

# Prevention of Lung Pathology and Mortality in Rabbit *Staphylococcus aureus* Pneumonia with Cytotoxin-Neutralizing Monoclonal IgGs (ASN100) that Penetrate Epithelial Lining Fluid

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## Introduction

*Staphylococcus aureus* pneumonia is associated with high mortality, irrespective of antibiotic susceptibility. Up to six cytolytic toxins are produced by *S. aureus*: alpha-hemolysin (Hla) and the five bi-component leukocidins (HlgAB, HlgCB, LukED, LukSF, and LukGH/LukAB) that are important for pathogenesis. We previously described two human IgG1 monoclonal antibodies (mAb), ASN-1 and ASN-2, that in combination (as ASN100) neutralize these six toxins. Unlike mice, rabbits are sensitive to all of these toxins and therefore, the prophylactic efficacy and key pharmacokinetic (PK) parameters of ASN100 were determined in this species.

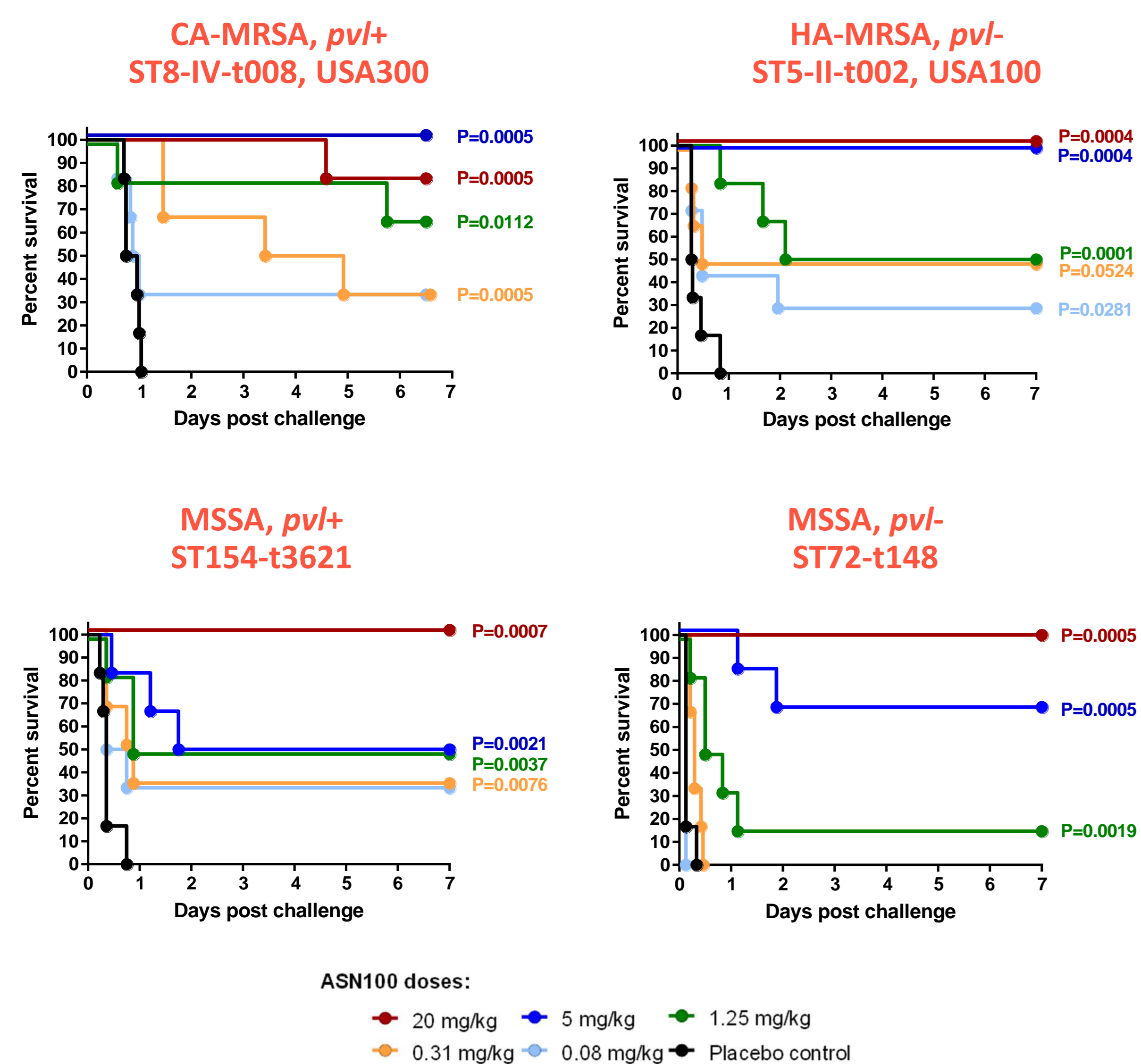
