Title

Protective Efficacy of Monoclonal Antibodies Neutralizing Alpha-Hemolysin and Bicomponent Leukocidins in a Rabbit Model of Staphylococcus aureus Necrotizing Pneumonia.

Journal

Antimicrobial Agents and Chemotherapy, 64(3)

ISSN

0066-4804

Authors

Vu, Trang TT
Nguyen, Nhu TQ
Tran, Vuvi G
et al.

Publication Date

2020-02-21

DOI

10.1128/aac.02220-19

Peer reviewed

AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

EXPERIMENTAL THERAPEUTICS

Protective Efficacy of Monoclonal Antibodies Neutralizing Alpha-Hemolysin and Bicomponent Leukocidins in a Rabbit Model of *Staphylococcus aureus* Necrotizing Pneumonia

Trang T. T. Vu,[a] Nhu T. Q. Nguyen,[a] Vuvi G. Tran,[a] Emmanuelle Gras,[a,b] Yanjie Mao,[a,c] David H. Jung,[a] Christine Tkaczyk,[d] Bret R. Sellman,[d] Binh An Diep[a]

[a]Division of HIV, Infectious Diseases, and Global Medicine, Department of Medicine, University of California, San Francisco, San Francisco, California, USA
[b]Université François Rabelais, Tours, France
[c]Department of Orthopaedic Surgery, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, People's Republic of China
[d]Microbial Sciences, AstraZeneca, Gaithersburg, Maryland, USA

ABSTRACT *Staphylococcus aureus* is a major human pathogen that causes a wide range of infections by producing an arsenal of cytotoxins. We found that passive immunization with either a monoclonal antibody (MAb) neutralizing alpha-hemolysin or a broadly cross-reactive MAb neutralizing Panton-Valentine leukocidin, leukocidin ED, and gamma-hemolysins HlgAB and HlgCB conferred only partial protection, whereas the combination of those two MAbs conferred significant protection in a rabbit model of necrotizing pneumonia caused by the USA300 methicillin-resistant *S. aureus* epidemic clone.

KEYWORDS *Staphylococcus aureus*, therapeutic antibodies, necrotizing pneumonia, acute lung inflammation

Staphylococcus aureus is a major public health concern because of its notorious antibiotic-resistant capability (1). *S. aureus* employs a variety of secreted membrane-damaging toxins to counter-attack the host immune defenses and to disseminate from the primary site of infection, making its treatment difficult and complicated (2, 3). Loss of effectiveness of antibiotic therapies leads to the urgent need to develop alternative control agents, such as monoclonal antibodies (MAbs) (4–7). Engineering of human IgG1 MAbs, such as the triple-amino-acid YTE substitution in the Fc region, results in extended serum half-lives of 80 to 112 days, thus making MAbs ideal for prophylaxis (8).

Alpha-hemolysin (Hla) is a major virulence factor, and its neutralization by Hla-specific MAbs, including MEDI4893*, conferred significant protection in mouse and ferret pneumonia models (9–14) but partial protection in a phase 2 trial that showed 31.9% (90% confidence interval, −7.5% to 56.8%) relative risk reduction for pneumonia development in patients who were mechanically ventilated and colonized with *S. aureus* (15). MEDI4893* alone was not sufficient to confer full protection in a rabbit necrotizing pneumonia model (14), which may be due to the fact that rabbits are also susceptible to other lung-damaging toxins, including Panton-Valentine leukocidin (LukSF-PV) (16, 17). Rabbit is a more relevant animal species for evaluating the effects of neutralizing MAbs against staphylococcal bicomponent leukocidins, because rabbit and human neutrophils are similarly susceptible to these toxins (16, 18, 19), whereas mouse neutrophils are resistant to LukSF-PV, HlgAB, and HlgCB and are lysed by leukocidin ED (LukED) but to a lesser extent (16, 18).

Here, we report protective efficacy of MEDI4893* and two cross-neutralizing leukocidin MAbs, SAN177 and SAN481, in the rabbit model of necrotizing pneumonia caused by USA300/SF8300, a community-associated methicillin-resistant *S. aureus* (MRSA) ep-

Citation Vu TTT, Nguyen NTQ, Tran VG, Gras E, Mao Y, Jung DH, Tkaczyk C, Sellman BR, Diep BA. 2020. Protective efficacy of monoclonal antibodies neutralizing alpha-hemolysin and bicomponent leukocidins in a rabbit model of *Staphylococcus aureus* necrotizing pneumonia. Antimicrob Agents Chemother 64:e02220-19. https://doi.org/10.1128/AAC.02220-19.

