

# Merck Investigator Studies Program (MISP) Protocol Template

## Requirements for Submitting a Full Proposal

### Section #1 - MISP Protocol Identification

<b>Study Title:</b>	Tissue Penetration of Ceftolozane/Tazobactam in Diabetic Patients with Lower Limb Infections
<b>Request Date:</b>	04-SEP-2015
<b>Version Date:</b>	27-JUN-2016
<b>Institution Name</b>	Center for Anti-Infective Research and Development Hartford Hospital
<b>Investigator Contact Information:</b> <ul style="list-style-type: none"> <li>– Full address</li> <li>– Phone No.</li> <li>– Fax No.</li> <li>– e-mail address</li> </ul>	David P. Nicolau, PharmD, FCCP, FIDSA 80 Seymour Street, Hartford, CT 06102 Tel: 860.972.3941 Fax: 860.545.3992 Email: david.nicolau@hhchealth.org

## Section #2- Core Protocol

<b>2.1 Objectives &amp; Hypotheses</b>	<p><b>2.1.1 Primary Objectives:</b></p> <p>To describe the pharmacokinetic profile of intravenous ceftolozane/tazobactam in the plasma and subcutaneous tissue of diabetic patients with ongoing wound infections of the lower extremities (i.e., below the knee) using <i>in vivo</i> microdialysis and compare the profile with that of a control group of healthy volunteer participants.</p> <p><b>2.1.2 Secondary Objectives:</b></p> <p>To describe the safety and tolerability of intravenous ceftolozane/tazobactam in diabetic patients with diabetic foot infections and in healthy participants.</p>
<b>2.2 Background &amp; Rationale, Significance of Selected Topic &amp; Preliminary Data</b>	<p>The annual incidence of foot ulcers among diabetics has been estimated to be between 6% to 11.5%, and 10 to 15% of those with diabetes will have at least one foot ulcer during their lifetime.<sup>1</sup> The majority (upwards of 60%) of these diabetic ulcers become infected.<sup>2</sup> Treatment of these complicated diabetic wound infections primarily involves surgical intervention, topical antiseptics, and systemic antibiotic therapy.<sup>3</sup> However, despite advances in these areas, outcomes from such infections remain poor and often lead to limb amputation in 15% to 20% of participants within 5 years.<sup>4</sup></p> <p>Different pathogens are implicated in diabetic wound infections, and infections due to antibiotic-resistant pathogens are on the rise. Such pathogens include methicillin-resistant <i>Staphylococcus aureus</i> (MRSA), extended-spectrum <math>\beta</math>-lactamase (ESBL)–producing Gram-negative bacilli, as well as highly resistant <i>Pseudomonas aeruginosa</i>.<sup>5</sup> Other pathogens also isolated from these infections include <i>Streptococcus</i> spp. Therefore, antibiotics targeting these pathogens are often prescribed either as monotherapy or in combination. The emergence of resistance in pathogens causing diabetic wound infections challenges treatment since limited treatment options are available.</p> <p>Ceftolozane/tazobactam (Zerbaxa<sup>®</sup>, Cubist Pharmaceuticals Inc., Lexington, MA), is a <math>\beta</math>-lactam antibiotic and member of the cephalosporin class that demonstrates potent activity against antibiotic-resistant Enterobacteriaceae, <i>P. aeruginosa</i>, and <i>Streptococcus</i> spp.<sup>6</sup></p> <p>The pharmacokinetic profile of ceftolozane/tazobactam has been described in healthy adult volunteers with some studies being focused on pulmonary penetration of the drug.<sup>7</sup> However, penetration into skin tissue of an active infection has yet to be elucidated. The most accurate method to calculate overall penetration is to evaluate the entire drug exposure, or AUC, in the tissue of interest and compare this with the overall AUC in blood. In the setting of clinical infection, our group has successfully applied a technique called <i>in vivo</i> microdialysis to assess the interstitial (i.e., extracellular) concentration profile of antimicrobials in comparison with their systemic exposures.<sup>8-11</sup> This approach involves placing a probe (with a semi-permeable membrane at the tip) into tissue and constantly perfusing the probe with a physiologic solution via a pump; this approach allows for the continuous collection</p>

