In-vitro activity of diverse β-lactam/AAI101 combinations *vs.* multidrug-resistant Gram-negative clinical strains

S. Mushtaq,[1] A. Chaudhry,[1] R. Adkin,[1] N. Woodford,[1] N. Benedict,[1] R. Pypstra,[2] and S. Shapiro[2]

[1]Antimicrobial Resistance & Healthcare Associated Infections Reference Unit, Public Health England, UK; [2]Allecra Therapeutics SAS, St-Louis, France

Introduction

AAI101 is a novel extended-spectrum β-lactamase inhibitor (BLI), belonging to the penicillanic acid sulfone family, which is currently in Phase I clinical trials.

β-Lactam resistance in Gram-negative pathogens principally involves production of a wide diversity of β-lactamases, and may be exacerbated by auxiliary non-β-lactamase mechanisms such as porin mutations and upregulated efflux. Whilst BLIs like tazobactam (Tazo) have helped preserve the clinical value of β-lactams by inhibiting β-lactamases before they can inactivate the antibiotic, emergence of more aggressive β-lactamases, especially when complemented by auxiliary non-β-lactamase resistance mechanisms, have restricted treatment options for infections caused by multidrug-resistant (MDR) Enterobacteriaceae and non-fermentative bacilli.[1]

The purpose of this study was to assess the *in-vitro* activity of AAI101 in combination with different β-lactam antibiotics against 61 Gram-negative clinical isolates representing a variety of β-lactam resistance mechanisms.

Materials and Methods

- Agar dilution MICs were determined for 61 bacterial strains, each with a defined mechanism of β-lactam resistance (Table 1).

Table 1. Resistotypes of "challenge panel"*

Species	Represented β-lactamases (and other resistance mechanism)
E. coli (n = 22)	TEM-3, TEM-10, 3 CTX-M-2, 3 CTX-M-14, 3 CTX-M-15; 3 KPC, NMC-A; 5 pAmpCs (ACC, 2 CIT/CMYs, DHA, FOX); 2 OXA-48
K. pneumoniae (n = 15)	2 ESBL + porin loss; 11 KPC (including 6 ST258 phenotype); 2 OXA-48
Enterobacter spp. (n = 9)	TEM-24; cAmpC, ciAmpC, 2 cdAmpC + porin loss, 3 KPC + cdAmpC; IMI
P. aeruginosa (n = 6)	PER-1, VEB; 2 cAmpC, 2 upregulated efflux
A. baumannii (n = 5)	OXA-51, OXA-58, 2 OXA-23, OXA-24/40
Others (n = 4)	K1 hyperproducer (*K. oxytoca*), SME (*S. marcescens*), 2 cAmpC AmpCs (*M. morganii, S. odorifera*)

*AmpC prefixes: c, chromosome-encoded; cd, chromosome-encoded derepressed; ci, chromosome-encoded inducible; p, plasmid-encoded

- Agents tested were piperacillin (Pip), ceftriaxone (Cro), cefepime (Fep), and meropenem (Mem), alone or combined with AAI101 4, 8, or 16 mg/L; and Pip/Tazo 4 mg/L.
- Control strains *E. coli* ATCC25922 and *P. aeruginosa* ATCC27853 were tested in all assay runs.
- MICs were interpreted according to 2014 CLSI breakpoints (Cro breakpoints for *P. aeruginosa* not assigned, assumed identical to those for *Acinetobacter* spp.).[2]

Results

- AAI101 generally lacked intrinsic antibacterial activity (MIC_{50} > 128 mg/L).
- Increasing concentrations of AAI101 generally increased the activity of the partnered β-lactam antibiotics.

KPC producers: 11 *K. pneumoniae*, 3 *E. coli*, 3 *E. cloacae* + cdAmpC

- All KPC-producing *E. coli* and *Enterobacter* spp. were susceptible (^{S}) to β-lactam/AAI101.
- Generally, KPC-producing *K. pneumoniae* were β-lactam^S/AAI101, but ST258 strains were not.

ESBL producers: 11 *E. coli*, 2 *K. pneumoniae* + porin loss, 1 *K. oxytoca*, 1 *E. aerogenes*, 2 *P. aeruginosa*

- ESBL-producing Enterobacteriaceae were generally susceptible to Pip/BLI. The geometric mean MIC (GMM) for Pip/Tazo (15.4 mg/L) was reduced to 7.1 mg/L for Pip/AAI101 4 mg/L.

