



ORIGINAL ARTICLE

Characterisation of anti-alpha toxin antibody levels and colonisation status after administration of an investigational human monoclonal antibody, MEDI4893, against *Staphylococcus aureus* alpha toxin

Alexey Ruzin, Yuling Wu, Li Yu, Xiang-Qing Yu, David E Tabor, Hoyin Mok, Christine Tkaczyk, Kathryn Jensen, Terramika Bellamy, Lorin Roskos, Mark T Esser & Hasan S Jafri

MedImmune, Gaithersburg, MD, USA

Correspondence

Alexey Ruzin, MedImmune, One MedImmune Way, Gaithersburg, MD 20878, USA.
E-mail: ruzina@medimmune.com

Present address

Xiang-Qing Yu, Janssen Pharmaceuticals, Inc., Johnson & Johnson, Spring House, PA, USA

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Abstract

Objectives. MEDI4893 is a novel, long-acting human monoclonal antibody targeting *Staphylococcus aureus* (SA) alpha toxin (AT). This report presents the results of the exploratory analyses from a randomised phase 1 dose-escalation study in healthy human subjects receiving single intravenous MEDI4893 doses or placebo. **Methods.** Anti-AT antibodies and AT expression were measured as described previously. Nasal swabs were analysed by culture and PCR. Data were summarised by treatment groups and visits by using SAS System Version 9.3. **Results.** Subjects receiving 2250 or 5000 mg of MEDI4893 had the highest serum anti-AT neutralising antibody (NAb) levels: approximately 180- to 240-, 70- to 100- and sevenfold to 10-fold higher than respective baseline levels at peak, 30 and 360 days, respectively. In these subjects, levels of serum anti-AT NAb were >3.2 International Units (IU) mL⁻¹ for at least 211 days. In the upper respiratory tract, anti-AT NAb levels increased with MEDI4893 dose. No apparent effect of MEDI4893 on SA nasal colonisation, *hla* gene sequence or AT expression was observed. Five AT variants were detected, their lytic activity was fully neutralised by MEDI4893. **Discussion.** Our results indicate that (1) MEDI4893 administration at 2250 and 5000 mg would provide effective immunoprophylaxis against systemic SA disease; (2) MEDI4893 distributes to the upper respiratory tract and retains neutralising activity against AT; and (3) potential for emergence of MEDI4893 resistance is low. **Conclusion.** Intravenous administration of MEDI4893 maintained levels of anti-AT NAb in serum and nasal mucosa that may provide effective immunoprophylaxis against SA disease and support continued clinical development of MEDI4893.

Keywords: alpha toxin, immunoprophylaxis, MEDI4893, *Staphylococcus aureus*.

INTRODUCTION

Staphylococcus aureus is a major bacterial pathogen that causes various infections ranging from mild skin and soft-tissue infections to serious invasive diseases such as endocarditis, osteomyelitis and necrotising pneumonia.^{1–3} Bacterial pneumonia is the second leading type of nosocomial infections and is the leading cause of death from nosocomial infection in the United States.⁴ Approximately one-quarter of mechanically ventilated patients in intensive care units develop *S. aureus* pneumonia; half of these cases are caused by methicillin-resistant *S. aureus* (MRSA).⁵

During infection, *S. aureus* releases a variety of different toxins. Alpha toxin (AT; also known as alpha haemolysin) is encoded by the *hla* gene and is a key virulence factor that contributes to *S. aureus* pathogenesis by causing tissue invasion and necrosis, promoting immune evasion and altering bacterial killing in macrophages.^{6–11} *Staphylococcus aureus* strains deficient in AT expression are less virulent in animal models of dermonecrosis, pneumonia, sepsis, endocarditis and mastitis.^{11–17} Targeted neutralisation of AT could therefore prevent or limit *S. aureus* disease. This hypothesis is supported by studies that demonstrated a reduction in *S. aureus* disease severity in murine infection models after active or passive immunisation directed against AT.^{8,11,14,18–21} In addition, high levels of anti-AT antibodies in patients with bacteremia, sepsis and endocarditis have been associated with improved outcomes.^{22–24}