	<p>of extracellular fluid from the tissue and enables assessment of drug concentration over a specified time period (i.e., the entire dosing interval). Because foot infections are very common in the diabetic patients and given the in vitro potency of ceftolozane/tazobactam against Enterobacteriaceae, <i>P. aeruginosa</i>, and <i>Streptococcus</i> spp., a study examining the tissue penetration of the drug in this patient population would be beneficial to understand its pharmacokinetics in the inflamed tissue of the diabetic foot, and hence provide a basis for future clinical studies on the drug’s efficacy for this type of infection. A control group of healthy volunteer participants will be used to compare penetration and tissue exposure.</p>															
2.3 Study Design	<p><b>2.3.1 Sites</b></p> <p>The study will be conducted at Hartford Hospital, Hartford, CT. The Study Group will be admitted to an inpatient unit while the Control Group will be studied in the Clinical Research Center at Hartford Hospital.</p> <p><b>2.3.2 Inclusion Criteria</b></p> <p><u>2.3.2.1 Study Group</u></p> <p>Participants with a documented medical history of Type 1 or Type 2 diabetes (for which they are receiving insulin or oral anti-hyperglycemic agents), and a suspected complicated skin and skin structure infection will be included. The suspected infection will be an ongoing and infection will be mild or moderate or Grade 2 or 3 as defined by the Infectious Diseases Society of America (Table 1).<sup>5</sup> Other anti-infective agents besides the study drug will be permitted for the purposes of treatment except members of the β-lactam group of antibiotics.</p> <p><b>Table 1.</b> Clinical Classification of Infections</p> <table><tr><th>Clinical manifestations of infection</th><th>Infection severity</th><th>PEDIS grade</th></tr><tr><td>No symptoms or signs of infection present, as defined by the presence of at least 2 of the following items:<ul style="list-style-type: none"><li>Local swelling or induration</li><li>Erythema</li><li>Local tenderness or pain</li><li>Local warmth</li><li>Purulent discharge (thick, opaque to white or sanguineous secretion)</li></ul></td><td>Uninfected</td><td>1</td></tr><tr><td>Local infection involving only the skin and the subcutaneous tissue (without involvement of deeper tissues and without systemic signs as described below). If erythema, must be &gt; 0.5 cm to ≤ 2 cm around the ulcer</td><td>Mild</td><td>2</td></tr><tr><td>Local infection (as described above) with erythema &gt; 2 cm, or involving structures deeper than skin and subcutaneous tissues (e.g., abscess, osteomyelitis, septic arthritis, fasciitis), and no systemic inflammatory response signs (as described below)</td><td>Moderate</td><td>3</td></tr><tr><td>Local infection (as described above) with the signs of SIRS, as manifested by ≥ 2 of the following:<ul style="list-style-type: none"><li>Temperature &gt; 38°C or &lt; 36°C</li><li>Heart rate &gt; 90 beats/min</li><li>Respiratory rate &gt; 20 breaths/min or PaCO2 &lt; 32 mm Hg</li><li>White blood cell count &gt; 12,000 or &lt; 4,000 cells/μL or ≥ 10% immature (band) forms</li></ul></td><td>Severe</td><td>4</td></tr></table>	Clinical manifestations of infection	Infection severity	PEDIS grade	No symptoms or signs of infection present, as defined by the presence of at least 2 of the following items: <ul style="list-style-type: none"><li>Local swelling or induration</li><li>Erythema</li><li>Local tenderness or pain</li><li>Local warmth</li><li>Purulent discharge (thick, opaque to white or sanguineous secretion)</li></ul>	Uninfected	1	Local infection involving only the skin and the subcutaneous tissue (without involvement of deeper tissues and without systemic signs as described below). If erythema, must be > 0.5 cm to ≤ 2 cm around the ulcer	Mild	2	Local infection (as described above) with erythema > 2 cm, or involving structures deeper than skin and subcutaneous tissues (e.g., abscess, osteomyelitis, septic arthritis, fasciitis), and no systemic inflammatory response signs (as described below)	Moderate	3	Local infection (as described above) with the signs of SIRS, as manifested by ≥ 2 of the following: <ul style="list-style-type: none"><li>Temperature &gt; 38°C or &lt; 36°C</li><li>Heart rate &gt; 90 beats/min</li><li>Respiratory rate &gt; 20 breaths/min or PaCO2 &lt; 32 mm Hg</li><li>White blood cell count &gt; 12,000 or &lt; 4,000 cells/μL or ≥ 10% immature (band) forms</li></ul>	Severe	4
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### 2.3.2.2 Control Group

Male or female healthy adult ( $\geq 18$  years of age) volunteer participants who will be identified via hospital and local advertisements (newspaper, postings, and internet) in the Greater Hartford area, Connecticut region will be eligible for enrollment.

