As described herein, a series of hollow-fiber infection model studies were undertaken to determine the tebipenem exposure required to prevent on-therapy drug-resistance and to evaluate the tebipenem regimen evaluated in the recently-completed Phase 3 clinical trial.

**METHODS**

**Antimicrobial Agent and Challenge Isolates**

- A panel of 35 E. coli isolates were selected based upon their known resistance mechanisms and tebipenem minimum inhibition concentration (MIC) values. All isolates were either purchased from ATCC Laboratories (North Liberty, IA) or provided by the National Collection of Type Cultures (Table 1).

**Tebipenem was provided by Spero Therapeutics (Cambridge, MA).**

**RESULTS**

**Table 1.** Known resistance mechanisms and tebipenem MIC values of isolates utilized in the hollow-fiber in vitro infection model studies.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>MIC (mg/L)</th>
<th>Age</th>
<th>Known resistance mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli 998822</td>
<td>0.005</td>
<td>18</td>
<td>β-lactamase (ESBL) and AmpC-β-lactamase</td>
</tr>
</tbody>
</table>

**Hollow-Fiber In Vitro Model**

- 10 mL of each E. coli isolate were inoculated into the hollow-fiber in vitro infection model cartridge (Ribebern Systems, Frederick, MD) at an inoculum of 10^8 colony forming units (CFU/mL) using Mueller-Hinton broth medium (Table 1).

Each challenge isolate was subjected to concentration-time profiles simulating free-drug plasma concentrations observed after tebipenem administration in healthy volunteers, assuming a protein binding value of 45%. (8)

**Hollow-Fiber In Vitro Model**

- A series of 10-day dose-ranging studies were completed using two isolates (E. coli 998822 and NCTC 13441) exposed to tebipenem regimens of 4.6 to 1200 mg q8h, linearly scaled from the tebipenem 600 mg q8h regimen evaluated in healthy volunteers.

- A single isolate (E. coli 4443) was evaluated over a 10-day period using only the 600 mg q8h regimen evaluated in the Phase 3 trial.

For each study, samples were collected for the enumeration of the total and resistant isolates, with samples obtained at 0 and 5 hours after start of the experiment, and on Days 1, 2, 3, 4, 6, and 8, respectively.

- Samples for bacterial enumeration were washed twice with sterile saline, serially diluted and plated on both drug-supplemented and agar-supplemented tebipenem concentrations representing four-times the baseline aminoglycoside MIC.

- MIC values were determined for a subset of isolates found in the drug-supplemented agar plates.

- 1 mL samples were collected for the evaluation of the simulated pharmacokinetic profile via liquid chromatography-tandem mass spectrometry on a Sciex 5500 with an Exion LC AC front-end.

- All hollow-fiber in vitro infection model studies were completed in duplicate and compared to a no-treatment control.

**Figure 1.** Schematic of the hollow-fiber in vitro infection models utilized in the studies described herein.

**Figure 2.** The relationship between targeted and observed tebipenem concentrations simulated in the hollow-fiber in vitro infection model.

- The targeted concentration-time profiles for tebipenem were well simulated in the hollow-fiber in vitro model for all dosing regimens.

- The above-described agreement was supported by the coefficient of determination (r^2) of 0.98 and a slope value of 0.93, representing a deviation from 1 to 4% for the agreement observed between targeted and observed concentration-time profiles.

**Figure 3.** Average E. coli NCTC 13441 total and drug-resistant subpopulations observed in the 10-day hollow-fiber in vitro infection model dose-ranging studies.

- A 4-log dose-response, ranging from treatment failure to reductions in bacterial burden from baseline, was observed in the 10-day hollow-fiber in vitro infection model dose-ranging studies for E. coli 998822 and NCTC 13441 as shown in Figure 3 and 4, respectively. A summary of the results is provided below.

- For E. coli 998822 shown in Figure 3, amplification of resistant subpopulations to densities greater than that observed in the no-treatment control was observed for tebipenem doses ranging from 37.5 mg to 150 mg q8h.

- Tebipenem 600 mg q8h successfully reduced the bacterial burden to ≤ 3 log CFU/mL with no amplification of resistance over the 10-day duration of the study.

**Figure 4.** Average E. coli NCTC 13441 shown in Figure 4, amplification of resistant subpopulations was observed for tebipenem dosing regimens of 150 and 300 mg q8h.

- Tebipenem 600 mg q8h with an average bacterial burden between 4 and 8 log CFU/mL over the 10-day period, with amplification of the resistant population only seen in one replicate on Day 10.

**Figure 5.** Average E. coli NCTC 13441 total and drug-resistant subpopulations observed in the 10-day hollow-fiber in vitro infection model dose-ranging studies.

- As shown in Figure 5 for E. coli 9443, a 2.3 log CFU/mL reduction over the course of the 10-day period, with amplification of resistance occurring in one dose, was observed after administration of tebipenem 600 mg q8h.

**Figure 6.** Average E. coli 4443 total and drug-resistant subpopulations observed for tebipenem 600 mg q8h hollow-fiber infection models based on the study for tebipenem 600 mg q8h dose.

**CONCLUSIONS**

- The stability of tebipenem 600 mg q8h, the dosing regimen studied in a Phase 3 study of patients with cUTI [4], to suppress the amplification of a pre-existing drug-resistant E. coli subpopulation was evaluated over 10 days in a study conducted using a hollow-fiber in vitro infection model.

- When challenged with a bacterial inoculum of 1 x 10^6 CFU/mL, tebipenem exposures ranging from 40 to 900 mg q8h reduced bacterial burdens below the level observed on the initial inoculum over a 10-day study period, with intermittent amplification of pre-existing drug-resistant subpopulations towards the end of the study.

- These data support the selection of tebipenem 600 mg q8h dosing regimen that minimizes the potential for on-therapy drug-resistance and maximizes the growth of bacteria occurring in one of two replicates, was observed after administration of tebipenem 600 mg q8h.

**REFERENCES**