The emergence and spread of antibiotic-resistant *S. aureus* strains complicate the management of *S. aureus* infections and highlight the need to consider novel approaches such as immunoprophylaxis. As AT plays an important role in *S. aureus* pathogenesis, pre-emptive targeting of AT by a specific monoclonal antibody may prevent *S. aureus* disease in at-risk populations, such as mechanically ventilated patients colonised with *S. aureus* in the lower respiratory tract. MEDI4893 is a potent and long-acting human immunoglobulin G1 kappa (IgG1 κ) monoclonal antibody that neutralises *S. aureus* AT.²⁵ In animal models, MEDI4893 has been shown to prevent lethal *S. aureus* pneumonia, reduce the pathology associated with infection and accelerate pulmonary bacterial clearance even in the absence of concurrent antimicrobial therapy.²⁶

Results from a phase 1, first-in-human, randomised, double-blind, placebo-controlled,

dose-escalation study demonstrated the safety and tolerability of MEDI4893. Pharmacokinetic analyses from this study demonstrated approximately dose-dependent increases in MEDI4893 levels in serum and nasal wash after a single intravenous (IV) administration and a serum half-life of 80–112 days.²⁷ This report extends the findings of the phase 1 study with results of exploratory analyses evaluating the levels of anti-AT antibody in serum and nasal wash samples and the effect of MEDI4893 administration on *S. aureus* colonisation status, bacterial load, AT expression, and *hla* gene and AT protein sequence.

RESULTS

Levels of anti-AT IgG and neutralising antibodies in serum

Serum anti-AT IgG and anti-AT neutralising antibodies were detected in all treatment groups prior to infusion, and their baseline levels were similar across all treatment groups. No significant changes in the baseline anti-AT IgG and neutralising antibody levels were observed in the placebo group during the entire 360-day follow-up period, which suggests that AT-specific humoral immunity remains stable in healthy individuals for a prolonged time.

Upon administration of MEDI4893, serum anti-AT IgG and neutralising antibody levels increased; the highest levels were observed at the end of infusion. The magnitude of increase from the baseline to the peak levels correlated with MEDI4893 dose (Figure 1; Table 1). Thereafter, the concentrations declined in a biexponential manner through 360 days after dosing. In MEDI4893 cohorts 3 (2250 mg) and 4 (5000 mg), the serum anti-AT neutralising antibody levels were approximately 70- to 100- and sevenfold to 10-fold higher than baseline levels on days 31 and 361, respectively. In subjects that received either 2250 or 5000 mg of MEDI4893, the levels of serum AT neutralising antibodies exceeded the threshold of 3.2 IU mL⁻¹ for at least 211 days after MEDI4893 administration.

Levels of anti-AT IgG and neutralising antibodies in the upper respiratory tract

Anti-AT IgG and anti-AT neutralising antibody levels in the upper respiratory tract were measured on days 1 (MEDI4893 predose), 8 and 31. These antibody levels were MEDI4893 dose-dependent