### **2.3.3 Exclusion Criteria**

#### 2.3.3.1 General Criteria

Participants will be excluded if any of the following criteria are met:

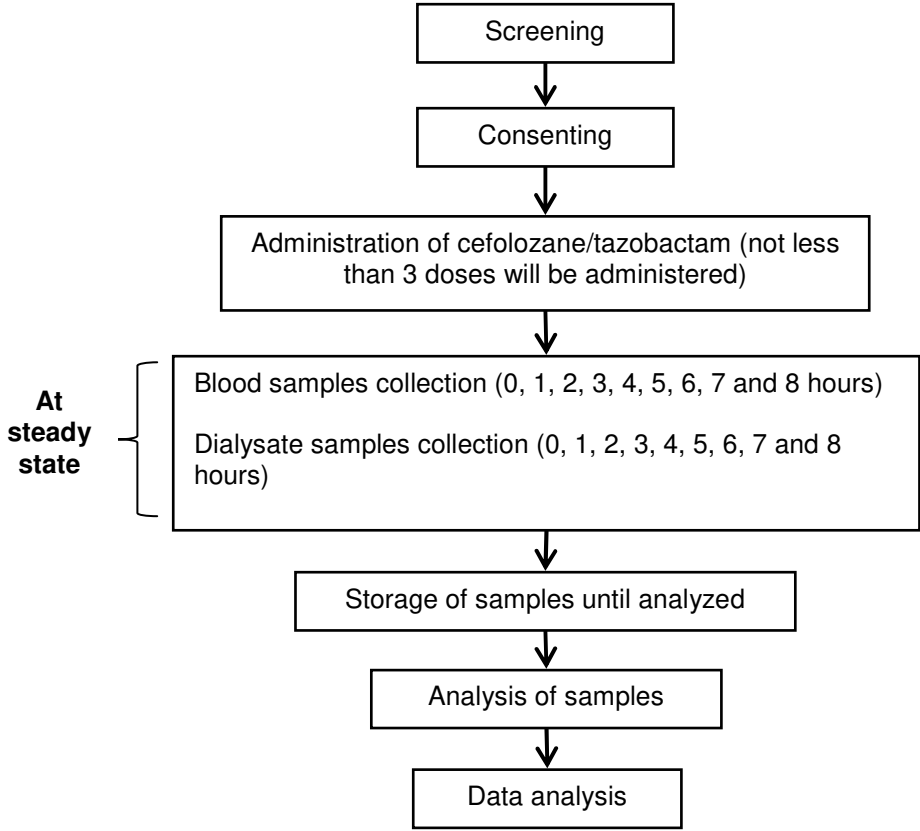
1. Less than 18 years of age
2. History of any reported hypersensitivity or allergy to ceftolozane/tazobactam, piperacillin/tazobactam, or any  $\beta$ -lactam antibiotic
3. History of hypersensitivity to lidocaine or lidocaine derivatives
4. Females who are pregnant or breastfeeding
5. Concomitant receipt of any  $\beta$ -lactams antibiotic
6. Concomitant receipt of probenecid
7. Reduced kidney function defined as creatinine clearance of  $\leq 50$  mL/min, as calculated by Cockcroft-Gault
8. Any other reason felt by the investigator to potentially affect the outcomes of the study

#### 2.3.3.2 Additional Exclusion Criteria for Study Group

1. Participants likely to require multiple surgical interventions during the study period, which therefore could affect placement of the microdialysis catheter

#### 2.3.3.3 Additional Exclusion Criteria for Control Group

1. Positive urine drug screen (cocaine, THC, opiates, benzodiazepines, and amphetamines)
2. History of regular alcohol consumption exceeding 7 drinks/week for females or 14 drinks/week for men (1 drink = 5 ounces of wine or 12 ounces of beer or 1.5 ounces of hard liquor) within 6 months of screening.
3. Use of tobacco- or nicotine-containing products in excess of the equivalence of 5 cigarettes per day.
4. Use of prescription or nonprescription drugs, vitamins, or dietary supplements within 7 days or 5 half-lives (whichever is longer) prior to the first dose of study medication, with the exception of acetaminophen at doses of  $\leq 1$  g/day. Herbal supplements, hormonal methods of contraception (including oral and transdermal contraceptives, injectable progesterone, progestin subdermal implants, progesterone-releasing IUDs, postcoital contraceptive methods), and hormone replacement therapy must be discontinued at least 14 days prior to the first dose of study medication. Depo-Provera® must be discontinued at least 6 months prior to the first dose of study medication.

