**TITLE: Level 2 Therapeutic Award for Further** Development of CPZEN-45 for Treatment of Nontuberculous Mycobacterial (NTM) Lung Infections

**Relevance to FY23 PRMP: Portfolio: *Respiratory Health*; Topic Areas: *Prevention and Treatment; Strategic Goals: prevent lung injury caused by infection and development and testing of novel treatment to slow progression or reverse lung injury***. Chronic lung disease is the most frequent disorder caused by non-tuberculosis mycobacteria (NTM); moreover, NTM lung infections not uncommonly complicate individuals with chronic obstructive pulmonary disease (COPD). COPD is the fifth leading cause of death in the U.S. and accounts for more than $40 billion annually in direct and indirect health care costs. COPD affects approximately 6% of civilians and at least 8% of the Veteran population. When veterans with respiratory complaints are evaluated with spirometry and questionnaires, rates of COPD may be as high as 43%. Mortality rates in COPD patients with NTM infection in the U.S. Veterans Health Administration is 1.43 times higher compared to those that are uninfected. Treatment of NTM lung disease often requires treatment 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.

**Rationale and Supporting Data:** Members of our team at the Institute for Microbial Chemistry (IMC) in Tokyo have discovered a **new** **chemical entity**, CPZEN-45, with a **unique mechanism of action-inhibition of Wec A, which inhibits the first step in cell wall biosynthesis**. Based upon our inter- and intra-species protein and genetic variation studies, regions of the target protein have 100% conservation, suggesting **these regions may have high selective pressures not to mutate**. We have shown in animal models that development of resistance to CPZEN-45 during treatment is indeed rare. We have shown CPZEN-45: (i) to directly kill both drug sensitive and drug resistant NTM, (ii) to have efficacy in experimentally infected mice, and (iii) to be able to be delivered directly to the lungs as a dry powder or aerosol. In summary, we have achieved critical milestones in characterizing the physical properties of CPZEN-45 that support its clinical development for NTM - including defining its pharmacokinetic (PK) properties and toxicology profile *in vitro* and *in vivo* in multiple species: human cells and microsomes, mice, rats, guinea pigs, and dogs - and found nothing to impede translational development of CPZEN-45. The FDA has agreed with our development proposal to phase 1 clinical trials. However, before CPZEN-45 can be evaluated in patients, 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 GLP toxicology testing in animals to further ensure safety.

**Specific Aims of Level 2 Funding:** CPZEN-45 HCl is a semi-synthetic antibiotic produced from caprazamycins found in the fermentation broth of *Streptomyces* sp.During the fermentation of *Streptomyces sp. MK730-62F2,* multiple caprazamycins are produced. For the production of CPZEN-45 HCl, the caprazamycins are converted to caprazene directly in the **fermentation** broth by acid hydrolysis and caprazene is then extracted by dichloromethane or methanol. Caprazene is then converted to CPZEN-45 HCl by a **synthetic** process. To date, our efforts to optimize the production of caprazene have been focused on developing an IND-enabling, robust, scalable process to produce CPZEN-45 on a multi-kilo scale.

For the synthetic process our API manufacturer (Cambrex) has achieved a non-GMP CPZEN-45 HCl batch at the 800-gram scale which was tested and released for use in the formulation of drug product. The manufacturer has also focused on the development and validation of numerous phase-appropriate analytical methods for API release and stability activities. Currently, Cambrex is completing analytical method development and validation activities; establishing specifications for starting materials, finished product and intermediates; and planning for a cGMP API campaign at the 5 kg scale for a Phase 1 clinical study. **Specific Aim:** With the Level 2 DOD Therapeutic Development Award, Cambrex will focus on further scale up of the cGMP API manufacture of CPZEN-45 HCl to support nonclinical animal studies including toxicology studies as well as supplies to produce drug product for a Phase 2 clinical study, up to approximately 40 kg. This work will include further phase-appropriate method development and validation of analytical methods, stability studies and appropriate documentation to support the cGMP process.

