A-048

Pharmacodynamic evaluation of AAI101, a novel extended-spectrum beta-lactamase inhibitor (BLI), with piperacillin against ESBL producers in a hollow fiber infection model

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Abstract

Background: AAI101 is a novel extended-spectrum BLI with potent activity against diverse beta-lactamases. To explore dosing and frequency of administration of AAI101, dose range-finding studies in combination with piperacillin (PIP) were performed with ESBL-producing *K. pneumoniae* strains. The pharmacodynamic (PD) driver for AAI101 associated with efficacy was determined by dose fractionation.

Methods: MICs and mutation frequencies (MFs) were obtained for CTX-M-15- and SHV-producing *K. pneumoniae* strains. Ten-day dose range studies for PIP 4 g q6h, 30-min boluses, alone or combined with 2-fold increments of continuous infusions (CIs) of AAI101, identified doses promoting bacterial killing and preventing amplification/emergence of resistance. The lowest bactericidal PIP-AAI101 dose for the SHV producer was selected for a dose fractionation study.

Results: PIP MICs for the CTX-M-15 and SHV producers were 8 and 4mg/L, respectively, in the presence of 8 mg/L of AAI101, *vs.* >256 mg/L and 128 mg/L resp. for PIP alone. MFs to 3 x MIC were ~7.57 and <~8.84, resp. For the CTX-M-15 producer, resistance emerged with PIP alone or with PIP + AAI101 CIs ≤8 mg/L, whereas AAI101 concentrations ≥16 mg/L prevented resistance. For the SHV producer, PIP alone failed but PIP + AAI101 CIs ≥4 mg/L resulted in bacterial killing and prevented resistance. Dose fractionation of AAI101 with the SHV producer showed that time above a threshold concentration of AAI101 (T > Th) is the PD driver associated with efficacy. To achieve killing and prevent resistance, the minimal time the threshold of 4 mg/L of AAI101 needed to be exceeded was between 27% and 38% of the dosing interval.

Conclusions: At clinically achievable concentrations, AAI101 combined with piperacillin was efficacious against PIP-resistant ESBL-producing *K. pneumoniae* and prevented amplification/emergence of resistance in a 10-day hollow fiber infection model. For the SHV producer, the AAI101 pharmacodynamic driver for bacterial eradication and prevention of resistance was time above a threshold concentration (T > Th) of 4 mg/L, with the minimal T > Th for efficacy lying between 27% and 38% of the dosing interval.

Background

- Enterobacteriaceae that express extended-spectrum beta-lactamases (ESBLs), including CTX-Ms and SHVs, are important causes of hospital-associated infections. These microbes are resistant to multiple beta-lactam antibiotics, including piperacillin, ceftazidime, and cefepime. Certain beta-lactamase inhibitors can restore the activity of the beta-lactam antibiotics against ESBL-producing bacteria. The pharmacodynamic index that predicts the activity of the commercially-licensed beta-lactamase inhibitors, including tazobactam, and sulbactam, is time above a threshold (T > Th) concentration.

- AAI101 is an investigational beta-lactamase inhibitor with broader activity against class A, class C and class D beta-lactamases compared to tazobactam. In phase I pharmacokinetic studies in human volunteers AAI101 demonstrated dose-proportional increases in plasma Cmax and 24 h-AUC values for single doses of 1,000 to 4,000 mg IV. Multi-dose AAI101 of 1 and 2 g IV Q6h had Cmax values of 63.9 and 136.5 mg/L and $AUC_{0\text{-}6h}$ of 146.9 and 292.8 mg·h/L, respectively. The mean plasma half-life was approximately 2 hours. The PK of AAI101 was similar when examined alone and in combination with PIP 4 g IV q6h.

- The purpose of this project was to conduct dose-range and dose-fractionation studies in an *in vitro* hollow fiber infection model to identify the exposure intensity and frequency of administration of AAI101 that maximizes the activity of piperacillin 4 g IV q6h for killing *Klebsiella pneumoniae* strains producing CTX-M or SHV ESBLs and prevents the amplification of bacterial subpopulations with reduced susceptibilities to the beta-lactam/beta-lactamase combination.

Methods

- **Bacteria.** *K. pneumoniae* (Kpn) 24-1314a produces a CTX-M-15 ESBL and Kpn 3331 expresses a SHV ESBL. The strains were obtained from JMI Laboratories (North Liberty, IA).

- **Drugs**. The structures of AAI101 and tazobactam are shown in **Figure 1.** Vials of AAI101 powder were provided by Allecra Therapeutics SAS (St-Louis, France) on dry ice. The vials were stored at -80°C in a desiccator. AAI101 powder was dissolved in sterile water and then with medium. Piperacillin sodium (PIP) was purchased from MP Biomedical, LLC (Solon, OH). PIP powder was dissolved in water, aliquoted, and stored at -80°C. For each study an aliquot of the PIP was thawed and diluted in medium or water.