<p><b>2.4 Study Flowchart</b></p>	 <pre> graph TD     A[Screening] --&gt; B[Consenting]     B --&gt; C[Administration of ceflozane/tazobactam (not less than 3 doses will be administered)]     C --&gt; D["At steady state: Blood samples collection (0, 1, 2, 3, 4, 5, 6, 7 and 8 hours) Dialysate samples collection (0, 1, 2, 3, 4, 5, 6, 7 and 8 hours)"]     D --&gt; E[Storage of samples until analyzed]     E --&gt; F[Analysis of samples]     F --&gt; G[Data analysis] </pre>
<p><b>2.5 Study Procedures</b></p>	<p><b><i>2.5.1 Screening Procedure</i></b></p> <p><u><i>2.5.1.1 Study Group</i></u> Electronic medical records of admitted diabetic patients who are being followed by podiatrists for infections of the lower limbs will be screened to identify patients eligible for study enrollment through evaluating each patient’s profile for the meeting of inclusion and exclusion criteria. Screening will involve accessing patient’s age, past medical history, physician’s notes, laboratory and imaging data, and medication administration record.</p> <p><u><i>2.5.1.2 Control Group</i></u> Adult healthy volunteer participants will be identified via hospital and local advertisements (newspaper, postings, and internet) and will be initially screened via phone interview and if eligible all interested volunteers will be screened by a study physician within 28 days of the scheduled study period to confirm that inclusion and exclusion criteria are met after providing written informed consent.</p> <p><b><i>2.5.2 Informed Consent</i></b></p> <p>Prior to study enrollment, healthy volunteer participants and patients identified as eligible for the study will be approached for consenting, where they will be provided with written informed consent that will document their election and agreement to participate in the study. The consenting process will be conducted by the principal investigator, co-investigators, or trained research staff members.</p>

### **2.5.3 Study Drug**

All participants will receive intravenous ceftolozane/tazobactam 1.5 gram (g) every 8 hours to achieve steady-state prior to the initiation of the pharmacokinetic study. Admitted patients will receive between 3 and 9 doses (1 – 3 days) prior to pharmacokinetic sampling; this is to permit flexibility with microdialysis catheter insertion and sampling while minimizing interference with standard clinical care. Effort will be made to complete the pharmacokinetic sampling as soon as clinically feasible after the third dose. This is not a treatment study; all patients will also receive standard intravenous antibiotic therapy to treat their diabetic foot infections. All healthy volunteer participants will receive exactly 3 doses of ceftolozane/tazobactam, which is sufficient to achieve steady-state. Ceftolozane/tazobactam will be provided by Merck & Co.

### **2.5.4 Procedures**

#### **2.5.4.1 Baseline Participant Evaluations**

At screening, eligibility will be verified using inclusion/exclusion criteria. A general physical examination, including pedal pulses, a medical history and vital signs will be conducted by the study physician. Clinical laboratory tests, including serum electrolyte panel, serum creatinine, liver function panel, complete blood count, glycosylated hemoglobin (HbA1c), albumin, and urinalysis will be performed unless drawn within the last three days and the participant is clinically stable. Urine or serum pregnancy tests will be required for women of childbearing potential. Concomitant medications will be recorded.

#### **2.5.4.2 Microdialysis Procedure**

Prior to the final scheduled dose, a microdialysis probe (63 MD catheter; MDialysis Inc., N. Chelmsford, MA) with a membrane length of 30 mm and molecular cut-off of 20 kDa will be inserted into subcutaneous tissue near the margin of the wound (study group) or healthy thigh tissue (control group) via a guidance cannula, following a local injection of lidocaine 0.5% solution to minimize pain.<sup>8</sup> Specifically, a puncture hole will be made 10 cm away from the margin of the wound, in order to place the semi-permeable probe at the tip of the catheter within 5 cm of the wound (i.e., peri-ulcer area).<sup>13</sup> The guidance cannula will be removed, leaving the microdialysis probe implanted subcutaneously.

The microdialysis system will be connected and constantly perfused with lactated Ringer's solution (perfusate) at a flow rate of 2 µL/min with a microinfusion pump (CMA 107 microdialysis pump, CMA Microdialysis AB, Solna, Sweden). After a 30-minute baseline period, sampling of the interstitial fluid will begin before the start of the final dose of intravenous ceftolozane/tazobactam for baseline values. At the beginning of the final dose, sampling will occur as noted in section 5.3.6. Once dialysate sampling is complete, the probe will be calibrated by the retrodialysis technique over a 1-hour interval to assess recovery of the antibiotic through the dialysis membrane. A calibration standard concentration of ceftolozane/tazobactam 300/150 µg/mL will be added to the perfusate and its rate of disappearance through the membrane will determine the recovery rate by obtaining a dialysate sample. Recovery of ceftolozane/tazobactam via retrodialysis will be calculated as follows:  
$$\% \text{ Recovery} = 100 - (\text{Concentration}_{\text{dialysate}} / \text{Concentration}_{\text{perfusate}} \times 100).$$

#### 2.5.4.3 Study Drug Administration

Ceftolozane/tazobactam 1.5 g will be administered by the intravenous route every 8 hours, with each dose infused over 1 hour. The doses will be administered by the nursing staff with a record log of the date and time.

