figure 3: HPLC purity analysis of CPZEN-45 Lot S180401

Sample name: CPZEN-45 HCl Lot.S180401



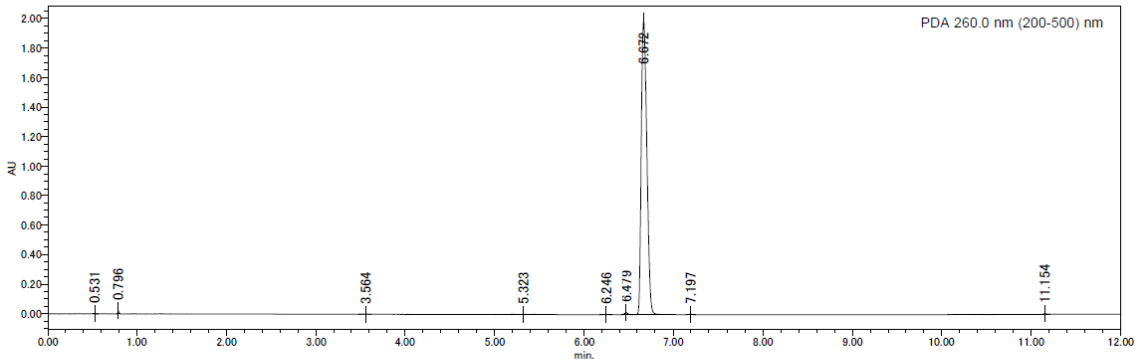
Table

Retension Time (min)	Area	Height	% (area)
0.533	8721	6808	0.10
0.796	12667	20373	0.15
3.565	2438	1132	0.03
5.432	38342	3160	0.45
6.250	3585	1383	0.04
6.481	36977	12993	0.44
6.678	8339783	1914666	98.53
7.200	7978	2996	0.09
11.163	13480	4498	0.16

Waters Acquity UPLC H Class System with PDA
Sample diluent: 1000µg/mL, 50%MeCN
Injection: 3µL
Column: Waters, Symmetry C18 3.5µm 2.1 mm x 150 mm
Flow rate: 520µL/min
Temperature: 30°C
Gradient: A : H_2O + TFA 0.01%
B : MeCN + TFA 0.01 %
B = 5% (0min) - 50% (12min)

Figure 4: HPLC purity analysis of CPZEN-45 Lot S180501-1

Sample name: CPZEN-45 HCl Lot.S180501-1



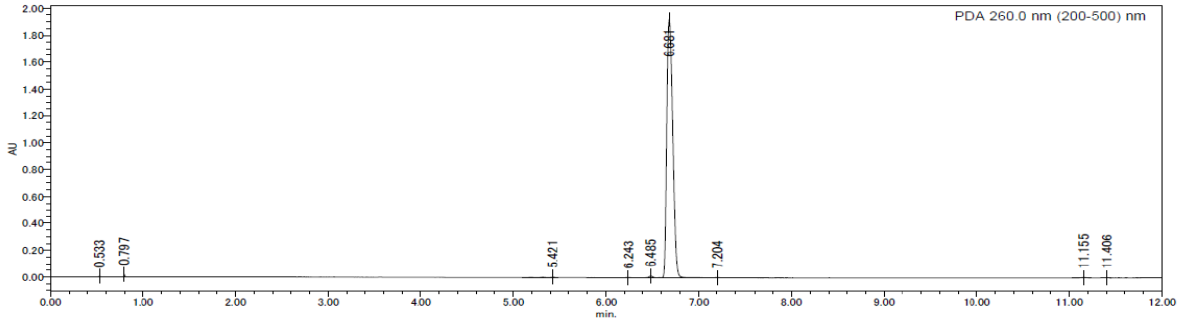
Table

Retension Time (min)	Area	Height	% (area)
0.531	3735	2900	0.04
0.796	13819	22378	0.16
3.564	2609	1097	0.03
5.323	13574	2097	0.16
6.246	4984	1545	0.06
6.479	34448	13815	0.40
6.672	8627454	1985317	98.96
7.197	6403	2723	0.07
11.154	10795	4036	0.12

