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

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3. *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 cytolytins (ASN-1: Hla, HgAB, HgCB, 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 ASN1 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.

### Methods

**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 USA300 CA-MRSA isolates (upper and lower panel, respectively) and subcutaneously (s.c.) treated with oxacillin (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 (5 mg/kg) as a single bolus. Survival was monitored for 10 days post challenge or alternatively, organs were harvested at 16 hours post challenge.

**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 800 mg/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.

**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 (intermittent i.v. infusion q4h for 48h). Survival was monitored for 168 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.

### Acknowledgements

We thank Sandrine Albac and Davy Hayez for their technical support.

### Disclosure and Contact

Disclosure: All authors are employees and/or shareholders in the companies performing this work.
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