#### 2.5.4.4 Plasma Collection and Concentration Determination

Blood samples to assess ceftolozane/tazobactam concentrations will be conducted over an 8-hour period after the administration of the final dose. Blood samples of 10 mL each will be collected through a Jelco® catheter or a peripherally inserted central catheter (PICC), if available, at pre-determined time points. Blood sample times will be at 0 (just before administration of the final dose), 1, 2, 3, 4, 5, 6, 7, and 8 hours. Blood samples will be collected in a 10-mL BD Vacutainer® containing sodium heparin (green top). All blood samples will be immediately centrifuged ( $2,000 \times g$  for 10 min) to collect the separated plasma, which will be stored in amber-colored polypropylene tubes to protect from light at -80 °C until concentration determination.

Concentrations of both ceftolozane and tazobactam in plasma will be assessed using a validated high performance liquid chromatography (HPLC) at the Center for Anti-Infective Research and Development of Hartford Hospital.

#### 2.5.4.5 Dialysate Collection and Concentration Determination

Interstitial fluid concentrations of the soft tissue in the lower extremity will be assessed by *in vivo* microdialysis. Participants will refrain from excessive movement during the sampling process. After catheter insertion, the probe will be perfused with lactated Ringer's solution for 30 minutes prior to sampling. A baseline dialysate sample (120 µL) will be taken over an hour after placement of the microdialysis catheters just before the administration of the final dose. Further dialysate samples of approximately 120 µL each will be collected at 0 (just before administration of the final dose of ceftolozane/tazobactam), 1, 2, 3, 4, 5, 6, 7, and 8 hours. After sampling is complete, recovery of the antibiotic through the dialysis filter will be assessed by the retrodialysis technique over a 1-hour interval (Section 2.5.4.2). All dialysate samples will be collected in 200 µL microvials (CMA Microdialysis AB, Solna, Sweden), which will be additionally stored within amber-colored polypropylene tubes at -80 °C to protect against light and evaporation until concentration determination. The catheter will then be removed by trained study personnel.

Both ceftolozane and tazobactam concentrations in dialysate will be assessed using HPLC at the Center for Anti-Infective Research and Development of Hartford Hospital.

#### 2.5.4.6 Protein Binding Analysis and Determination

Protein binding studies will be conducted by a minimum of three independent tests using Centrifree® Ultrafiltration devices (Millipore Corporation, Billerica, MA) with 30 kDa molecular cut-off filters, as per the manufacturer's package insert. Ultrafiltration of an aqueous standard will be performed to assess binding of the drug to the ultrafiltration membrane. An additional blood sample of 10 mL will be collected at the peak concentration (1 hour after final dose administration) into a 10-mL BD Vacutainer® containing sodium heparin (green top). Samples will be immediately centrifuged to collect the separated plasma. Exactly 0.9 mL of plasma