Address correspondence to Bret R. Sellman, bret.sellman@astrazeneca.com, or Binh An Diep, binh.diep@ucsf.edu.

Received 4 November 2019
Returned for modification 5 December 2019
Accepted 9 December 2019
Accepted manuscript posted online 16 December 2019
Published 21 February 2020

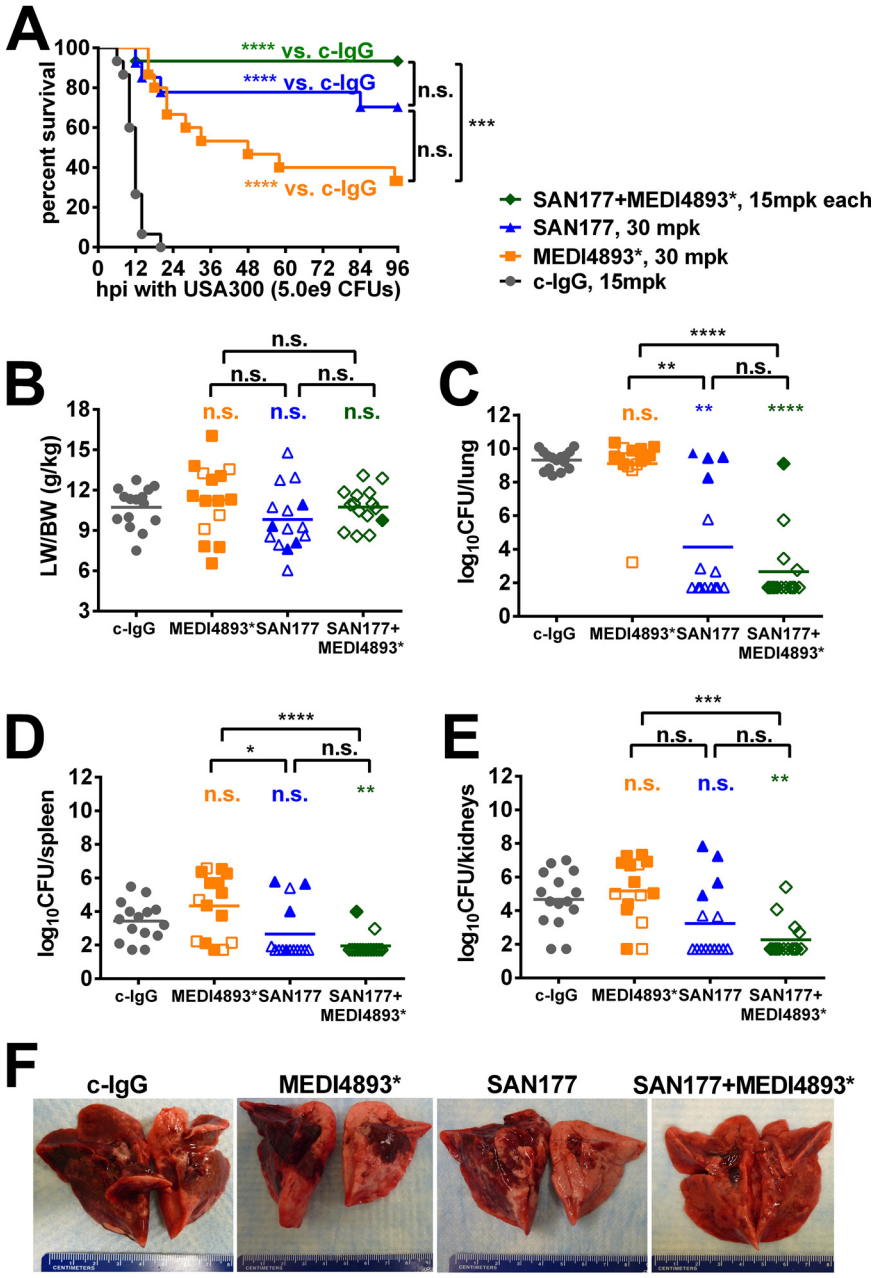

FIG 1 Passive immunization with an anti-Hla MAb or an anti-F subunit broadly cross-reactive antibody (SAN177) that neutralizes LukSF-PV, LukED, and gamma-hemolysin, in a rabbit model of necrotizing pneumonia. (A to E) Kaplan-Meier survival curves (A), LW/BW ratios (B), $\log_{10}$CFU/lung (C), $\log_{10}$CFU/spleen (D), and $\log_{10}$CFU/kidneys (E) were compared for rabbits pretreated intravenously with 30 mg/kg c-IgG ($n = 15$), 30 mg/kg MEDI4893* ($n = 15$), 30 mg/kg SAN177 ($n = 15$), or the combination of 15 mg/kg SAN177 and 15 mg/kg MEDI4893* ($n = 15$) at 24 h before infection with 5.0×10^9 CFU of USA300/SF8300 in the rabbit model of necrotizing pneumonia. The two-sided log-rank (Mantel-Cox) test was used to evaluate differences in survival for rabbits pretreated with c-IgG versus MEDI4893* ($P < 0.0001$), c-IgG versus SAN177 ($P < 0.0001$), c-IgG versus SAN177 plus MEDI4893* ($P < 0.0001$), SAN177 versus MEDI4893* ($P = 0.0142$), SAN177 versus SAN177 plus MEDI4893* ($P = 0.099$), and MEDI4893* versus SAN177 plus MEDI4893* ($P = 0.0006$), with a P value of <0.0083 (significance level of 0.05 divided by 6 different comparisons) being considered statistically significant, to account for multiple comparisons using the Bonferroni method. Open symbols represent surviving animals, while closed symbols represent animals that were found dead or were euthanized after becoming moribund and were recorded as nonsurviving (B to E). For LW/BW ratios and bacterial counts, all 6 pairwise comparisons were analyzed by nonparametric one-way analysis of variance with the Kruskal-Wallis test followed by Dunn's multiple-comparison test. n.s., not significant ($P > 0.05$); *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ****, $P \leq 0.0001$. (F) Photographs depict gross pathology of representative lungs; these lungs were selected because they had median LW/BW ratios for their respective experimental groups, as shown in panel B.