## Methods

Male New Zealand White rabbits were passively immunized with ASN100 at five dose levels between 0.08 to 20 mg/kg total mAb (ASN-1 and ASN-2 at 1:1 ratio), 24 hours prior to intratracheal challenge with lethal doses of USA300 CA-MRSA, USA100 HA-MRSA, or two MSSA strains. Control animals were treated with placebo. Survival was monitored for 7 days post challenge.

Alternatively, animals were sacrificed at 12 hours post challenge and gross necropsy as well as microbiological analyses were performed.

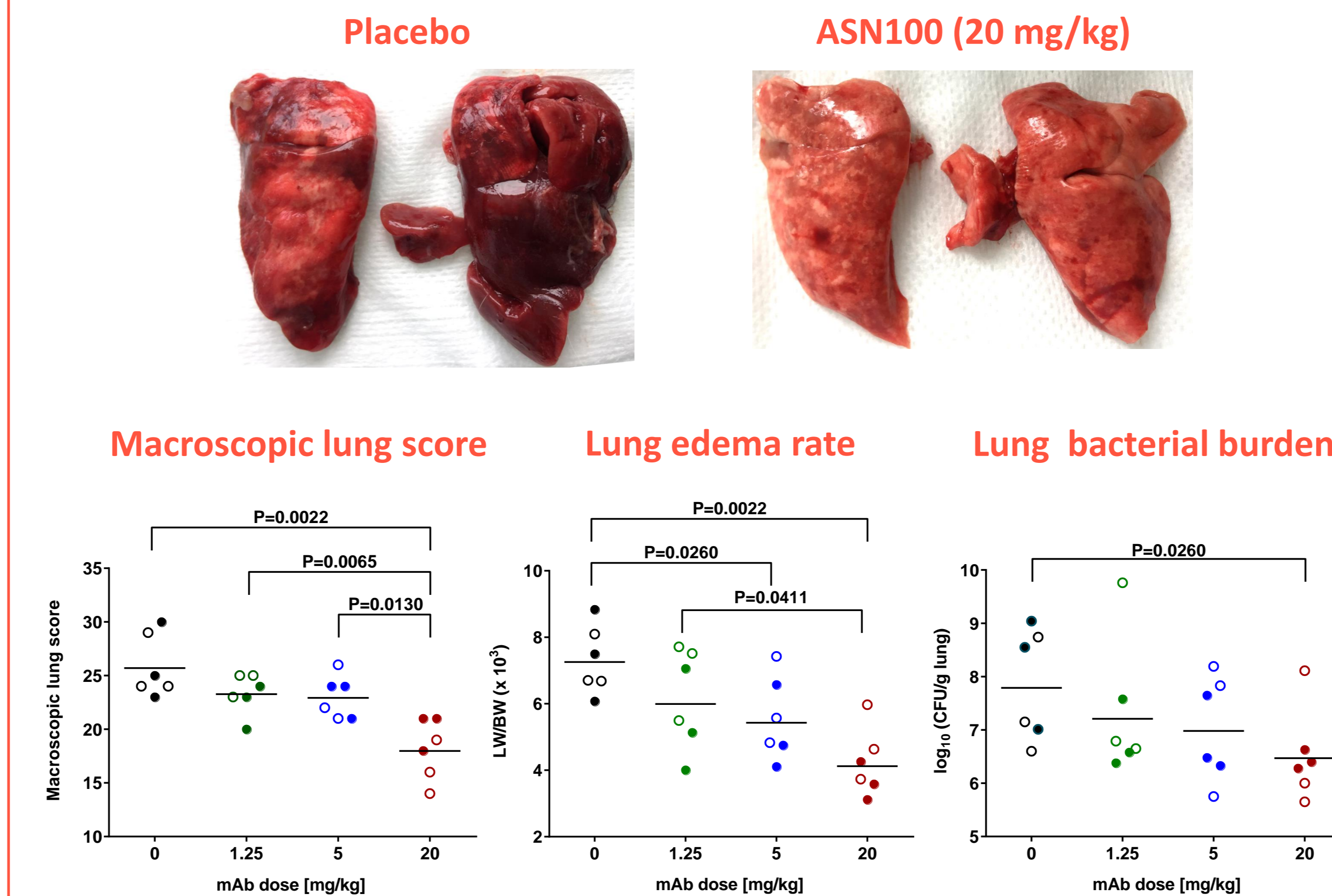
In the PK studies, serum and bronchoalveolar lavage fluid (BALF) samples were serially collected from uninfected and infected animals immunized with 40 mg/kg of ASN100. Total human and rabbit IgG concentrations were determined by sandwich ELISA using human or rabbit specific capture and detection antibodies. ASN-1 and ASN-2 (free drug) concentrations were measured with anti-idiotypic mAbs. Urea levels in BALF and serum were quantified in ELISA and used to determine antibody concentrations in epithelial lining fluid (ELF).