	<p>will be transferred into three separate ultrafiltration devices and centrifuged for 45 minutes at 25 °C at <math>2,000 \times g</math> to generate an ultrafiltrate volume of approximately 250 <math>\mu</math>L.</p> <p>Drug concentration in the initial aliquot of plasma and ultrafiltrates will be determined by HPLC at the Center for Anti-Infective Research and Development. In addition, non-specific protein binding of ceftolozane/tazobactam will be determined in aqueous fluid using a concentration that is similar to the peak concentration of the compound.</p> <p>Individual protein binding percentages will be applied to each participant's data at all time points to determine free plasma concentrations. The percentage of protein binding will be calculated using the following formula: % Protein Binding = <math>100 - (\text{Concentration}_{\text{ultrafiltrate}} / \text{Concentration}_{\text{plasma}} \times 100)</math>.</p> <p><b><u>2.5.4.7 Study Exit Evaluation</u></b> A full physical examination, including vital signs and clinical lab tests will be performed on the last day of the study. All abnormal determinations will be followed until resolution unless follow-up is determined to be unnecessary by the study physician.</p>												
<b>2.6 Study Duration</b>	<table> <tr> <td>IRB Submissions and Approval</td><td>2 months</td></tr> <tr> <td>Enrollment and Sampling of 10 Patients</td><td>6-12 months</td></tr> <tr> <td>Enrollment and Sampling of 6 Healthy Volunteers</td><td>4 months</td></tr> <tr> <td>Ceftolozane/Tazobactam HPLC</td><td>1 month</td></tr> <tr> <td>Pharmacokinetic Analysis</td><td>1 month</td></tr> <tr> <td>Final Report</td><td>2 months</td></tr> </table>	IRB Submissions and Approval	2 months	Enrollment and Sampling of 10 Patients	6-12 months	Enrollment and Sampling of 6 Healthy Volunteers	4 months	Ceftolozane/Tazobactam HPLC	1 month	Pharmacokinetic Analysis	1 month	Final Report	2 months
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<b>2.7 Statistical Analysis and Sample Size Justification</b>	<p><b><u>2.7.1 Data Validation and Analysis</u></b> The PI will be responsible for the review, validation, and analysis of these data as collected and recorded by the study staff.</p> <p><b><u>2.7.2 Primary Endpoint</u></b> The primary endpoints of the study are to assess the penetration of ceftolozane/tazobactam into infected skin tissue and compare it with the penetration into healthy skin tissue.</p> <p><b><u>2.7.2.1 Primary Endpoint Definition</u></b> Percentage of tissue penetration of ceftolozane/tazobactam will be defined based on the AUC in tissue and in plasma. This will be calculated as: <math>\text{AUC}_{\text{tissue}} / \text{freeAUC}_{\text{plasma}} \times 100</math>. Plasma pharmacokinetic parameters that will be estimated include <math>\text{AUC}_{0-8(\text{plasma})}</math>, elimination rate constant (<math>K_e</math>), half-life (<math>t_{1/2}</math>), total plasma clearance (<math>\text{CL}_T</math>), and Volume of distribution (<math>V_d</math>).</p> <p><b><u>2.7.3 Statistical Analysis</u></b> This is a descriptive pharmacokinetics study to evaluate the exposure and penetration ratio of ceftolozane/tazobactam into the interstitial fluid of infected tissue of diabetic patients compared with healthy volunteer participants.</p> <p>Pharmacokinetic analyses for ceftolozane/tazobactam will be conducted using Phoenix (version 6.3, Pharsight Corporation, Mountain View, CA). Non-compartmental pharmacokinetics analysis will be used. Pharmacokinetic parameters</p>												



	<p>for plasma will be determined using each individual's plasma concentration-time profile. The maximum concentration (<math>C_{max}</math>) for each participant will be estimated by visual inspection of the concentration-time profiles. The log-linear trapezoidal method will be used to determine <math>AUC_{0-24 (plasma)}</math> for each participant. The elimination rate constant (<math>K_e</math>) will be estimated by the slope of the terminal portion of the concentration-time profile; half-life (<math>t_{1/2}</math>) will be calculated by <math>0.693/K_e</math>. Total plasma clearance (<math>CL_T</math>) will be calculated by <math>Dose/AUC_{0-24 (plasma)}</math>. Volume of distribution (<math>V_d</math>) will be calculated by <math>CL_T/K_e</math>.</p> <p>All microdialysis concentrations will be corrected for recovery before pharmacokinetic analysis as follows: <math>Concentration_{tissue} = 100 \times (Concentration_{sample} / \% \text{ in vivo recovery})</math>. The <math>AUC_{0-24 (tissue)}</math> will be assessed by the log-linear trapezoidal rule. Percentage of penetration into tissue will be calculated as follows: <math>AUC_{tissue} / freeAUC_{plasma} \times 100</math>.</p> <p>Student's <i>t</i>-test will be used to compare the penetration ratio and pharmacokinetic parameter values of ceftolozane and tazobactam between study and control groups.</p> <p><b>2.7.4 Sample Size Determination</b></p> <p>Ten patients with diabetic foot infection and six healthy volunteers are to be enrolled in the study. This is primarily a descriptive, controlled study to observe the exposure and penetration ratio of ceftolozane/tazobactam into the interstitial fluid of infected tissue of diabetic patients and compare it to that of healthy volunteers. In similar study designs, 10 study patients were sufficient to describe the central tendency of these penetration estimates and some confidence of dispersion. Given reduced variability in healthy participants, 6 volunteers will be utilized.</p>
<b>2.8 Specific Drug Supply Requirements</b>	<p>Ceftolozane/tazobactam 1.5 g vials will be provided by Merck &amp; Co. as an open label supply.</p>
<b>2.9 Adverse Experience Reporting</b>	<p>Participants will be monitored for any sign or symptom of adverse events throughout the course of the study. Unanticipated, life-threatening or fatal adverse events will be reported to the IRB, the manufacturer, and the Food and Drug Administration according to federal guidelines. All adverse events requiring medical attention will be treated by the study physician and will be recorded by the investigator.</p> <p><b>2.9.1 Definitions</b></p> <p>For the purpose of this study, an adverse event will be defined as any pathologic or unintended change in the structure (signs), function (symptoms), or chemistry (laboratory values) of the body associated with the use of the study drug, whether or not considered drug related, and will be categorized as one of the following:</p> <ul style="list-style-type: none"> <li>• MILD – present, but easily tolerated</li> <li>• MODERATE – discomfort that interferes with usual activities</li> <li>• SEVERE – incapacitating, inability to work or do usual activities</li> </ul> <p>A serious adverse event will be defined as any of the above which results in death or is immediately life-threatening, requires in-participant hospitalization, or is an</p>