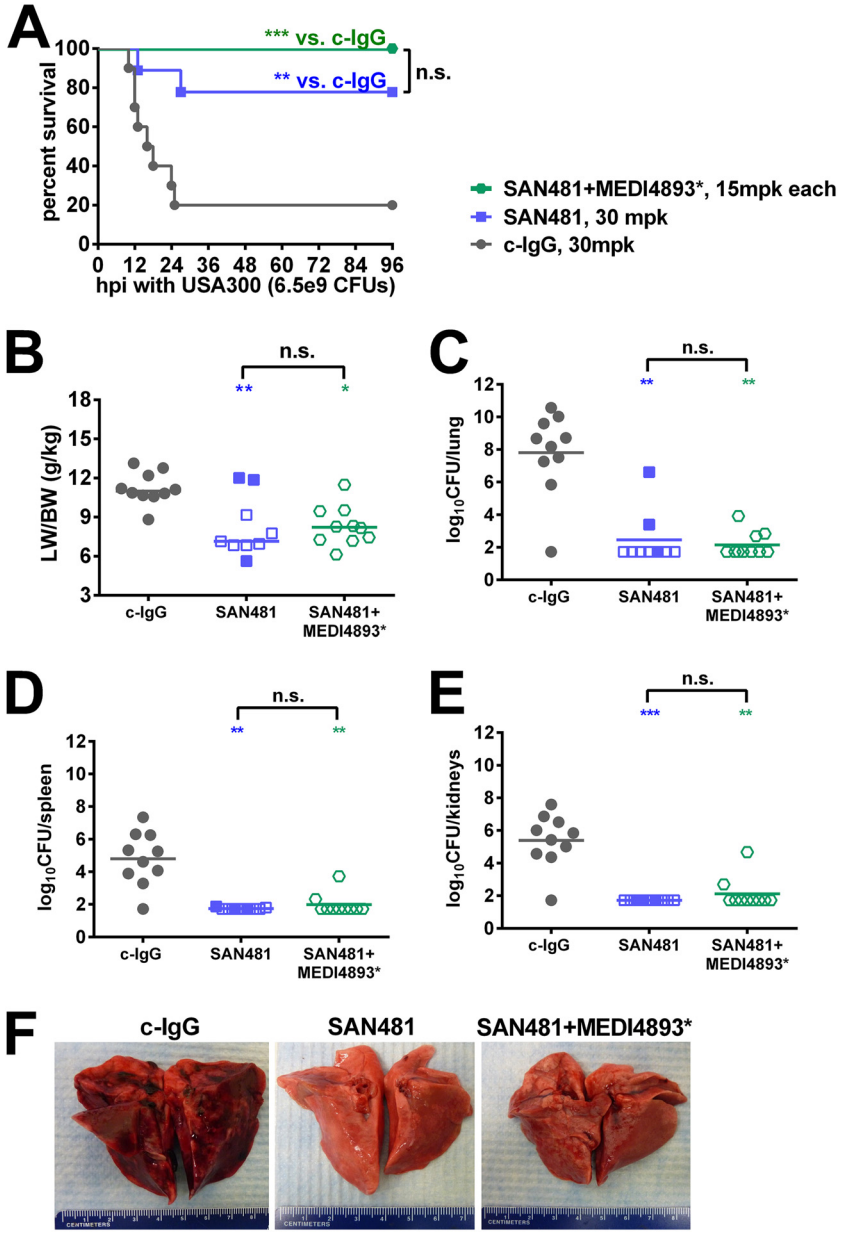

FIG 2 Passive immunization with an anti-Hla MAb or an anti-F subunit broadly cross-reactive antibody (SAN481) that neutralizes LukSF-PV, LukED, and gamma-hemolysin, in a rabbit model of necrotizing pneumonia. (A to E) Kaplan-Meier survival curves (A), LW/BW ratios (B), $\log_{10}$CFU/lung (C), $\log_{10}$CFU/spleen (D), and $\log_{10}$CFU/kidneys (E) were compared for rabbits pretreated intravenously with 30 mg/kg c-IgG ($n = 10$), 30 mg/kg SAN481 ($n = 10$), or the combination of 15 mg/kg SAN481 and 15 mg/kg MEDI4893* ($n = 10$) at 24 h before infection with 6.5×10^9 CFU of USA300/SF8300 in the rabbit model of necrotizing pneumonia. One rabbit pretreated with SAN481 experienced anesthesia-related death immediately before bacterial inoculation; therefore, the rabbit was excluded from subsequent analysis. The two-sided log-rank (Mantel-Cox) test was used to evaluate differences in survival for rabbits pretreated with c-IgG versus SAN481 ($P = 0.008$), c-IgG versus SAN481 plus MEDI4893* ($P = 0.0002$), and SAN481 versus SAN481 plus MEDI4893* ($P = 0.1246$), with a P value of <0.0167 (significance level of 0.05 divided by 3 different comparisons) being considered statistically significant, to account for multiple comparisons using the Bonferroni method. Open symbols represent surviving animals, while closed symbols represent animals that were found dead or were euthanized after becoming moribund and were recorded as nonsurviving (B to E). For LW/BW ratios and bacterial counts, all 3 pairwise comparisons were analyzed by nonparametric one-way analysis of variance with the Kruskal-Wallis test followed by Dunn's multiple-comparison test. n.s., not significant ($P > 0.05$); *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. (F) Photographs depict gross pathology of representative lungs; these lungs were selected because they had median LW/BW ratios for their respective experimental groups, as shown in panel B.