Fig 1. Structure of tazobactam and AAI101

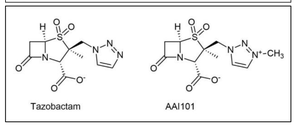
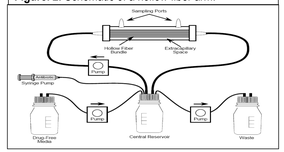


Figure. 2. Schematic of a hollow fiber arm.



- **Susceptibility studies**. Microdilution broth and agar dilution MICs for two-fold dilutions of PIP alone and in combination with fixed concentrations of 4 and 8 mg/L of AAI101 were determined in cation-adjusted Mueller Hinton II broth and in Mueller Hinton agar. MICs for PIP in combination with 4 mg/L of tazobactam were also examined. Final bacterial densities in the broth and on agar were 5 x 10^5 CFU/mL and 10^4 CFU/spot, respectively. Cultures were incubated at 35°C, ambient air, for 16-20 h before the MICs were read.

- **Mutation frequencies studies.** The mutation frequency for 3 x MIC of PIP in combination with a fixed concentration of 8 mg/L of AAI101 were conducted using standard methods.

- **Hollow fiber infection model (HFIM).** HFIMs **(Figure 2)** examine the effect of simulated drug PK profiles on the killing of bacteria and on the amplification of drug resistance. HFIM experimental arms containing ~10^7 CFU/mL (10 mL) of late log-phase growth bacteria were treated with simulated regimens of PIP 4 g IV Q6h (steady state free Cmax: 85.7 mg/L, free C_{trough} 12.7 mg/L , protein binding of 30%, half-life 2 h) given as 30-min infusions. For the dose-range studies, AAI101 was given as continuous infusions following a loading dose. For the dose fractionation studies, AAI101 was given using different schedules of administration. The bolus-dosed regimens for AAI101 were administered as 30-min infusions, simulating a 2 h half-life (no protein binding). Treatment was for 10 days.

- Throughout the experiments bacterial specimens taken from the study arms were cultured quantitatively on drug-free agar and agar supplemented with 3 x MIC PIP in combination with 8 mg/L of AAI101 in order to evaluate the effect of each regimen on the total and less-susceptible bacterial populations.

Results

- **Susceptibility studies.** The broth MIC values are shown in Table 1. CLSI breakpoints for PIP: S ≤ 16, I = 32 -64, and R ≥ 128 mg/L.

Table 1. Broth MICs (mg/L) for PIP alone, AAI101 alone, and PIP combined with fixed concentrations of 4 and 8 mg/L of AA101 (AAI). PIP combined with a fixed conc. of 4 mg/L of tazobactam (tazo) was the comparator.

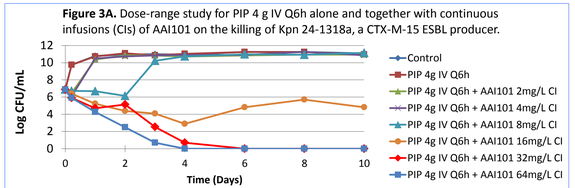
Strain	ESBL type	PIP	AAI	PIP + AAI 4 mg/L	PIP + AAI 8 mg/L	PIP + tazo 4 mg/L
Kpn 24-1318a	CTX-M-15	>256	>256	8	8	8
Kpn 3331	SHV	128	>256	4	4	4

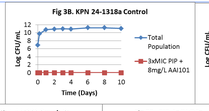
- **Mutation frequency values.** Mutation frequency to 3 x MIC of PIP plus 8 mg/L of AAI101 was ~7.57 log CFU for Kpn 24-1318a, a CTX-M-15 producer. For Kpn 3331 (SHV ESBL) the mutation frequencies were < ~8.84 log CFU in 2 trials and ~8.89 log CFU in 1 trial. Isolates that grew on the drug-containing agars had PIP MICs (when tested with a fixed conc. of 8 mg/L of AAI101) of >128 mg/L for Kpn 24-1318a and 16 mg/L for Kpn 3331.

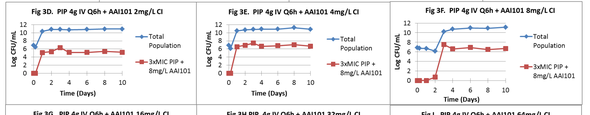
Results (Continued)

- **Dose-range study for Kpn 24-1318a (CTX-M-15). Figure 3A** shows the dose-response effect of PIP alone and combined with 2-fold increments of continuous infusions (CIs) of AAI101 on the total bacterial population. **Figures 3B-3I** show the effect of these regimens on the less-susceptible bacterial populations. Drug resistance was seen for PIP alone and in combination with CIs of AAI101 ≤ 8 mg/L, whereas PIP together with AAI101 CIs ≥ 16 mg/L prevented resistance. The resistant populations had MICs for PIP ≥ 64 mg/L when tested with a fixed AAI101 conc. of 8 mg/L.

Figure 3A. Dose-range study for PIP 4 g IV Q6h alone and together with continuous infusions (CIs) of AAI101 on the killing of Kpn 24-1318a, a CTX-M-15 ESBL producer.