	<p>important medical event that may jeopardize the participant or require medical intervention to prevent one of the previously mentioned outcomes.</p> <p><b>2.9.2 Relationship to Study Medication</b>  Relationship of the AE to the study medication (i.e., causality) will be evaluated according to the investigator's opinion, as one of the following:</p> <ul style="list-style-type: none"> <li>• Concurrent condition – unrelated to study drug</li> <li>• REMOTE adverse drug event – little or no temporal relationship to study drug</li> <li>• POSSIBLE adverse drug event – temporal relationship to study drug</li> <li>• PROBABLE adverse drug event – commonly associated with study drug</li> <li>• DEFINITE adverse drug event – reappeared on re-challenge of study drug</li> </ul> <p><b>2.9.3 Expectedness of AE</b>  As AEs are expected with the study medication, patients will be closely monitored throughout the study for any AE occurrence and will be managed accordingly by the study physician. All AEs will be recorded by the investigator as described in Section 2.9.4 below.</p> <p><b>2.9.4 Recording and Reporting an AE</b>  All adverse events requiring medical attention will be recorded by the investigator as such: categorization by severity (mild, moderate, or severe), established time frame (start and stop time), complete description of event, all interventions (medical and pharmacological), and causality.</p> <p><b>2.9.5 Recording and Reporting a SAE</b>  All serious adverse events will be reported to the Institutional Review Board, sponsor, and the Food and Drug Administration according to Federal guidelines. A serious adverse event will be defined as any adverse event that results in death, is immediately life-threatening, requires or prolongs hospitalization, or is an important medical event that may jeopardize the participant or may require medical intervention to prevent one of the previously mentioned outcomes.</p>																														
<p><b>2.10 Itemized Study Budget</b></p>	<p><b>2.10.1 Staff Related Costs</b></p> <table> <tr><td>Principal Investigator</td><td>\$15,000</td></tr> <tr><td>Study Coordinator</td><td>\$26,250</td></tr> <tr><td>Research Assistant (1)</td><td>\$13,000</td></tr> <tr><td>Research Assistant (2)</td><td>\$6,500</td></tr> <tr><td>Study Physician</td><td>\$10,000</td></tr> <tr><td>Nursing</td><td>\$7,250</td></tr> <tr><td>Phlebotomy</td><td>\$480</td></tr> <tr><td>Clinical Research Center Medical Coverage (PA, APRN)</td><td>\$3,600</td></tr> </table> <p><b>2.10.2 Participant Fess</b></p> <table> <tr><td>Drug Toxicity Screening</td><td>\$3,900</td></tr> <tr><td>Patient Payments</td><td>\$5,000</td></tr> <tr><td>Healthy Volunteer Fees</td><td>\$6,000</td></tr> <tr><td>Healthy Volunteer Housing</td><td>\$1,440</td></tr> <tr><td>Healthy Volunteer Parking</td><td>\$600</td></tr> <tr><td>Healthy Volunteer Consumables</td><td>\$3,000</td></tr> </table> <p><b>2.10.3 Supplies</b></p> <table> <tr><td>Sample Storage Supplies</td><td>\$1,920</td></tr> </table>	Principal Investigator	\$15,000	Study Coordinator	\$26,250	Research Assistant (1)	\$13,000	Research Assistant (2)	\$6,500	Study Physician	\$10,000	Nursing	\$7,250	Phlebotomy	\$480	Clinical Research Center Medical Coverage (PA, APRN)	\$3,600	Drug Toxicity Screening	\$3,900	Patient Payments	\$5,000	Healthy Volunteer Fees	\$6,000	Healthy Volunteer Housing	\$1,440	Healthy Volunteer Parking	\$600	Healthy Volunteer Consumables	\$3,000	Sample Storage Supplies	\$1,920
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	Blood Sampling Supplies	\$4,000
	Peripherally Inserted Central Catheters	\$8,000
	Lidocaine 0.5%, 50ml flip top, pack of 25	\$60
	Millipore Ultrafiltration Devices	\$2,880
	CMA 60 Microdialysis Probe (\$1950 pack of 4)	\$7,800
	CMA 107 Pump (\$3450 per pump, n=2)	\$6,900
	CMA 107 Pump Batteries (package of 5)	\$700
	CMA 20/107 Sample Collection Vials/Syringes	\$2400
	HPLC Analysis of Samples	\$29,120
	Laboratory Services	\$16,000
	<b>2.10.4 Administrative Fees</b>	
	Pharmacy Dispensing Fess	\$3,200
	Institutional Document Preparation and Submission	\$2,000
	Publication Cost (Medical Writing)	\$4,500
	IRB Fees	\$2,500
	Meeting-Related Expenses	\$2,500
	<b>2.10.5 Overhead Costs</b>	
	25%	
	(excluding IRB Fees and Meeting-Related Expenses)	\$47,875
	<b>Total Direct Cost (Less Overhead)</b>	\$196,500
	<b>TOTAL</b>	<b>\$244,375</b>
<b>2.11 References</b>	<ol style="list-style-type: none"> <li>1. Margolis D, Malay DS, Hoffstad OJ, et al. Incidence of diabetic foot ulcer and lower extremity amputation among Medicare beneficiaries, 2006 to 2008. Data Points #2 (prepared by the University of Pennsylvania DEcIDE Center, under Contract No. HHSA29020050041I). Rockville, MD: Agency for Healthcare Research and Quality. January 2011. AHRQ Publication No. 10(11)-EHC009-1-EF</li> <li>2. Lipsky BA. New developments in diagnosing and treating diabetic foot infections. <i>Diabetes Metab Res Rev</i> 2008; electronically published ahead of print.</li> <li>3. Lipsky BA. Medical treatment of diabetic foot infections. <i>Clin Infect Dis</i> 2004; 39:S104-114.</li> <li>4. Ramsey SD, Newton K, Blough D, et al. Incidence, outcomes, and cost of foot ulcers in participants with diabetes. <i>Diabetes Care</i> 1999; 22:382-387.</li> <li>5. Lipsky BA, Berendt AR, Cornia PB, et al. 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. <i>Clin Infect Dis</i>. 2012;54(12):e132-73.</li> <li>6. Zerbaxa<sup>®</sup> (ceftolozane/tazobactam) Prescribing Information, Cubist Pharmaceuticals Inc. Lexington, MA. 2014.</li> <li>7. Zhanel GG, Chung P, Adam H, et al. Ceftolozane/tazobactam: a novel cephalosporin/β-lactamase inhibitor combination with activity against multidrug-resistant gram-negative bacilli. <i>Drugs</i>. 2014;74(1):31-51.</li> <li>8. Müller M, Haag O, Burgdorff T, et al. Characterization of peripheral-compartment kinetics of antibiotics by in vivo microdialysis. <i>Antimicrob Agents Chemother</i> 1996; 40:2703-2709.</li> <li>9. Bhalodi AA, Housman ST, Shepard A, Nugent J, Nicolau DP. Tissue pharmacokinetics of cefazolin in patients with lower limb infections. <i>Antimicrob Agents Chemother</i>. 2013;57(11):5679-83.</li> </ol>	