idemic clone producing multiple leukocidins. The rabbit experimental pneumonia protocol was reviewed and approved by the University of California, San Francisco, Institutional Animal Care and Use Committee and was conducted in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. SAN177 and SAN481 (20) were selected from human tonsillar memory B cells, as described previously (21), for their ability to cross-neutralize HlgAB, HlgBC, LukSF-PV, and LukED by binding the F subunits of the leukocidins (C. Tkaczyk and B. R. Sellman, unpublished data). Two animal efficacy studies were performed. In the first study, rabbits were randomly assigned to prophylaxis with 30 mg/kg isotype-matched control MAb (c-IgG), 30 mg/kg MEDI4893*, 30 mg/kg SAN177, or a combination of 15 mg/kg SAN177 and 15 mg/kg MEDI4893*, which were administered intravenously at 24 h before endobronchial challenge with 5.0×10^9 CFU of SF8300 (in 1.8 ml of saline solution), as described previously (17, 19). All rabbits (15 of 15 rabbits) that were pretreated with c-IgG had lethal pneumonia between 6 and 20 h after infection (Fig. 1A). Mortality rates were 67% (10/15 rabbits; $P < 0.0001$ versus c-IgG by log-rank test) for rabbits pretreated with MEDI4893* and 27% (4/15 rabbits; $P < 0.0001$ versus c-IgG by log-rank test) for those pretreated with SAN177. Rabbits pretreated with SAN177 plus MEDI4893* had the lowest mortality rate of 7% (1/15 rabbits; $P < 0.0001$ versus c-IgG by log-rank test), indicating the importance of neutralization of both Hla and bicomponent leukocidins. Lung weight (LW)/body weight (BW) ratios, a quantitative marker of pulmonary edema, were not significantly different among the four experimental groups (Fig. 1B). Lung bacterial counts were significantly lower in rabbits pretreated with SAN177 or SAN177 plus MEDI4893* than in those pretreated with MEDI4893* or c-IgG (Fig. 1C), and spleen and kidney bacterial counts were significantly reduced only in rabbits pretreated with SAN177 plus MEDI4893* (Fig. 1D and E). Gross images of lungs harvested at the time of death or euthanasia at 96 h postinfection demonstrated areas of severe lung necrosis in rabbits pretreated with c-IgG, but areas were reduced in rabbits pretreated with MEDI4893*, SAN177, or SAN177 plus MEDI4893* (Fig. 1F).

Although SAN177 conferred significant protection in the rabbit model, there were developability issues associated with this molecule, due to aggregation at high concentrations (unpublished data). Therefore, we identified SAN481 (20), another candidate MAb that binds to LukF, LukD, and HlgB, and evaluated its protective efficacy, with and without MEDI4893*, in the rabbit model of necrotizing pneumonia. In the second study, rabbits were randomly assigned to prophylaxis with 30 mg/kg isotype-matched c-IgG, 30 mg/kg SAN481, or a combination of 15 mg/kg SAN481 plus 15 mg/kg of MEDI4893*, which were administered intravenously 24 h before endobronchial challenge with 7.0×10^9 CFU of USA300 (in 1.8 ml of saline solution), as described previously (17, 19). Eighty percent of rabbits (8/10 rabbits) pretreated with c-IgG had lethal pneumonia between 10 and 25 h after infection (Fig. 2A). Mortality rates were 22% (2/9 rabbits; $P = 0.008$ versus c-IgG by log-rank test) for rabbits pretreated with SAN481 and 0% (0/10 rabbits; $P = 0.0002$ versus c-IgG by log-rank test) for those pretreated with SAN481 plus MEDI4893*. The groups pretreated with SAN481 and SAN481 plus MEDI4893* showed significantly reduced LW/BW ratios of 8.24 and 8.33, respectively, compared to 11.22 for the c-IgG-pretreated rabbits, suggesting that SAN481 is potent in reducing acute lung injury. Interestingly, rabbits pretreated with Hla-F#5 MAb, which cross-neutralizes Hla, HlgAB, HlgCB, LukSF-PV, and LukED, showed an increased LW/BW ratio, compared to those pretreated with a control MAb, although it should be noted that Hla-F#5 protected all rabbits from lethal pneumonia (16). Lung, spleen, and kidney bacterial counts were significantly lower in rabbits pretreated with SAN481 or SAN481 plus MEDI4893* than in those pretreated with c-IgG (Fig. 2C to E). Gross images of lungs harvested at the time of death or euthanasia at 96 h postinfection demonstrated areas of severe necrosis in rabbits pretreated with c-IgG, but areas were reduced in rabbits pretreated with SAN481 or SAN481 plus MEDI4893* (Fig. 2F).

Taking these data together, we found that addition of SAN177 or SAN481 to MEDI4893* resulted in enhanced efficacy in the rabbit model of USA300 necrotizing

pneumonia. These preclinical data support the continued development of antileukocidin MAbs in combination with an anti-Hla MAb to extend strain coverage to *S. aureus* strains expressing leukocidins and to prevent life-threatening pneumonia. Future rabbit studies will further evaluate the efficacy of MEDI4893* and SAN481 MAbs for the prevention and adjunctive treatment of MRSA ventilator-associated pneumonia, which are primary indications for these antitoxin MAbs.