Results (continued)

- AAI101 increased Cro and Fep coverage of enterobacterial ESBL producers, and rendered the *P. aeruginosa* PER-1 producer Fep^S but not Cro^S.

AmpC producers: 5 pAmpC (*E. coli*), 8 cAmpC (2 *E. cloacae*, 2 *E. cloacae* + porin loss, 1 *M. morganii*, 1 *S. odorifera*, and 2 *P. aeruginosa*)

- AAI101 had little effect on the coverage of Pip, Cro, or Mem.
- AAI101 increased substantially Fep coverage, from 44% susceptible for Fep alone (GMM, 1.3 mg/L) to 94% susceptible for Fep/AAI101 4 mg/L (GMM, 0.5 mg/L).

OXA producers: 2 *E. coli*, 2 *K. pneumoniae*, 5 *A. baumannii*

- No OXA-48 producers were Pip^S or Mem^S ± BLI, but + AAI101 8 mg/L 3/4 were Cro^S, and 4/4 Fep^S.
- *A. baumannii* producing OXA-51 and OXA-58 were Pip^S (+ AAI101 16 mg/L) and Fep^S (+ AAI101 4 mg/L).

Efflux: 2 *P. aeruginosa*

- Both efflux isolates were Pip^S and Mem^S; one became Fep^S with AAI101 8 mg/L.

Table 2. Number of susceptible strains/total strains tested according to 2014 CLSI breakpoints

β-Lactam/BLI combination	Class A	Class A + porin loss	Class C	Class C + porin loss	Classes A + C	Class D	Efflux	% Overall (n = 61)
Pip	2/32	0/2	1/11	0/2	0/3	0/9	2/2	8
Pip/Tazo	14/32	0/2	4/11	1/2	0/3	0/9	2/2	34
Pip/AAI101 (4)	21/32	0/2	3/11	0/2	1/3	0/9	2/2	44
Pip/AAI101 (8)	20/32	1/2	8/11	0/2	2/3	0/9	2/2	54
Pip/AAI101 (16)	26/32	2/2	9/11	0/2	2/3	2/9	2/2	70
Fep	9/32	0/2	7/11	0/2	0/3	0/9	1/2	28
Fep/AAI101 (4)	25/32	0/2	11/11	1/2	3/3	5/9	1/2	75
Fep/AAI101 (8)	25/32	1/2	11/11	1/2	3/3	6/9	2/2	80
Cro	3/32	0/2	1/11	0/2	0/3	0/9	0/2	7
Cro/AAI101 (4)	18/32	0/2	2/11	0/2	0/3	2/9	0/2	36
Cro/AAI101 (8)	21/32	2/2	2/11	0/2	1/3	3/9	0/2	48
Mem	15/32	0/2	11/11	0/2	0/3	0/9	2/2	46
Mem/AAI101 (4)	23/32	0/2	11/11	0/2	2/3	0/9	2/2	62
Mem/AAI101 (8)	25/32	1/2	11/11	0/2	3/3	0/9	2/2	69

Conclusions

- Addition of AAI101 to Pip, Cro, Fep and/or Mem enhanced activity and generally restored susceptibility for a broad variety of MDR Gram-negative isolates, including:
  - ESBL-producing Enterobacteriaceae and *P. aeruginosa;*
  - *K. pneumoniae*, *E. coli,* and *Enterobacter* spp. with acquired class A or class D carbapenemases (KPC, IMI, OXA-48);
  - chromosomally-encoded AmpC-producing Enterobacteriaceae and *P. aeruginosa*;
  - *A. baumannii* with intrinsic or acquired class D carbapenemases; and
  - *P. aeruginosa* strains with upregulated β-lactam efflux.
- AAI101 has the potential to restore the clinical utility of well-established β-lactam for the treatment of infections caused by MDR Gram-negative pathogens.

References

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Correspondence to: Stuart Shapiro, Allecra Therapeutics SAS, 13, rue de Village-Neuf, F-68300 St-Louis, France. E-mail : srs@allecra.com