Pharmacokinetic-Pharmacodynamic Analyses for GT-1 (LCB10-200) Using Data from a Murine-Thigh Infection Model

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REVISED ABSTRACT

Background: GT-1 is a novel siderophore-cephalosporin (co-developed by LegoChem Biosciences and Geom Therapeutics) with *in vitro* activity against resistant Gram-negative bacteria, including *P. aeruginosa, A. baumannii,* and Enterobacteriaceae. Given this activity, GT-1 is being developed for the treatment of patients with urinary and other serious hospital infections. These studies were undertaken to characterize the PK-PD of GT-1.

Methods: PK was determined in infected mice after SQ administration (4 to 1200 mg/kg). A series of studies were conducted to identify the PK-PD index associated with efficacy (dose-fractionation study) and the magnitude of that PK-PD index needed for various levels of efficacy (dose-ranging studies). Mice were rendered neutropenic by 2 injections of cyclophosphamide 4 days (150 mg/kg) and 1 day (100 mg/kg) prior to the start of therapy. Infection was produced by injection of ~1 X 10^6 CFU into the posterior thighs of mice 2 hours prior to treatment. The challenge isolate in the dose-fractionation study (4.69 to 1200 mg/kg daily doses administered in equal divided doses q3, 6, or 12h) was *E. coli* ATCC 25922 (MIC = 0.25 mg/L). The challenge isolates (n=10) in the dose-ranging studies (1.17 to 300 mg/kg q6h) included *E. coli* (n=4), *K. pneumoniae* (n=3) and *P. aeruginosa* (n=3) with MIC values ranging from 0.06 to 2 mg/L. Relationships between change in $\log_{10}$ CFU from baseline and free-drug plasma AUC:MIC and Cmax:MIC ratio and %T>MIC were evaluated using Hill-type models.

Results: A linear 3-compartment model with 1st-order absorption fit the plasma PK data well (r^2 = 0.969). Like other ß-lactams, the PK-PD index that best described *in vivo* efficacy was %T>MIC (Figure 3). Across the 10 Gram-negative isolates, the free-drug %T>MIC associated with net bacterial stasis, and 1- and 2-$\log_{10}$ CFU reductions from baseline was 40.2, 58.3, and 85.8, respectively (r^2 = 0.800).

Conclusions: These data will be useful to support GT-1 dose selection for future clinical studies.

INTRODUCTION

- GT-1 (co-developed by LegoChem Biosciences and Geom Therapeutics) is a novel cephalosporin with *in vitro* activity against multi-drug resistant Gram-negative bacteria, including *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and Enterobacteriaceae.
  - Activity towards these organisms is in-part attributable to a siderophore-like moiety in GT-1's chemical structure.
    - Siderophores are iron chelating compounds released by numerous pathogens, which are responsible for scavenging iron and delivering it to these pathogens.
- Given its activity to highly resistant Gram-negative bacteria, GT-1 is being developed for the treatment of patients with urinary and other serious hospital infections.
- The studies described herein were undertaken to characterize the pharmacokinetics-pharmacodynamics (PK-PD) of GT-1.

OBJECTIVES

- To determine the PK-PD index most associated with GT-1 efficacy using a murine-thigh infection model; and
- To identify PK-PD targets associated with net bacterial stasis and 1- and 2-$\log_{10}$ colony forming units (CFU) reductions from baseline.

METHODS

- Six-week old, pathogen-free, female mice from the Institute for Cancer Research (ICR) weighing 23 to 27 g were used for all studies.

Pharmacokinetic Study

- PK samples were collected, at the times displayed in **Table 1**, post-dose via tail vein puncture from neutropenic mice assigned to receive one of six subcutaneous GT-1 single dose regimens (4 to 1200 mg/kg).
- Plasma concentrations were determined using liquid chromatography-tandem mass spectrometry (LC-MS/MS).
- A PK model was fit to the dataset using NONMEM.

Table 1. Pharmacokinetic sampling scheme

Time Point	Number of Samples					
	4 mg/kg	16 mg/kg	64 mg/kg	256 mg/kg	600 mg/kg	1200 mg/kg
0.5	3	3	6	3	3	3
1	3	3	6	3	3	3
1.5	-	-	3	-	3	3
2	3	3	6	3	3	3
3	3	3	6	3	3	3
4	3	3	6	3	3	3
6	-	-	3	-	3	3
8	3	3	6	3	3	3
12	3	3	6	3	3	3

In Vitro Susceptibility Testing

- Susceptibility-testing studies were completed in duplicate using Clinical and Laboratory Standards Institute (CLSI) methods for the 11 bacterial isolates used in the dose-fractionation and dose-ranging studies.
- MHBII treated with Chelex and supplemented with magnesium and calcium (CMHBII) was utilized.

Neutropenic Murine-Thigh Infection Model

- Animals were rendered neutropenic by two intraperitoneal (IP) injections of cyclophosphamide, the first at 150 mg/kg 4 days before infection (Day–4) and the second at 100 mg/kg 1 day prior to inoculation (Day–1).
- On Day 0, animals were inoculated intramuscularly (0.1 mL/thigh) into the right thigh.
- Treatment (with either GT-1 or a vehicle) was administered beginning 2 hours post-inoculation.
- Animals were euthanized at 2 and 26 hours post-inoculation. Each animal's right thigh muscle was then harvested and subsequently weighed, homogenized, and plated for determination of CFU per gram.