For our initial toxicology studies and our planned Phase 1 clinical trial drug substance, non-cGMP starting material, caprazene, was sourced from Zhejiang Hisun Pharmaceutical Co. Ltd (China). However, during the pre-IND meeting held with the FDA on April 26, 2021, FDA indicated that while the use of non‑cGMP fermentation produced caprazene as the starting material for cGMP chemical conversion to CPZEN-45 HCl was acceptable for Phase 1, from Phase 2 forward, drug product must use caprazene starting material produced by fermentation using a cGMP compliant process. Unfortunately, Hisun cannot produce caprazene under cGMP compliance. As a result, we have identified Abbvie North Chicago (USA) Fermentation Operations as the CMO for future cGMP caprazene production activities based on their strong expertise and experience in cGMP fermentation. **Specific Aim:** With the Level 2 DOD Therapeutic Development Award, IMC will provide technology transfer to AbbVie. Abbvie will scale up the cGMP fermentation process to produce caprazene for use in the manufacture of cGMP CPZEN-45 HCl. This will include development and validation of appropriate testing methods to support the process as well as generation of documents to demonstrate compliance with cGMP procedures (e.g., specifications, batch records). It is expected that Abbvie will produce up to approximately 160 kg of caprazene for use in cGMP production of CPZEN‑45 HCl by Cambrex.

In order to meet the market demands for treatment of US Veterans with COPD and NTM infection we plan to develop an easily deployed, nebulized drug product based upon our work performed to date utilizing a dry powder inhalation approach. **Specific Aim**: With the Level 2 DOD Therapeutic Development Award, we plan to develop, optimize, scale up, manufacture and package a nebulizer formulation of CPZEN-45 for use in a vibrating mesh nebulizer to achieve an appropriate particle size at the desired output rate. This will include development and phase-appropriate validation of analytical methods to demonstrate aerodynamic performance as well as solution stability. Stability studies will follow regulatory guidelines (e.g., ICH) for storage conditions and duration.

To support clinical studies for NTM infection, we plan to conduct additional nonclinical studies to support the CPZEN-45 program. **Specific Aim**: With the Level 2 Therapeutic Development Award, we plan to conduct the following studies: PK/PD studies to better define the pharmacokinetic and pharmacodynamics of nebulized CPZEN-45 and 3-month toxicological studies with TK via nebulizer formulation in two species to support a clinical study. Finally, we plan to file an IND. **Specific Aim**: With the Level 2 Therapeutic Development Award, we plan to compile the required documentation, write, review and submit an IND to the FDA for a nebulized CPZEN-45 drug product in support of a clinical trial in humans. We anticipate that accomplishment all of our specific aims outlined in this proposal will require 4 years and $4M in direct cost.

Our work to date related to manufacturing of CPZEN-45 has been funded by DOD Therapeutic Award W81XWH-18-1-0765: Aerosol Delivery of CPZEN-45 for Treatment of Nontuberculous Mycobacterial (NTMs) Infections Period of Performance End Date: 09/29/2023; PI Gail Cassell. The DOD Award has two objectives: 1) to optimize fermentation and scale-up manufacturing processes for high yield of CPZEN-45, including spray dried CPZEN-45; and 2) to define and characterize *in vitro* and *in vivo* efficacy of CPZEN-45 against clinical NTM isolates and further define its mechanism of action. On 07/01/2023 we will request a final No Cost Extension (NCE) will be requested for an additional 6-12 mos. However, **there will be no overlap with Level 2 funding being requested in this proposal**. If approved, work under the NCE will be limited to technology transfer by IMC to AbbVie for fermentation and extraction of caprazene. Level 2 funding will be used in collaboration with Cambrex for cGMP API manufacture of CPZEN-45 HCl to support further toxicology studies as well to production of drug product for a Phase 2 clinical trial. Other specific aims of the Level 2 Award are not included in the present DOD Award.