## Prophylactic efficacy of ASN100 against different *S. aureus* strains in rabbit pneumonia models



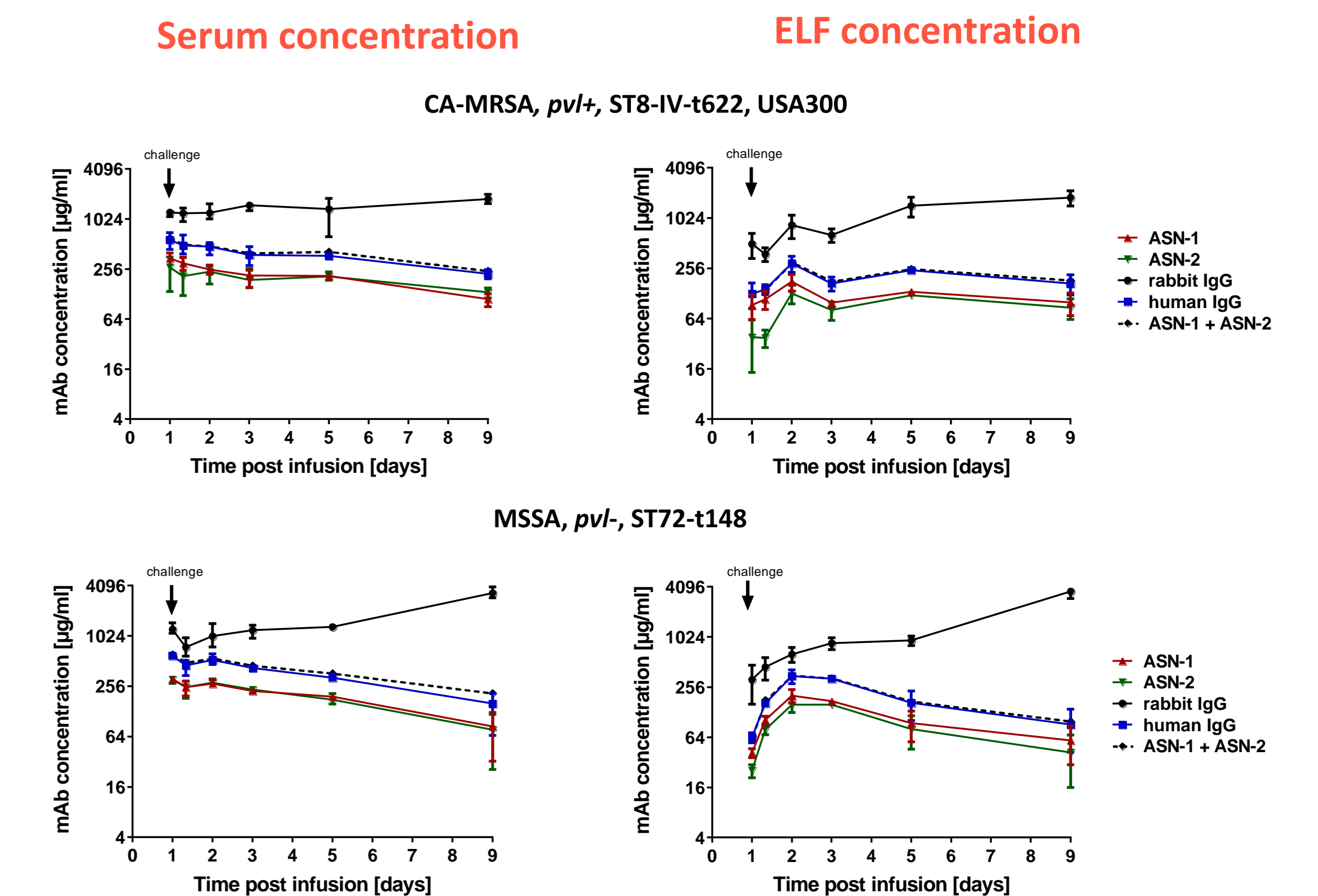
## ASN100 dose-dependent effect on lung pathology

