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Potentiating Antibiotics with Cytotoxin-Neutralizing Monoclonal Antibodies in Lethal Murine and Rabbit Models of *Staphylococcus aureus* Pneumonia

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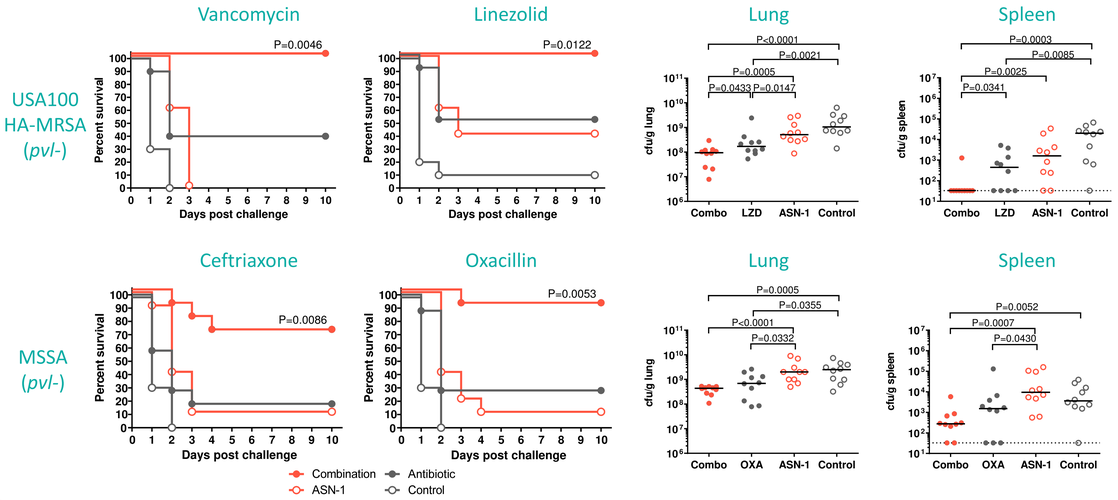
Introduction

S. aureus pneumonia is associated with high mortality, irrespective of antibiotic susceptibility. The pathogenesis is increasingly recognized to be driven by powerful toxins. Up to six cytolytic toxins, namely alpha-hemolysin (Hla) and five bi-component leukocidins, can be produced by *S. aureus*. ASN100 is an equimolar combination of two human monoclonal antibodies (ASN-1 and ASN-2) that together neutralize these six cytolysins (ASN-1: Hla, HlgAB, HlgCB, LukED and LukSF; ASN-2: different LukGH/LukAB variants). We previously reported that mice are mainly susceptible to Hla and LukED, whereas rabbits are sensitive to all these toxins (Diep *et al.*, AAC, 2016). We therefore evaluated the therapeutic efficacy of ASN-1 in murine models and that of ASN100 in rabbits. Since antibiotics lack anti-toxin mechanisms and are even reported to upregulate toxin production of *S. aureus*, we also determined the therapeutic synergy of the cytotoxin neutralizing mAbs with different classes of antibiotics against MRSA and MSSA strains in both animal species.

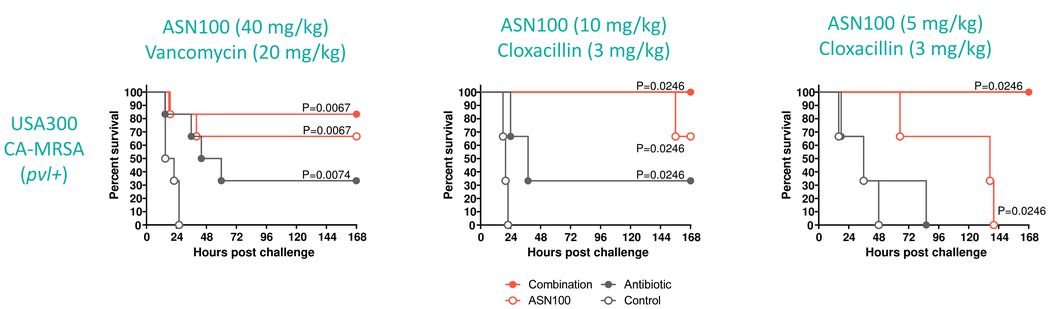
1. ASN-1 synergizes with clinically relevant antibiotics to protect mice from MRSA and MSSA pneumonia

Mice were intranasally (i.n.) challenged with USA100 HA-MRSA or MSSA strains (upper and lower panel, respectively) and subcutaneously (s.c.) treated with vancomycin (100 mg/kg), linezolid (4 mg/kg), ceftriaxone (20 mg/kg) or oxacillin (100 mg/kg) alone or in combination with intraperitoneally (i.p.) administered ASN-1 (5mg/kg) as a single bolus. Survival was monitored for 10 days post challenge or alternatively, organs were harvested at 16 hours post challenge.



