Synergistic inhibition of the concerted action of six *Staphylococcus aureus* cytotoxins with ASN100, a combination of two human monoclonal antibodies

Harald Rouha, Barbara Maierhofer, Karin Gross, Adriana Badarau, Ivana Dolezilkova, Susanne Weber, Lukas Stulík, Eszter Nagy
Arsanis Biosciences GmbH, Vienna, Austria

**Abstract**

Background: Staphylococcus aureus pathogenesis involves pore-forming cytotoxins capable of lysing a broad range of human cells. Alpha-hemolysin (Hla) is a key toxin responsible for lung epithelial cell lysis, facilitating pneumonia and systemic invasion. In addition up to five bi-component leukotoxins are produced by *S. aureus* that target immune cells, primarily neutrophils that are essential for bacterial clearance.

Two human monoclonal antibodies (mAbs) have been developed: ASN-1, which is cross-reactive to Hla and four leukotoxins (LukSF [PVL], LukED, HigAB, and HigCR), and ASN-2, targeting the fifth leukotoxin LukGH (LukAB). In this study, we tested the synergistic effect of the mAb combination (ASN100) in inhibiting the activity of *S. aureus* cytotoxins in vitro.

**Methods:** The two mAbs were tested alone or in combination in several in vitro toxin neutralization assays with recombinant toxins produced in *E. coli* or with native toxins either secreted into the culture supernatants by clinical *S. aureus* isolates or produced in situ during infection. The Hla-neutralizing effect was demonstrated in a human 3D lung epithelial tissue model. The protective effect towards human neutrophils was evaluated in luminescent cell viability assays, by microscopy, and in ex vivo infection assays with live bacteria.

**Results:** ASN300 was highly potent in neutralizing the tissue destructive effect of Hla in a human 3D lung tissue model upon infection with *S. aureus*. While ASN-1 alone was able maintain the integrity and barrier function of the epithelial cell layer, a combination of both mAbs was required to neutralize leukotoxins. ASN100 neutralized toxicity in diverse in vitro models with human leukocytes and a broad panel of clinically relevant *S. aureus* isolates.

**Conclusions:** ASN-1 and ASN-2 act synergistically to prevent epithelial tissue damage and phagocyte lysis neutralizing the concerted action of six different *S. aureus* cytotoxins.

**Both mAbs are required to prevent neutrophil death**

ASN100 neutralizes toxicity of diverse clinical isolates

**ASN-2 dominates during PMN ex vivo infection**

ASN100 spiked into plasma samples obtained from mechanically ventilated patients significantly increases neutralizing titers

**Conclusion and Outlook**

ASN-1 and ASN-2 act synergistically to prevent epithelial tissue damage and phagocyte lysis by neutralizing the concerted action of six different *S. aureus* cytotoxins. Although ASN-2 is only targeting one single leukocidin, it is a critical and highly important ASN100 component. 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*.

**Disclosure, References and Contact Info**

Disclosure: All authors are employees of Arsanis Biosciences GmbH and hold shares in the company. References: Rouha H, Badarau A, Viramí ZC, et al. mAbs 2015;7(1):243-54; Badarau A, Rouha H, Mafalla S, et al. mAbs 2016;8(7):1347-60. Contact: Harald Rouha, Arsanis Biosciences GmbH, Helmut-Quattinger-Gasse 2, 1030 Vienna, Austria; harald.rouha@arsanis.com