The three highest doses of ASN100 (1.25-20 mg/kg) were evaluated for reduction of lung pathology in infected animals sacrificed 12 hours post-challenge by a USA300 CA-MRSA (LAC) strain.



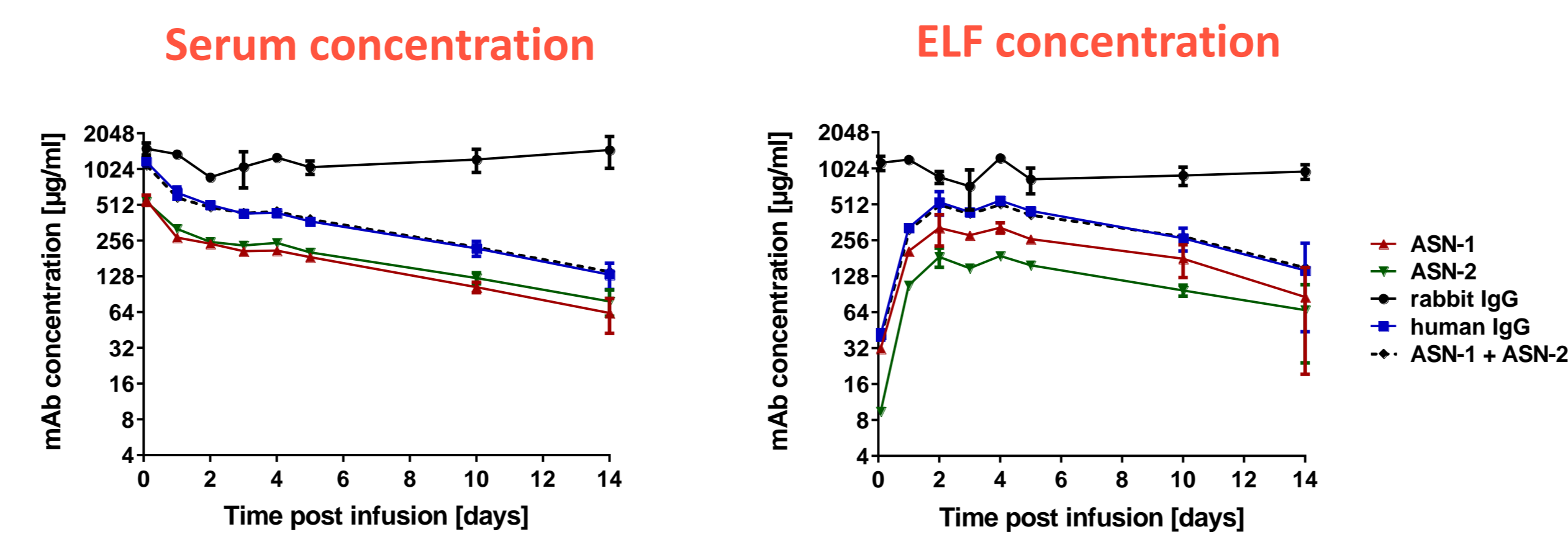
## Pharmacokinetics of ASN100 in infected rabbits

Serum and ELF concentrations of rabbits immunized with 40 mg/kg of ASN100 and challenged with lethal doses of USA300 CA-MRSA (TCH1516) or MSSA ST72 strains 24 hours later were determined at 6 time points (3 rabbits/time point) until day 9 post challenge.