Waters Acquity UPLC H Class System with PDA
Sample diluent: 1000µg/mL, 50%MeCN
Injection: 3µL
Column: Waters, Symmetry C18 3.5µm 2.1 mm x 150 mm
Flow rate: 520µL/min
Temperature: 30°C
Gradient: A : H_2O + TFA 0.01%
B : MeCN + TFA 0.01 %
B = 5% (0min) - 50% (12min)

Figure 5: HPLC purity analysis of CPZEN-45 Lot S180501-2

Sample name: CPZEN-45 HCl Lot.S180501-2



Table

Retension Time (min)	Area	Height	% (area)
0.533	4097	3118	0.05
0.797	11252	18402	0.13
5.421	22197	2789	0.26
6.243	4401	1409	0.05
6.485	34748	12577	0.41
6.681	8449399	1926210	98.87
7.204	6195	2316	0.07
11.155	10142	3585	0.12
11.406	3318	1393	0.04

Waters Acquity UPLC H Class System with PDA
Sample diluent: 1000µg/mL, 50%MeCN
Injection: 3µL
Column: Waters, Symmetry C18 3.5µm 2.1 mm x 150 mm
Flow rate: 520µL/min
Temperature: 30°C
Gradient: A : H_2O + TFA 0.01%
B : MeCN + TFA 0.01 %
B = 5% (0min) - 50% (12min)