ACKNOWLEDGMENTS

This work was supported by a grant from MedImmune, now part of AstraZeneca. B.A.D. was supported by U.S. Public Health Service grant NIH R01 AI087674.

Patent application 2014/0072,577, describing MEDI4893*, an anti-alpha-toxin human MAb used in this work, has been filed by MedImmune.

REFERENCES

1. Chambers HF, Deleo FR. 2009. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. Nat Rev Microbiol 7:629–641. https://doi.org/10.1038/nrmicro2200.
2. Spaan AN, van Strijp JAG, Torres VJ. 2017. Leukocidins: staphylococcal bi-component pore-forming toxins find their receptors. Nat Rev Microbiol 15:435–447. https://doi.org/10.1038/nrmicro.2017.27.
3. Berube BJ, Bubeck Wardenburg J. 2013. *Staphylococcus aureus* α-toxin: nearly a century of intrigue. Toxins (Basel) 5:1140–1166. https://doi.org/10.3390/toxins5061140.
4. Ventola CL. 2015. The antibiotic resistance crisis: part 1: causes and threats. P T 40:277–283.
5. Projan SJ, Shlaes DM. 2004. Antibacterial drug discovery: is it all downhill from here? Clin Microbiol Infect 10(Suppl 4):18–22. https://doi.org/10.1111/j.1465-0691.2004.1006.x.
6. Cheung GY, Otto M. 2012. The potential use of toxin antibodies as a strategy for controlling acute *Staphylococcus aureus* infections. Expert Opin Ther Targets 16:601–612. https://doi.org/10.1517/14728222.2012.682573.
7. DiGiandomenico A, Sellman BR. 2015. Antibacterial monoclonal antibodies: the next generation? Curr Opin Microbiol 27:78–85. https://doi.org/10.1016/j.mib.2015.07.014.
8. Yu XQ, Robbie GJ, Wu Y, Esser MT, Jensen K, Schwartz HI, Bellamy T, Hernandez-Illas M, Jafri HS. 2016. Safety, tolerability, and pharmacokinetics of MEDI4893, an investigational, extended-half-life, anti-*Staphylococcus aureus* alpha-toxin human monoclonal antibody, in healthy adults. Antimicrob Agents Chemother 61:e01020-16. https://doi.org/10.1128/AAC.01020-16.
9. Bubeck Wardenburg J, Patel RJ, Schneewind O. 2007. Surface proteins and exotoxins are required for the pathogenesis of *Staphylococcus aureus* pneumonia. Infect Immun 75:1040–1044. https://doi.org/10.1128/IAI.01313-06.
10. Menzies BE, Kernodle DS. 1996. Passive immunization with antiserum to a nontoxic alpha-toxin mutant from *Staphylococcus aureus* is protective in a murine model. Infect Immun 64:1839–1841.
11. Bubeck Wardenburg J, Schneewind O. 2008. Vaccine protection against *Staphylococcus aureus* pneumonia. J Exp Med 205:287–294. https://doi.org/10.1084/jem.20072208.
12. Hua L, Cohen TS, Shi Y, Datta V, Hilliard JJ, Tkaczyk C, Suzich J, Stover CK, Sellman BR. 2015. MEDI4893* promotes survival and extends the antibiotic treatment window in a *Staphylococcus aureus* immunocompromised pneumonia model. Antimicrob Agents Chemother 59:4526–4532. https://doi.org/10.1128/AAC.00510-15.
13. Hua L, Hilliard JJ, Shi Y, Tkaczyk C, Cheng LI, Yu X, Datta V, Ren S, Feng H, Zinsou R, Keller A, O'Day T, Du Q, Cheng L, Damschroder M, Robbie G, Suzich J, Stover CK, Sellman BR. 2014. Assessment of an anti-alpha-toxin monoclonal antibody for prevention and treatment of *Staphylococcus aureus*-induced pneumonia. Antimicrob Agents Chemother 58:1108–1117. https://doi.org/10.1128/AAC.02190-13.