	<ol style="list-style-type: none"> <li>10. Wiskirchen DE, Shepard A, Kuti JL, Nicolau DP. Determination of tissue penetration and pharmacokinetics of linezolid in patients with diabetic foot infections using <i>in vivo</i> microdialysis. <i>Antimicrob Agents Chemother</i>. 2011;55(9):4170-5.</li> <li>11. Bulik CC, Wiskirchen DE, Shepard A, Sutherland CA, Kuti JL, Nicolau DP. Tissue penetration and pharmacokinetics of tigecycline in diabetic patients with chronic wound infections described by using <i>in vivo</i> microdialysis. <i>Antimicrob Agents Chemother</i>. 2010;54(12):5209-13.</li> <li>12. Walker M, Hadgraft J, Lane ME. Investigation of the permeability characteristics of peri-ulcer and whole ischaemic skin tissue. <i>Int J Pharm</i> 2008; electronically published ahead of print.</li> </ol>
<b>2.12 Publication Plan</b>	The results of this study will be presented at an international congress such as the Interscience Conference on Antimicrobial Agents and Chemotherapy. A final publication would be submitted to a peer-reviewed journal such as Antimicrobial Agents and Chemotherapy, Journal of Antimicrobial Chemotherapy, or International Journal of Antimicrobial Agents. One abstract and one manuscript are anticipated to be presented at the conference and published.
<b>2.13 Curriculum Vitae</b>	A curriculum vita is uploaded online with this submission.
<b>2.13 Protocol Submission for Investigator-Initiated Studies</b>	<p>U.S. protocols should be submitted by US investigators directly or through the Global Research Specialist at <a href="http://www.merckisps.com">www.merckisps.com</a></p> <p>Non U.S. protocols should be submitted to the MSD office by the investigators.</p>