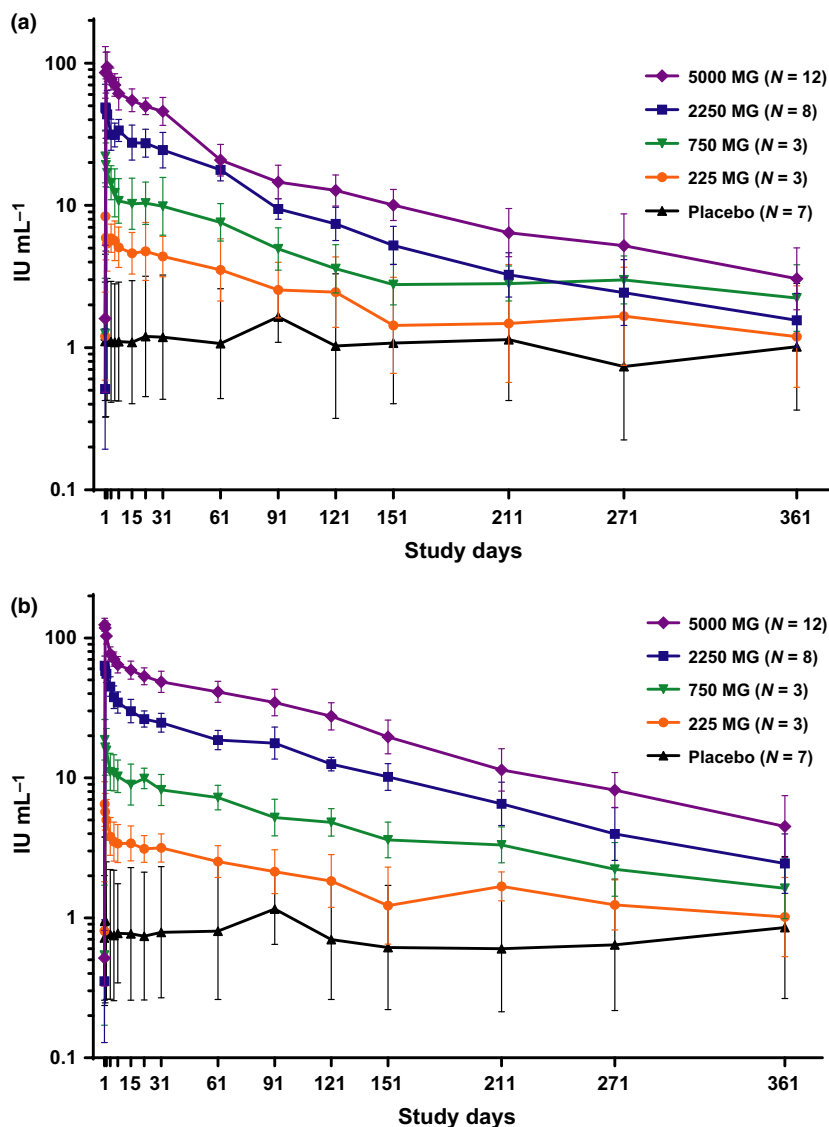


Figure 1. Anti-alpha toxin IgG and neutralising antibody levels in serum. Each sample was tested in duplicate. Anti-AT IgG (a) and neutralising antibody (b) in IU per millilitre of serum samples were summarised in mean and standard deviation in log scale for each treatment group at each visit time point.

and reached a plateau by day 8 (Figure 2). Compared with the baseline levels, the anti-AT neutralising antibody levels in the nasal wash samples were approximately eightfold to ninefold and 30-fold higher in MEDI4893 cohorts 3 (2250 mg) and 4 (5000 mg), respectively (Table 2).

Detection of *Staphylococcus aureus* nasal colonisation by PCR and culture

Both polymerase chain reaction (PCR) analysis and bacterial cultures were used to detect methicillin-susceptible *S. aureus* (MSSA) and MRSA nasal

colonisation in the different treatment groups. PCR detection was in agreement (96.3%) with the standard microbiological culture results. Approximately one-third of the subjects in the MEDI4893 total group were colonised by MSSA at all time points evaluated (range, 26.9–37.5% as measured by PCR and 29.2–38.5% as measured by culture), whereas fewer subjects in the placebo group were colonised by MSSA (range, 0–16.7% as measured by both PCR and culture). No subjects were positive for MRSA colonisation in either assay. No significant differences in the basal levels of anti-AT IgG and neutralising antibodies

Table 1. Geometric mean fold increase in serum anti-alpha toxin neutralising levels over baseline