Figure 6: NMR identity analysis of CPZEN-45 Lot S180301

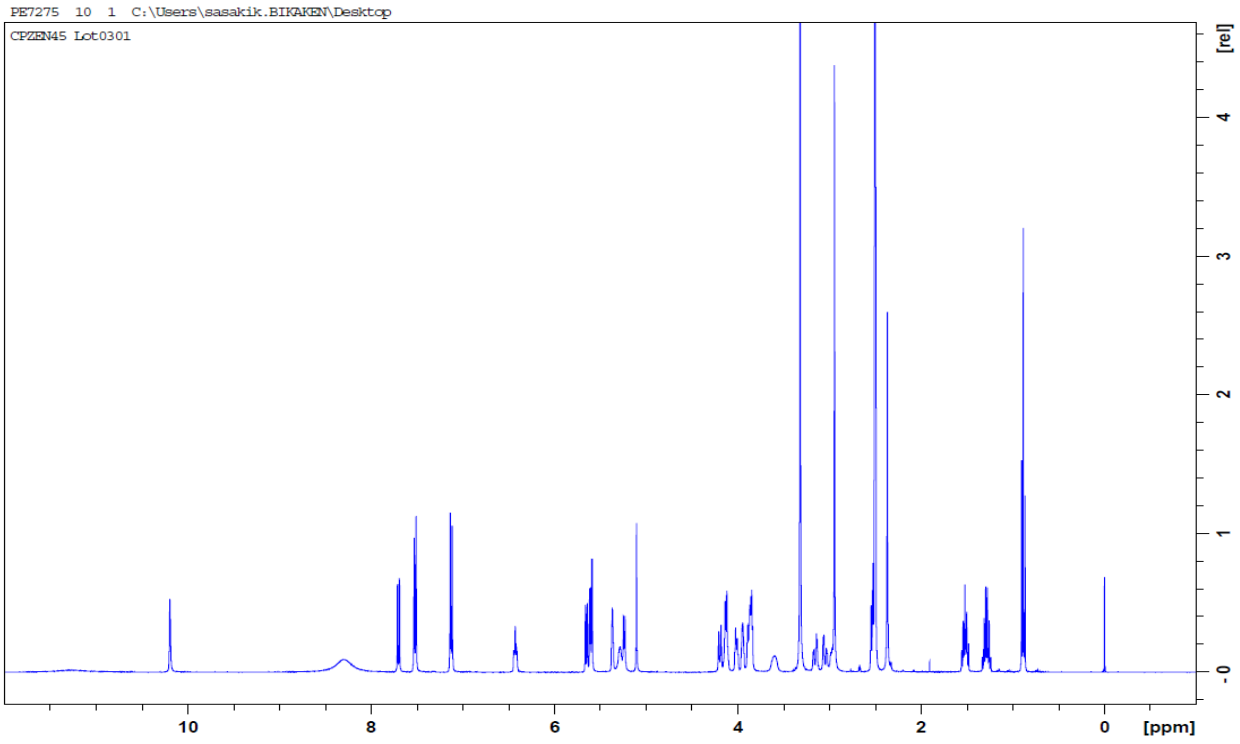


Figure 7: NMR identity analysis of CPZEN-45 Lot S180401

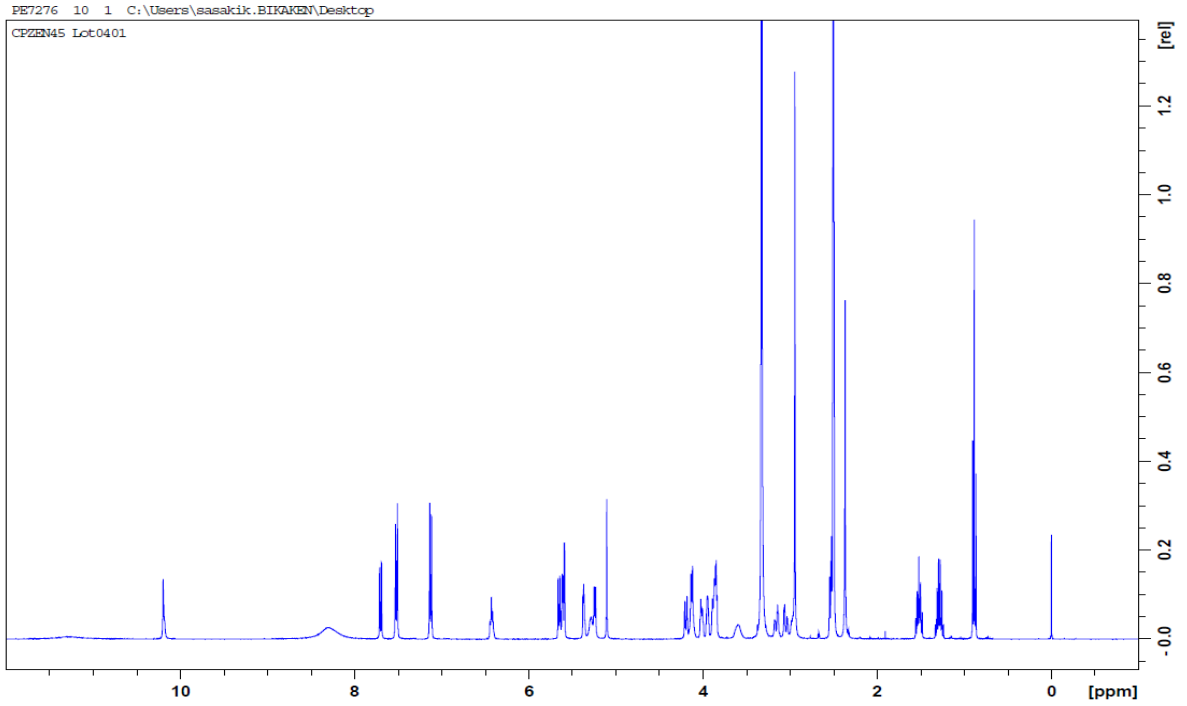


Figure 8: NMR identity analysis of CPZEN-45 Lot S180501-1

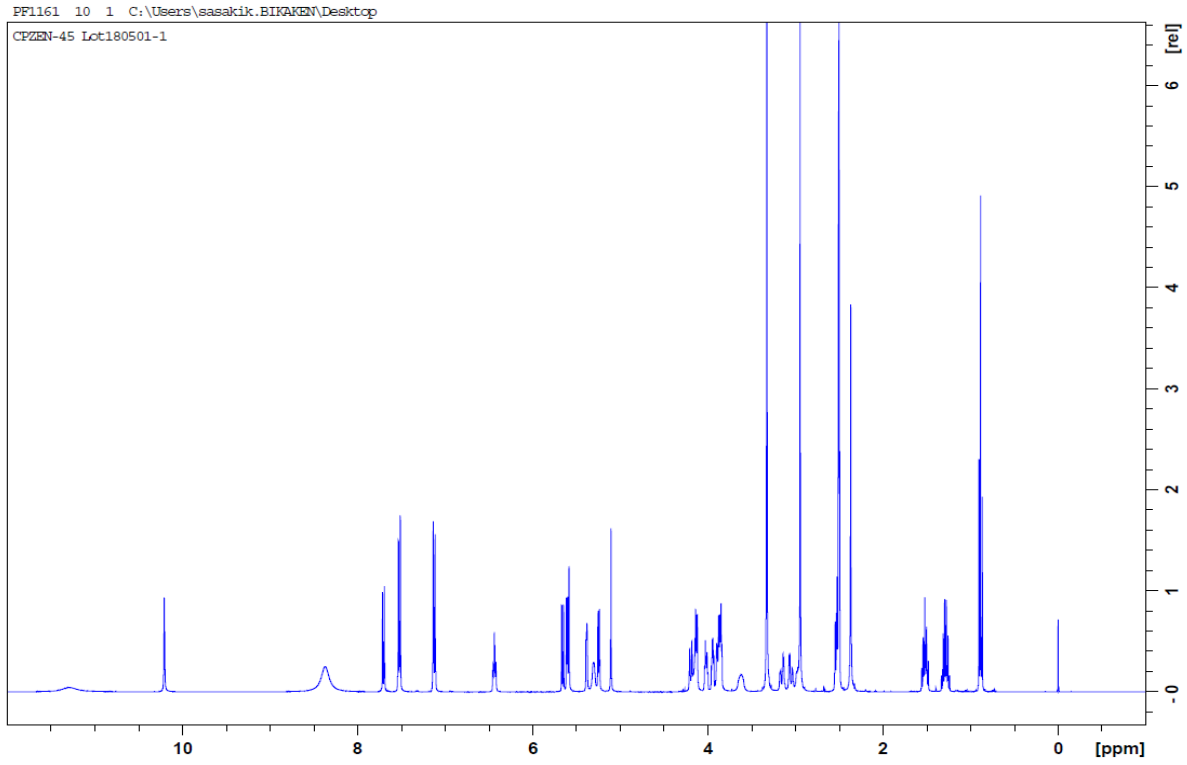


Table 1: Susceptibility of various NTM to CPZEN-45. Reported are Minimal Inhibitory Concentrations (µM)

TEST ORGANISMS	ERM	23S	CPZEN 0301	CPZEN 0401	CPZEN 0501-1	CPZEN 0501-2	CPZEN-45 HCL
M. abscessus subsp. abscessus DSM-43493			>128	>128	>128	>128	>128
M. abscessus subsp. abscessus DSM-43507			>128	>128	>128	>128	>128
M. abscessus subsp. abscessus DSM-44567			>128	>128	>128	>128	>128
M. abscessus subsp. abscessus JCM13569			>128	>128	>128	>128	>128
M. abscessus subsp. abscessus JCM13569	induced		>128	>128	>128	>128	>128
M. abscessus subsp. bolletii DSM-45149T			>128	>128	>128	>128	>128
M. abscessus subsp. massiliense DSM-45103T			>128	>128	>128	>128	>128