14. Diep BA, Hilliard JJ, Le VT, Tkaczyk C, Le HN, Tran VG, Rao RL, Dip EC, Pereira-Franchi EP, Cha P, Jacobson S, Broome R, Cheng LI, Weiss W, Prokai L, Nguyen V, Stover CK, Sellman BR. 2017. Targeting alpha toxin to mitigate its lethal toxicity in ferret and rabbit models of *Staphylococcus aureus* necrotizing pneumonia. Antimicrob Agents Chemother 61:e02456-16. https://doi.org/10.1128/AAC.02456-16.
15. François B, Garcia Sanchez M, Eggimann P, Dequin P, Laterre P, Huberlant V, Escudero D, Boulain T, Bretonniere C, Pugin J, Trenado Alvarez J, Ali O, Shoemaker K, Ruzin A, Vandamme D, Colbert S, Bellamy T, Dubovsky F, Jafri H. 2019. Suvratoxumab reduces *Staphylococcus aureus* pneumonia in high-risk ICU patients: results of the SAATELLITE study. Am J Respir Crit Care Med 199:A7358.
16. Diep BA, Le VT, Visram ZC, Rouha H, Stulik L, Dip EC, Nagy G, Nagy E. 2016. Improved protection in a rabbit model of community-associated methicillin-resistant *Staphylococcus aureus* necrotizing pneumonia upon neutralization of leukocidins in addition to alpha-hemolysin. Antimicrob Agents Chemother 60:6333–6340. https://doi.org/10.1128/AAC.01213-16.
17. Diep BA, Le VT, Badiou C, Le HN, Pinheiro MG, Duong AH, Wang X, Dip EC, Aguiar-Alves F, Basuino L, Marbach H, Mai TT, Sarda MN, Kajikawa O, Matute-Bello G, Tkaczyk C, Rasigade JP, Sellman BR, Chambers HF, Lina G. 2016. IVIG-mediated protection against necrotizing pneumonia caused by MRSA. Sci Transl Med 8:357ra124. https://doi.org/10.1126/scitranslmed.aag1153.
18. Loffler B, Hussain M, Grundmeier M, Bruck M, Holzinger D, Varga G, Roth J, Kahl BC, Proctor RA, Peters G. 2010. *Staphylococcus aureus* Panton-Valentine leukocidin is a very potent cytotoxic factor for human neutrophils. PLoS Pathog 6:e1000715. https://doi.org/10.1371/journal.ppat.1000715.
19. Diep BA, Chan L, Tattevin P, Kajikawa O, Martin TR, Basuino L, Mai TT, Marbach H, Braughton KR, Whitney AR, Gardner DJ, Fan X, Tseng CW, Liu GY, Badiou C, Etienne J, Lina G, Matthay MA, DeLeo FR, Chambers HF. 2010. Polymorphonuclear leukocytes mediate *Staphylococcus aureus* Panton-Valentine leukocidin-induced lung inflammation and injury. Proc Natl Acad Sci U S A 107:5587–5592. https://doi.org/10.1073/pnas.0912403107.
20. Ortines RV, Wang Y, Liu H, Dikeman DA, Pinsker BL, Miller RJ, Kim SE, Ackerman NE, Rizkallah JF, Marcello LT, Cohen TS, Tkaczyk C, Sellman BR, Miller LS, Ortines RV, Wang Y, Liu H, Dikeman DA, Pinsker BL, Miller RJ, Kim SE, Ackerman NE, Rizkallah JF, Marcello LT, Cohen TS, Tkaczyk C, Sellman BR, Miller LS. 2019. Efficacy of a multimechanistic monoclonal antibody combination against *Staphylococcus aureus* surgical site infections in mice. Antimicrob Agents Chemother 63:e00346-19. https://doi.org/10.1128/AAC.00346-19.
21. Tkaczyk C, Kasturirangan S, Minola A, Jones-Nelson O, Gunter V, Shi YY, Rosenthal K, Aleti V, Semenova E, Warrener P, Tabor D, Stover CK, Corti D, Rainey G, Sellman BR. 2017. Multimechanistic monoclonal antibodies (MAbs) targeting *Staphylococcus aureus* alpha-toxin and clumping factor A: activity and efficacy comparisons of a MAb combination and an engineered bispecific antibody approach. Antimicrob Agents Chemother 61:e00629-17. https://doi.org/10.1128/AAC.00629-17.