Days	Placebo (n = 7)	225 mg (n = 3)	750 mg (n = 3)	2250 mg (n = 8)	5000 mg (n = 12)
1 (pre-infusion)	1.0	1.0	1.0	1.0	1.0
1 (end of infusion)	1.3	8.1	34.7	179.4	240.5
1 (8 h postinfusion)	1.3	7.1	30.6	164.3	230.9
2	1.1	6.2	29.1	156.7	193.9
4	1.1	4.7	20.5	127.2	147.7
6	1.0	4.3	20.1	106.9	138.1
8	1.1	4.2	19.0	97.9	126.6
15	1.1	4.2	16.6	85.0	115.7
22	1.0	3.9	18.3	67.4	104.8
31	1.1	3.9	15.2	70.1	96.4
61	1.1	3.1	13.4	52.7	81.1
91	1.1	2.6	9.6	50.2	69.5
121	1.0	2.3	8.9	35.5	54.5
151	0.9	2.2	6.7	28.8	40.9
211	0.9	2.1	6.1	18.5	21.0
271	1.0	1.5	4.1	11.3	16.5
361	1.3	1.3	3.0	6.9	9.7

were observed between *S. aureus*-colonised and noncolonised subjects ($P > 0.05$).

The changes in PCR cycle threshold (Ct) values and culture scores relative to baseline (pre-infusion, day 1) were monitored over the course of 360 days (Figure 3). The results showed no consistent dose-dependent MEDI4893-mediated effect on nasal *S. aureus* colonisation.

AT gene and protein sequence in *Staphylococcus aureus* nasal isolates

Alpha toxin gene was detected in 54 of 56 nasal isolates by PCR amplification. Based on the amino acid sequences, five AT subtypes were identified in *S. aureus* isolates obtained from 14 subjects enrolled in this study (Table 3). AT protein sequence was identical to the USA300 reference sequence (subtype 1) in 37% of isolates. Amino acid substitutions were detected at positions 78, 113, 135, 155, 234, 265 and 301 in subtypes 4, 11, 45 and 58. None of the substitutions were detected in the MEDI4893 binding region that encompasses amino acid residues 203 through 226 and 287 through 297, which is consistent with the reported data that this region is highly conserved among a diverse collection of *S. aureus* clinical isolates.²⁸

AT protein expression in *Staphylococcus aureus* nasal isolates

No major changes in AT protein expression levels were observed among *S. aureus* isolates through day 361 across all treatment groups (Figure 4).

DISCUSSION

This randomised, dose-escalation phase 1 study of MEDI4893 in healthy subjects evaluated AT-specific IgG and neutralising antibody levels in serum and nasal wash samples after a single IV infusion, as well as the impact of MEDI4893 on *S. aureus* nasal colonisation, strain AT genotype and protein expression. Our results indicate that after MEDI4893 administration, anti-AT neutralising antibody levels in serum increased by up to 240.5-fold (MEDI4893 5000 mg cohort) compared with baseline and were maintained above the baseline levels for at least 121 days in all MEDI4893 cohorts. Previous studies have suggested that anti-AT antibody levels of ≥ 3.2 IU mL⁻¹ correlate with protection against *S. aureus* infections and represent ≥ 98 th percentile value in a healthy human population.²⁴ All subjects dosed with MEDI4893 had anti-AT neutralising antibody levels above 3.2 IU mL⁻¹ for at least 30 days after dosing. The neutralising antibody levels in subjects dosed with 2250 and 5000 mg were above 3.2 IU mL⁻¹ for at least 211 days, suggesting that MEDI4893 administration at these doses would provide effective immunoprophylaxis against systemic *S. aureus* disease.

After a single IV administration of MEDI4893 in healthy subjects, anti-AT neutralising antibody levels in the upper respiratory tract increased and reached a steady-state equilibrium between serum and nasal mucosa. This indicates that MEDI4893 distributes to the upper respiratory tract and