M. abscessus B-1690	–		>128	>128	>128	>128	>128
M. abscessus B-1691	–		128	128	128	128	128
M. abscessus B-1692	+		128	128	128	128	128
M. avium subsp. avium ATCC25291			0.5	0.5	0.5	0.5	0.5
M. avium subsp. avium 25291MacR08		A2058T	0.5	0.5	0.5	0.5	0.5
M. avium subsp. avium 25291MacR010		A2059G	1	1	1	1	1
M. avium subsp. avium DSM-43216			0.25	0.25	0.25	0.25	0.25
M. avium subsp. avium DSM-44157			2	2	2	2	2
M. avium subsp. avium DSM-44158			0.5	1	1	1	1
M. avium GTC 12472			4	4	4	4	4
M. avium GTC 12938			1	1	1	1	1
M. avium GTC 12957			2	2	2	2	2
M. avium GTC 12979			1	1	2	2	2
M. avium GTC 15120		WT	16	16	16	16	16
M. avium GTC 15122			8	8	8	8	8
M. avium GTC 15124		WT	4	4	4	4	4
M. avium GTC 15127			8	8	8	8	8
M. avium GTC 15128			4	4	4	4	4
M. avium GTC 15129		WT	8	8	8	8	8
M. avium B-1685			8	8	8	8	8
M. avium B-1686			8	8	8	8	8
M. avium B-1687			8	8	8	8	8
M. avium B-1688			4	4	4	4	4
M. avium B-1689			8	8	8	8	8
M. avium ATCC700897			4	4	4	4	4