3. ASN100 elicits therapeutic efficacy and enhances the efficacy of vancomycin and oxacillin in USA300 CA-MRSA rabbit pneumonia

Rabbits were intratracheally infected with a USA300 CA-MRSA strain and treated with ASN100 (single i.v. bolus) and/or vancomycin (continuous i.v. infusion for 48h) or cloxacillin (intermitted i.v. infusion q6h for 48h). Survival was monitored for 168 hours post challenge.

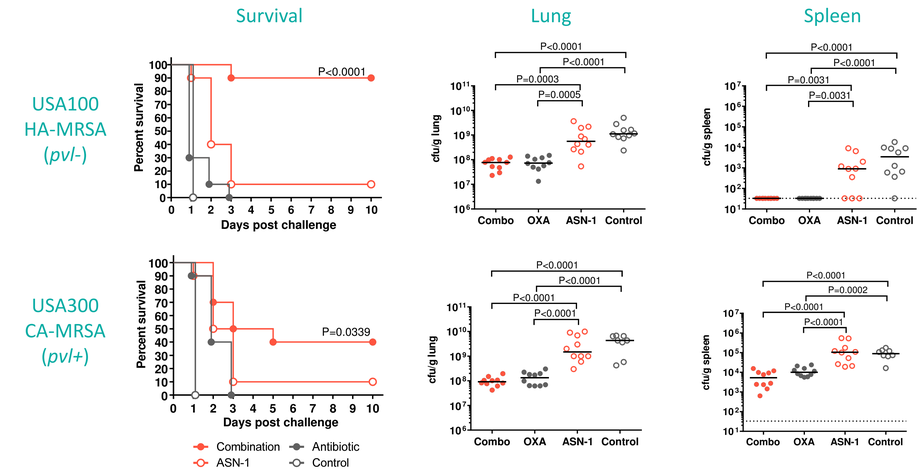


Methods

Challenge: Female BALB/cJRj mice were intranasally infected with lethal doses of USA100 HA-MRSA (ST5-II-t002), USA300 CA-MRSA (ST8-IV-t622; TCH1516) or MSSA (ST72-t148) strains. Male New Zealand White rabbits were intratracheally infected with a lethal dose of a USA300 CA-MRSA (ST8-IV-t008; LAC) strain.
Therapy: Two hours post infection, animals were treated with individually partially protective doses of antibiotics and/or ASN100. Study drugs were administered at doses and routes stated in the respective panels.
Readouts: Survival of animals was monitored for up to 10 days post challenge. As an alternative readout, bacterial organ loads and lung pathology were evaluated at 16 hours post infection.

2. ASN-1 synergizes with oxacillin to protect against USA100 and USA300 MRSA pneumonia in mice

Mice were i.n. challenged with the USA100 HA-MRSA or USA300 CA-MRSA isolates and treated with oxacillin (400 or 800mg/kg, respectively; s.c.) alone or in combination with ASN-1 (5 mg/kg; i.p.) as a single bolus. Survival was monitored up to 10 days post challenge or alternatively, organs were harvested at 16 hours post challenge.



Conclusions and Outlook

- The adjunct therapeutic treatment with cytotoxin neutralizing monoclonal antibodies (ASN-1 and ASN100) and different classes of antibiotics, synergistically improved the outcome of lethal *S. aureus* pneumonia in both mice and rabbits. Importantly, mAbs and beta-lactam antibiotics (oxacillin and cloxacillin) also synergized against MRSA strains in both species.
- In rabbit models, ASN100 alone elicited high level of therapeutic efficacy.
- Bacterial organ loads determined at early stages of infection did not predict survival. Antibiotics reduced the bacterial load in the lungs but only the combination with mAbs elicited significant protection, which highlights the importance of cytotoxin neutralization in these models.
- The high mortality observed in *S. aureus* pneumonia patients treated with antibiotics could be potentially reduced by adjunctive therapy with cytotoxin neutralizing monoclonal antibodies, such as ASN100.

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Disclosure and Contact

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