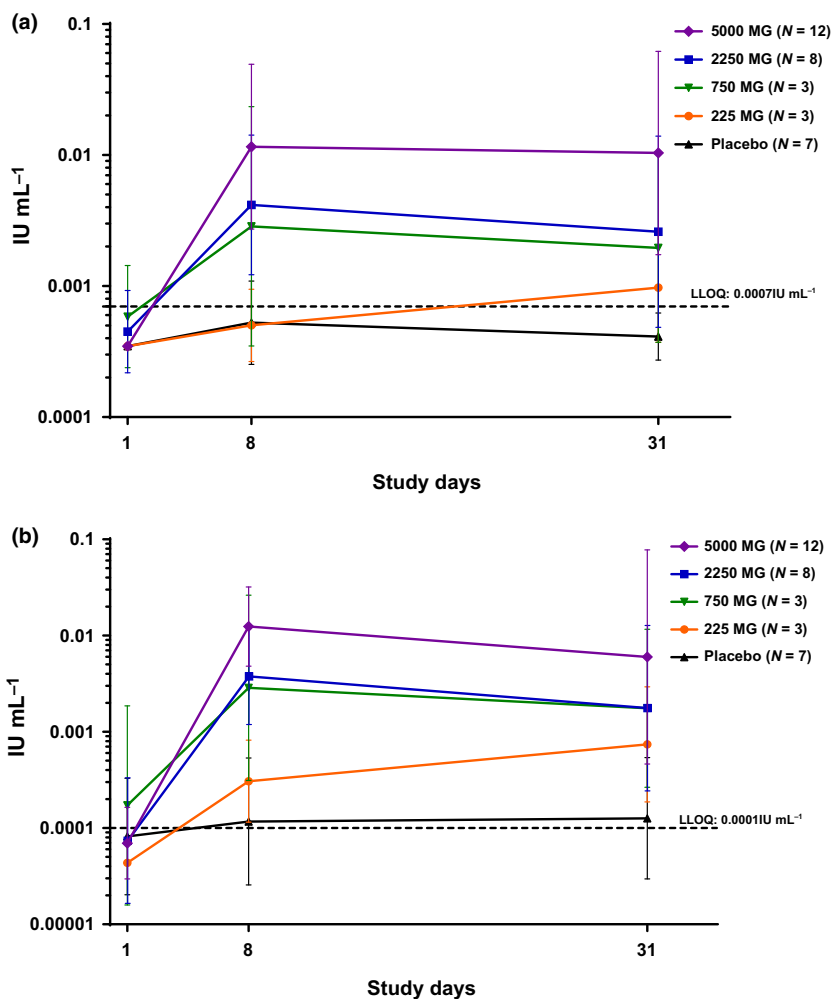


Figure 2. Anti-alpha toxin IgG and neutralising antibody levels in the upper respiratory tract. Each sample was tested in duplicate. Anti-AT IgG (a) and neutralising antibody (b) in IU per millilitre of nasal wash samples were summarised in mean and standard deviation in log scale for each treatment group at each visit time point. The dashed line represents the LLOQ of the assay, and LLOQ/2 was used for measurements below the LLOQ.

Table 2. Geometric mean fold increase in nasal anti-alpha toxin neutralising levels over baseline

Days	Placebo (n = 7)	225 mg (n = 3)	750 mg (n = 3)	2250 mg (n = 8)	5000 mg (n = 12)
8	1.2	1.4	4.9	9.3	33.3
31	1.2	2.8	3.4	7.5	29.8

retains neutralising activity against AT. As expression of *hla* gene increases steadily upon transition from nasal colonisation to bacteremia and heart lesions in animal models,²⁹ and elevated haemolytic activity by *S. aureus* strains is one of the risk factors that predict progression to ventilator-associated pneumonia,³⁰ sequestration

of AT by MEDI4893 at the site of colonisation (e.g. nares) is expected to contribute to the prevention of infection and dissemination of *S. aureus* to the rest of the body.

Although increased AT expression accompanies the transition of *S. aureus* from a commensal to a pathogenic bacterium, *hla* expression in the nares is low, and deletion of the *hla* gene does not affect *S. aureus* nasal colony counts.^{29,31} Exposure to MEDI4893 over the course of 360 days had no significant effect on nasal colonisation and caused no major changes in *in vivo* AT expression by *S. aureus* isolates. These results are consistent with published data showing that AT does not play a significant role in maintaining *S. aureus* nasal colonisation.