M. avium subsp. paratuberculosis ATCC43015			0.25	0.25	0.25	0.25	0.25
M. bovis BCG Pasteur			4	4	4	4	4
M. bovis BCG Tokyo 172-2			1	1	1	1	1
M. intracellulare JCM6384			0.125	0.125	0.125	0.125	0.125
M. intracellulare 6384MacR10		A2059G	0.125	0.125	0.125	0.125	0.125
M. intracellulare 6384MacR07		A2058G	0.125	0.125	0.125	0.125	0.125
M. intracellulare GTC 12944			0.5	0.5	0.5	0.5	0.5
M. intracellulare GTC 12966			4	4	4	4	4
M. intracellulare GTC 12981			4	2	4	4	4
M. intracellulare GTC 15454		WT	2	2	2	2	2
M. intracellulare GTC 15459			1	1	1	1	1
M. intracellulare GTC 15470			0.125	0.125	0.125	0.125	0.125
M. intracellulare GTC 15492		WT	2	2	2	2	2
M. intracellulare GTC 15505			0.25	0.25	0.25	0.25	0.25
M. intracellulare GTC 15525			0.25	0.125	0.25	0.25	0.25
M. intracellulare GTC 15526			0.125	0.125	0.125	0.125	0.125
M. intracellulare B-1693			0.125	0.125	0.125	0.125	0.125
M. intracellulare B-1694			2	2	2	2	2
M. intracellulare B-1695			4	4	4	4	4
M. intracellulare B-1696			4	4	4	4	4