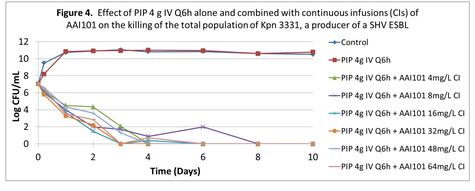






- **Dose-range study for Kpn 3331, a SHV ESBL producing strain. Figure 4** shows the effect of PIP 4 g IV Q6h alone and in combination with CIs of 4 to 64 mg/L of AAI101. Only the unprotected PIP arm failed. Bacteria in the PIP alone arm had PIP MICs of 128 mg/L. PIP in combination with CIs of AAI101 ≥ 4 mg/L was successful. Bacterial suspensions plated on agar containing 3 x MIC of PIP plus 8 mg/L of AAI101 were all negative for growth (data not shown).

Figure 4. Effect of PIP 4 g IV Q6h alone and combined with continuous infusions (CIs) of AAI101 on the killing of the total population of Kpn 3331, a producer of a SHV ESBL



Results (Continued)

- **Dose-fractionation study for AAI101 tested in combination with PIP 4 g IV Q6h against Kpn 3331, which produces a SHV ESBL.** PIP 4 g IV Q4h was examined alone and in combination with different dosing schedules for AAI101 **(Table 2).** The targeted 24h-AUC for AAI101 for all of the fractionated regimens was 96 mg·h/L, which is the 24h-AUC exposure for the lowest conc. of AAI101 (4 mg/L CI) that was examined in the dose-range study using Kpn 3331.

Table 2. PK/PD parameters for the dose fractionation study for PIP and AAI101 against Kpn 3331, a SHV ESBL producer. The PIP MIC for the strain was 4 mg/L when tested with a fixed conc. of AAI101 8 mg/L. The PK/PD targets for AAI101 were relative to a **threshold (Th) value of 4 mg/L**, which was the lowest concentration of AAI101 examined in the dose-range study which was successful.

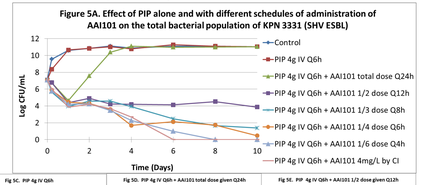
PIP Regimen	AUC/MIC	Cmax/MIC	Ctrough/MIC	Time > MIC	% Time above MIC
PIP 4g IV Q6h	233.24	21.415	3.183	24	100%

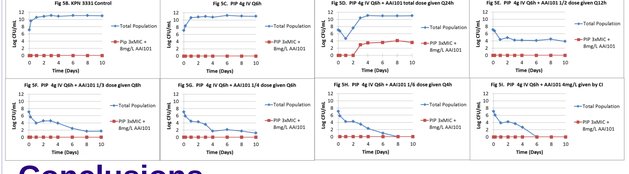
AAI Regimens	AUC/Th ratio	Cmax/Th ratio	Ctrough/Th ratio	Time > a Th of 4mg/L of AAI101	% Time > Th of 4 mg/L of AAI101
AAI101 AUC 96, total dose Q24h	24	7.617	0.003	6.13	26%
AAI101 AUC 96, ½ of the total dose Q12h	24	3.874	0.072	9	38%
AAI101 AUC 96, ⅓ of the total dose Q8h	24	2.724	0.203	10	42%
AAI101 AUC 96, ¼ of the total dose Q6h	24	2.204	0.033	10.5	44%
AAI101 AUC 96, ⅙ of the total dose Q4h	24	1.744	0.519	10.7	45%
AAI101 AUC 96/day (or 4 mg/L) by CI	24	1	1	24	100%

- PIP alone failed with bacteria that remained susceptible to 3 x MIC of PIP plus 8 mg/L of AAI101. PIP in combination with once-daily dosed AAI101 failed (**Figs. 5A and 5D**) due to isolates that had PIP/AAI101 MICs of 16/8 mg/L compared with a PIP/AAI101 MIC of 4/8 mg/L for the parent strain. All other PIP + AAI101 regimens were successful and showed increasing amounts of bacterial kill with increasing T > Th values.

- For Kpn 3331, a SHV ESBL producer, the AAI101 pharmacodynamic driver for bacterial eradication and prevention of resistance was time above a threshold conc. (T > Th) of 4 mg/L, with the minimal T > Th for efficacy between 27% and 38% of the dosing interval.

Figure 5A. Effect of PIP alone and with different schedules of administration of AAI101 on the total bacterial population of KPN 3331 (SHV ESBL)





Conclusions

- Piperacillin/AAI101 combinations at clinically achievable concentrations are efficacious and prevent amplification/emergence of resistance against ESBL-producing *K. pneumoniae.*

- The pharmacodynamic driver for AAI101 to achieve bacterial eradication and prevent resistance is time above a threshold concentration (T > Th). The minimal T > Th for maximal efficacy was 27 – 38% of the dosing interval.