Dose-Fractionation Study

- Challenge isolate was *E. coli* ATCC 25922.
- Five total GT-1 daily doses (1200, 300, 75, 18.75, and 4.69 mg/kg) were fractionated over four dosing intervals (every 3, 6, 12, and 24 hours).
- A protein binding estimate of 15.8% [Data on file, Geom Therapeutics] and the developed PK model were used to compute the free-drug AUC:MIC ratio, Cmax:MIC ratio, and %T>MIC for each dosing regimen.
- Hill-type models were then fit to the data. Both visual inspection of the data and the coefficient of determination (r^2) were used determine the PK-PD index most associated with GT-1 efficacy.

METHODS

Dose-Ranging Study

- Efficacy of GT-1 300, 75, 18.75, 4.69, and 1.17 mg/kg every six hours (q6h) against the 11 bacterial isolates (5 *Escherichia coli, 3 Klebsiella pneumoniae,* and 3 *P. aeruginosa)* was evaluated.
- Using the developed PK model and a protein binding estimate of 15.8%, free-drug %T > MIC values were computed for each regimen and corresponding isolate.
- A Hill-type model was fit to the data. PK-PD targets for net bacterial stasis and 1- and 2-$\log_{10}$ CFU reductions from baseline were determined.

RESULTS

Pharmacokinetic Study

- A linear 3-compartment model with 1st-order absorption best described GT-1 PK in mice following subcutaneous administration.
- Final model parameter estimates are shown in **Table 2**. As shown in **Figure 1**, the model provided excellent fits to the GT-1 plasma concentration-time profiles at multiple dose levels, as evidenced by the overall r^2 of 0.969 based on observed versus population predicted plasma concentrations.

Table 2. Pharmacokinetic model parameters

Parameter	Unit	Estimate
Volume central compartment	L/kg	0.161
Clearance	L/kg/h	2.04
Volume peripheral compartment	L/kg	0.0791
Distributional clearance	L/kg/h	0.0225
Volume peripheral compartment 2	L/kg	0.837
Distributional clearance 2	L/kg/h	1.67
Lag time	h	0.306
Absorption rate constant	h^{-1}	3.5

Figure 1. Goodness-of-fit plots for GT-1 concentration data grouped by dose on linear (A) and logarithmic (B) x-axes

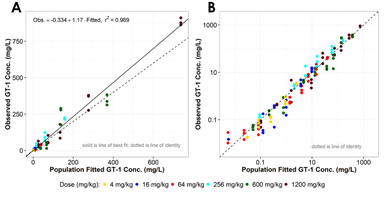


Table 3. GT-1 MIC values for the Gram-negative isolates evaluated

Isolate	MIC (mg/L)
E. coli NIH-J	≤ 0.03[a]
E. coli ATCC 25922	0.25
E. coli 1-894-1	0.25
E. coli 1-741-1	1
E. coli 355	1
K. pneumoniae ATCC 43816	0.06
K. pneumoniae 216	1
K. pneumoniae 7023	2
P. aeruginosa PO2	0.25
P. aeruginosa ATCC 27853	1
P. aeruginosa 4304A	2

a. MIC of 0.03 mg/L assumed for analyses

RESULTS

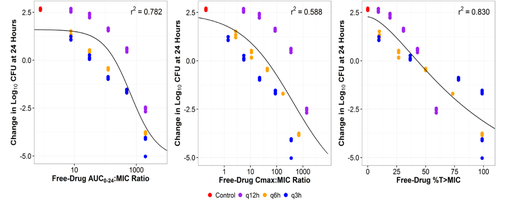
In Vitro Susceptibility Testing

- As displayed in **Table 3**, the MIC ranges for the *E. coli*, *K. pneumoniae*, and *P. aeruginosa* isolates were <0.03-1, 0.06-2 and 0.25-2 mg/L, respectively.

Dose-Fractionation Study

- Evaluation of the results of the dose-fraction study (**Figure 2**) revealed that the PK-PD index most associated with GT-1 efficacy is %T > MIC, as evidenced by the fairly heterogeneous spread of the data and good fit of the Hill-type model (r^2 = 0.830).

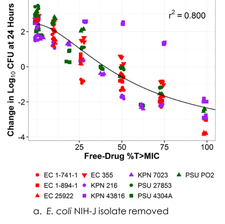
Figure 2. Relationships between change in $\log_{10}$ CFU from baseline at 24 hours and GT-1 free-drug $AUC_{0\text{-}24}$:MIC ratio, Cmax:MIC ratio, and %T > MIC



Dose-Ranging Study

- *E. coli* NIH-J isolate was excluded from the analysis because the MIC was off-scale low.
- The final data with a fitted Hill-type function is displayed in **Figure 3**.
  - *K. pneumoniae, E. coli,* and *P. aeruginosa* co-modeled well.
- Free-drug %T > MIC ratio targets associated with net bacterial stasis and 1- and 2-$\log_{10}$ CFU reductions of 40.2, 58.3, and 85.8, respectively, were identified based on the analysis excluding *E. coli* NIH-J.

Figure 3. Relationship between change in $\log_{10}$ CFU from baseline and GT-1 free-drug %T>MIC ratio based on data for various *K. pneumoniae*, *E. coli*, and *P. aeruginosa* isolates[a]



a. *E. coli* NIH-J isolate removed

CONCLUSIONS

- The results of the pooled PK-PD analysis based on data for all *E. coli*, *K. pneumoniae*, and *P. aeruginosa* isolates, with the exception of *E. coli* NIH-J isolate, demonstrated that the data co-modeled well (r^2=0.800).
- Based on the pooled analysis, free-drug %T > MIC targets associated with net bacterial stasis and 1- and 2-$\log_{10}$ CFU reductions for *E. coli, K. pneumoniae,* and *P. aeruginosa* isolates of 40.2, 58.3, and 85.8, respectively, were identified.

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