Figure 9. THP-1 Checkerboard

Approach: THP-1 cells were infected with 1:1 M. abscessus A for 2 hours, 1 day and 2 days. Optical density was evaluated with drugs alone in combination with CPZEN-45. Those drug combinations that resulted in a FIC value <0.5 indicative of CPZEN-45 synergies were then plated out to evaluate then kill curves. Based on the overall FIC values 2 treatment regimens were developed for the Chronic COPD SCID M. abscessus A infection mouse model studies.

To quantify the interactions between the antibiotics being tested (the FIC index), the following equation is used:

$$\frac{A}{MIC_A} + \frac{B}{MIC_B} = FIC_A + FIC_B = \text{FIC Index}$$

where A and B are the MIC of each antibiotic in combination (in a single well), and MIC_A and MIC_B are the MIC of each drug individually.

The FIC Index value is then used to categorize the interaction of the two antibiotics tested.

	FIC value
Synergy	<0.5
Antagonism	>4
Additive or indifference	0.5-4

Figure 10. Phylogenetic Analysis Clinical NTM

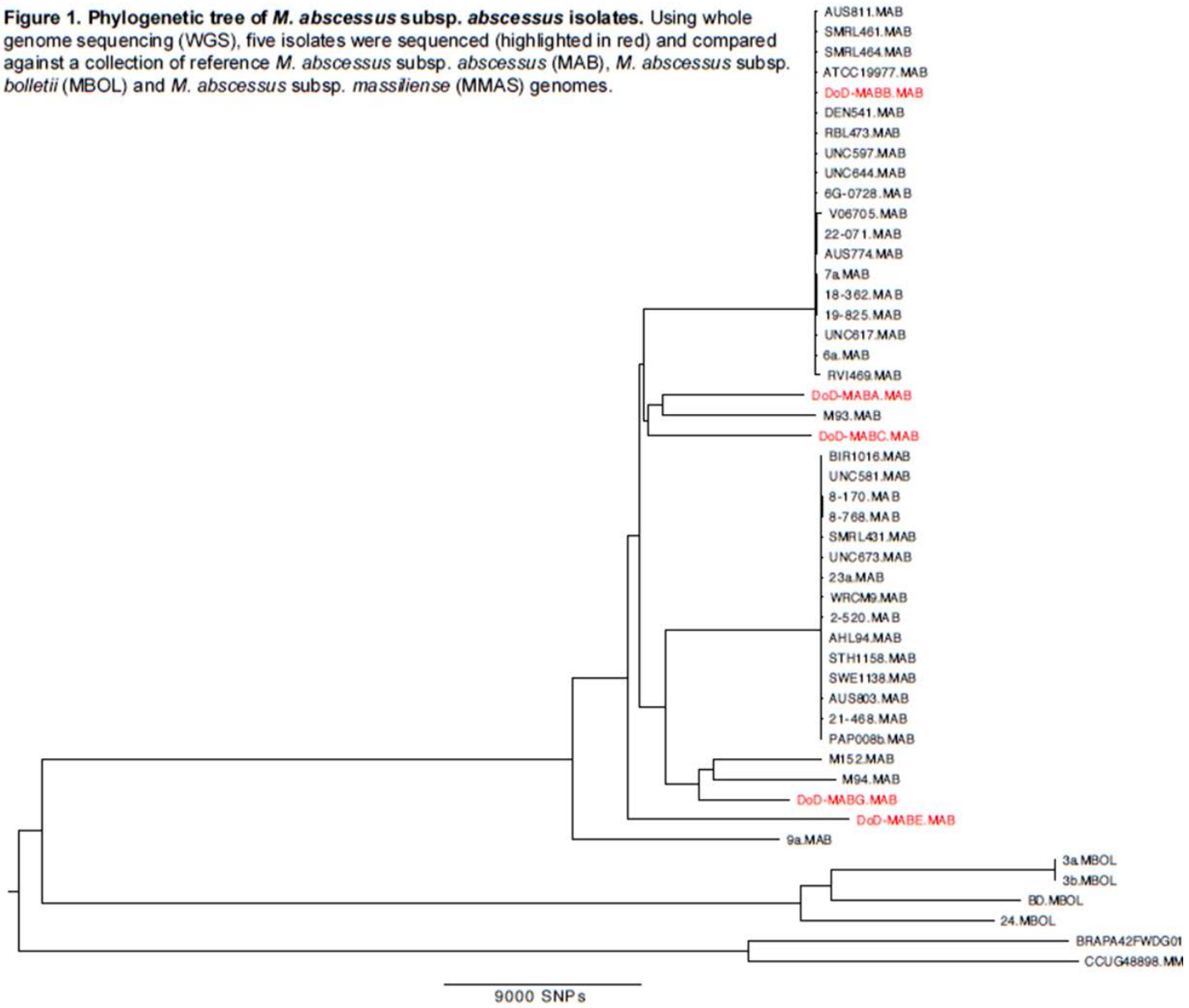


Figure 1. Phylogenetic tree of *M. abscessus* subsp. *abscessus* isolates. Using whole genome sequencing (WGS), five isolates were sequenced (highlighted in red) and compared against a collection of reference *M. abscessus* subsp. *abscessus* (MAB), *M. abscessus* subsp. *bolletii* (MBOL) and *M. abscessus* subsp. *massiliense* (MMAS) genomes.

Table 2. Minimum Inhibitory Concentration (THP-1 cells) against COPD Patient Strain A (M. abscessus subspecies abscessus (rough))

Compound	MIC (7H9 broth ug/ml, OD) *M. abscessus* strain A	Compound Combinations (ug/ml)	FIC Index value: THP-1 cells Day 2
CPZEN-45	1		1
Clofazimine	1.3	**CPZEN-45 +Clofazimine**	**0.41 (synergy)**
Amikacin	**16**	**CPZEN-45+Amikacin**	**1. (additive)**
Clarithromycin	**32 (R)**	ND	ND
Vancomycin		CPZEN-45+vancomycin	1.6 (additive)
Rifampicin	ND	CPZEN-45+Rifampicin	3.67 (Indifference)
Moxifloxacin	**16 (R)**	CPZEN-45+Moxifloxacin	5 (antagonism)
Isoniazid	ND	CPZEN-45+Isoniazid	1.6 (additive)
Cefepime	ND	CPZEN-45+Cefepime	.82 (additive)
Bedaquiline	1	**CPZEN-45+Bedaquiline**	**0.25 (synergy)**
Linolizid	8	CPZEN-45+Linolizid	3.67 (Indifference)
Ethambutol	ND	CPZEN-45+Ethambutol	1.3 (additive)