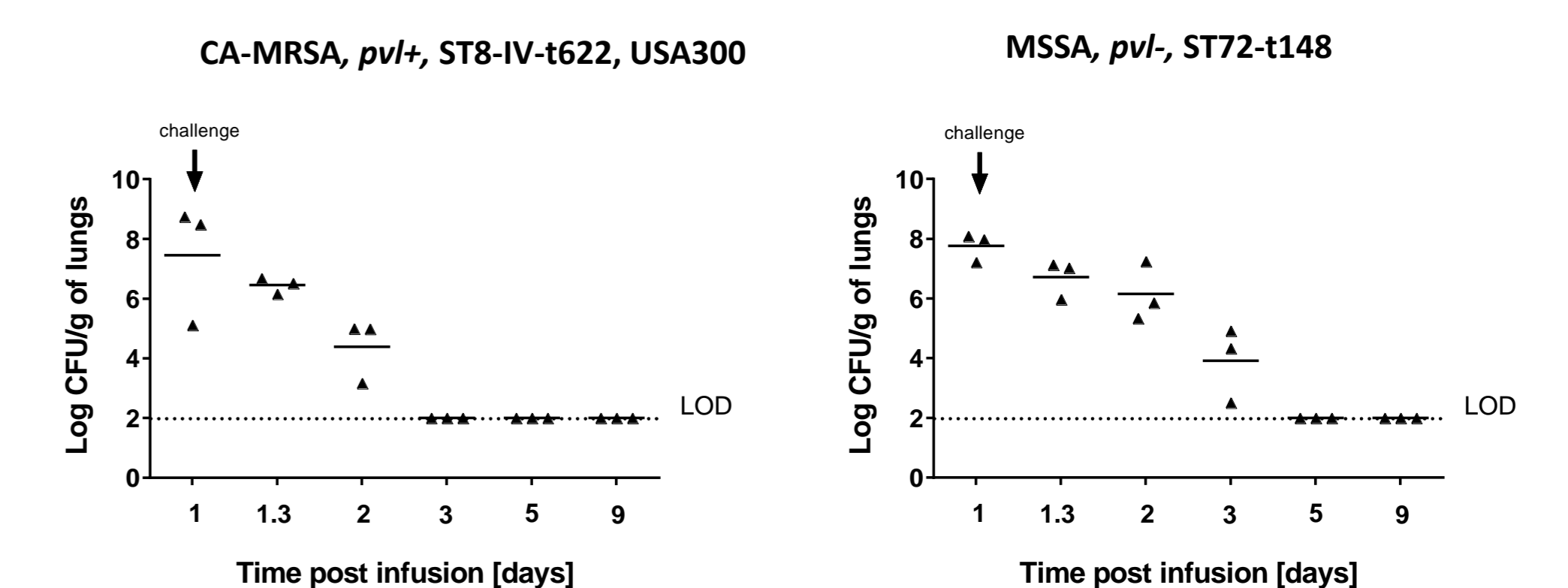
## Pharmacokinetics of ASN100 in non-infected rabbits

Serum and ELF concentrations of rabbits immunized with 40 mg/kg of ASN100 were determined at different time points after mAb administration (2 rabbits/time point).



## Clearance of *S. aureus* from the lungs of ASN100 protected rabbits

Bacterial lung burden was determined in rabbits immunized with 40 mg/kg of ASN100 and challenged with lethal doses of a USA300 CA-MRSA or an MSSA 24 hours after mAb administration.



## Conclusions

- ASN100 elicited high prophylactic efficacy against all *S. aureus* strains tested in lethal rabbit models of pneumonia irrespective of antibiotic susceptibility (MRSA and MSSA) and toxin expression (both *pvl-* and *pvl+*) profiles of the challenge strains.
- Full protection against the two MRSA strains was achieved by 5 mg/kg ASN100 (2.5 mg/kg each mAb), while the two MSSA strains required a higher dose (20 mg/kg).
- Reduction of bacterial counts, lung edema rate as well as macroscopic lung scores of infected animals were most pronounced with the 20 mg/kg ASN100 dose.
- PK-analyses of ASN100 in BALF from uninfected and *S. aureus* infected animals confirmed efficient mAb penetration into lung ELF within the first two days following intravenous administration; ASN100 ELF levels reached 50 % of the serum concentrations by 24 hours, and peak levels by 48 hours post-dosing in uninfected rabbits.
- ASN100 serum and ELF levels were not depleted in the lung of rabbits intratracheally infected with *S. aureus* despite the presence of bacteria for up to 3 days post challenge.

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