Efficacy of ASN100, a combination of two human monoclonal antibodies neutralizing six Staphylococcus aureus cytotoxins, in a CA-MRSA rabbit necrotizing pneumonia model

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Introduction
Cytotoxins of Staphylococcus aureus are implicated in the pathogenesis of pneumonia. Necrotizing pneumonia, a particularly severe form associated with high mortality, is most commonly caused by community-associated meticillin-resistant S. aureus (CA-MRSA). The role of α-haemolysin (Hla) is well established in S. aureus pneumonia pathogenesis, and the contribution of bicomponent leukocidins (such as the Panton-Valentine Leukocidin [PVL]) has been shown in rabbits that are, unlike mice, sensitive to all five bicomponent leukocidins produced by S. aureus. ASN100 is an equimolar combination of two human monoclonal antibodies (ASN-1 and ASN-2) that together neutralize six cytotoxins (ASN-1: Hla, HlgAB, HlgCB, LukED and LukSF; ASN-2: different LukGH/LukAB variants). ASN100 was tested for efficacy in a lethal USA300 CA-MRSA rabbit necrotizing pneumonia model. ASN100 is currently being studied in a Phase 2 clinical trial for the prevention of pneumonia in mechanically-ventilated patients who are heavily colonized with S. aureus.

Methods
Male New Zealand White rabbits were prophylactically treated with ASN100 at doses ranging from 0.08 to 20 mg/kg (six rabbits in each of the five dose groups) 24 hours prior to intratracheal instillation of a lethal dose of a PVL+ USA300 CA-MRSA strain (LAC). Survival was monitored for up to 156 hours post challenge. Gross necropsy, lung histopathology, and bacterial organ load analyses were performed in a blinded fashion at 12 hours post challenge. Statistical analyses: Kaplan-Meier plots with log-rank (Mantel-Cox) test; Group comparison by Mann-Whitney test.

Dose-dependent protection by ASN100
24 hours prior to lethal, intratracheal challenge with the USA300 CA-MRSA strain, rabbits were prophylactically treated with different doses of ASN100 and survival was subsequently monitored up to 156 hours post challenge.

ASN100 protected rabbits from lethal necrotizing pneumonia in a dose-dependent manner. Doses as low as 0.32 mg/kg significantly improved survival of animals challenged with the USA300 CA-MRSA, which is also reflected by the time-to-death analysis.

 ASN100 reduces the bacterial burden in different organs
The three highest doses of ASN100 (1.26-20 mg/kg) were evaluated for their contribution to improve the bacterial burden at early stages of infection. Rabbits were sacrificed at 12 hours post intratracheal challenge with the USA300 CA-MRSA.

Toxin neutralization improves macroscopic lung pathology
The lung pathology of infected animals was evaluated at 12 hours post challenge by means of macroscopic gross-necropsy.

ASN100 significantly reduced the bacterial burden in the lungs and systemically at the highest mAb dose, already at early stages of infection.

ASN100 preserves the lung architecture and immune cells
The lung pathology of infected animals was evaluated at 12 hours post challenge by histopathology (H&E and Giemsa staining of central lung lobes).

Conclusions and Outlook
- ASN100, a combination of two human monoclonal antibodies neutralizing six S. aureus cytotoxins, elicited a significant protective efficacy in this lethal rabbit model of necrotizing pneumonia caused by a USA300 CA-MRSA.
- The observed dose-dependent survival benefit was associated with a reduced bacterial burden in lungs and distal organs as well as a significantly improved lung pathology at the highest dose tested at early stages of infection.
- Ongoing preclinical studies are designed to corroborate this potent efficacy of ASN100 against a range of additional S. aureus isolates in such models.
- ASN100 is currently tested in a Phase 2 clinical trial, evaluating the prevention of S. aureus pneumonia in heavily colonized, mechanically ventilated patients.

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