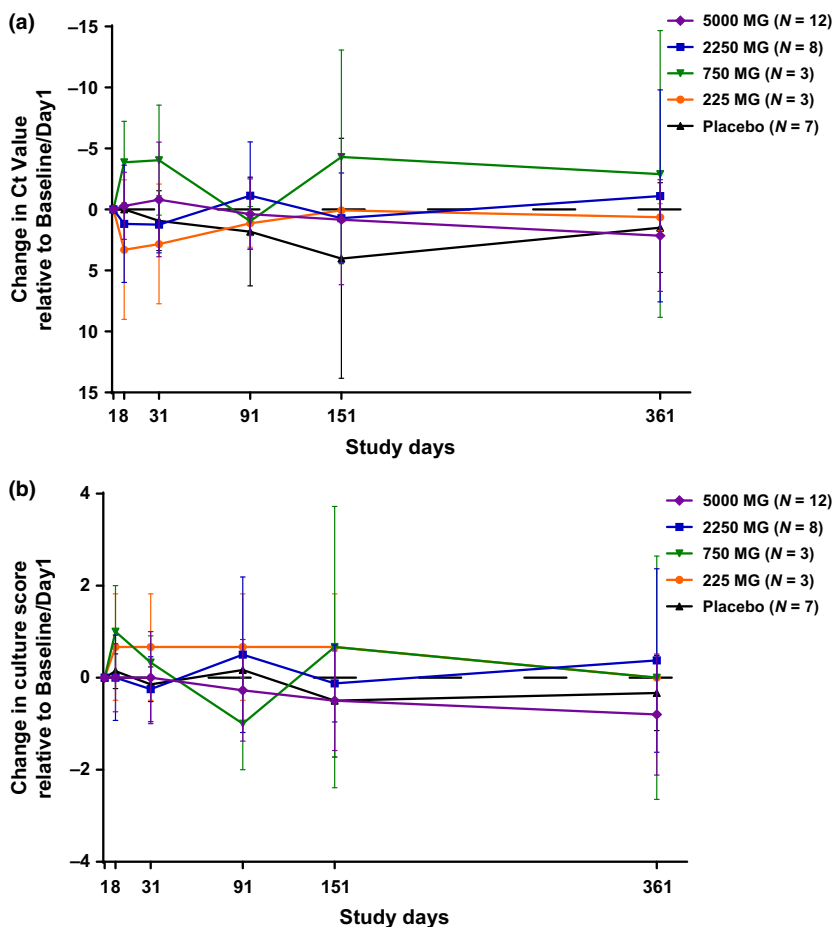


Figure 3. Changes in nasal *Staphylococcus aureus* colonisation relative to baseline in subject groups. Two nasal swabs, one from each nostril, were collected per study subject. One swab was used for *S. aureus* identification and enumeration by culture, another swab was used for Cepheid Xpert SA Nasal Complete PCR assay. Changes from the baseline measurement of *S. aureus* colonisation in PCR Ct values (a) and culture scores (b) of nasal swab samples were summarised in mean and standard deviation for each treatment group at each visit time point. The dashed line at 0 represents no change from baseline.

Table 3. Alpha toxin amino acid sequence subtypes from *Staphylococcus aureus* nasal isolates

AT subtype ^a	Isolates ^b (n = 54)		Subject ^c (n = 14)		Amino acid position				
	Count	%	Count	%	78	113	135	234	301
USA300 (reference)	N/A	N/A	N/A	N/A	L	Q	T	D	I
1	20	37	7	50	L	Q	T	D	I
4	7	13	2	14	L	Q	T	D	T
58	6	11	1	7	L	Q	A	D	I
11	16	30	5	36	L	Q	T	E	T
45	5	9	1	7	I	B	T	D	I

N/A, not applicable.

^aSubtypes 1, 4, 11 and 45 correspond to AT subtypes as defined previously.²⁸ Subtype 58 is novel and extends the previously reported list of AT subtypes.²⁸ Highlighted in bold are amino acid substitutions in subtypes 4, 11, 45 and 58 as compared to USA300 reference sequence (subtype 1). No amino acid changes are recorded downstream of stop codon at position 113 of subtype 45.

^bTwo MSSA-positive isolates did not yield AT-specific PCR product; thus, AT subtypes were not determined for those isolates.

^cTwo subjects with isolates from two AT subtypes (subject 1097401023: AT subtypes 1 and 4; subject 1097401024: AT subtypes 1 and 11).

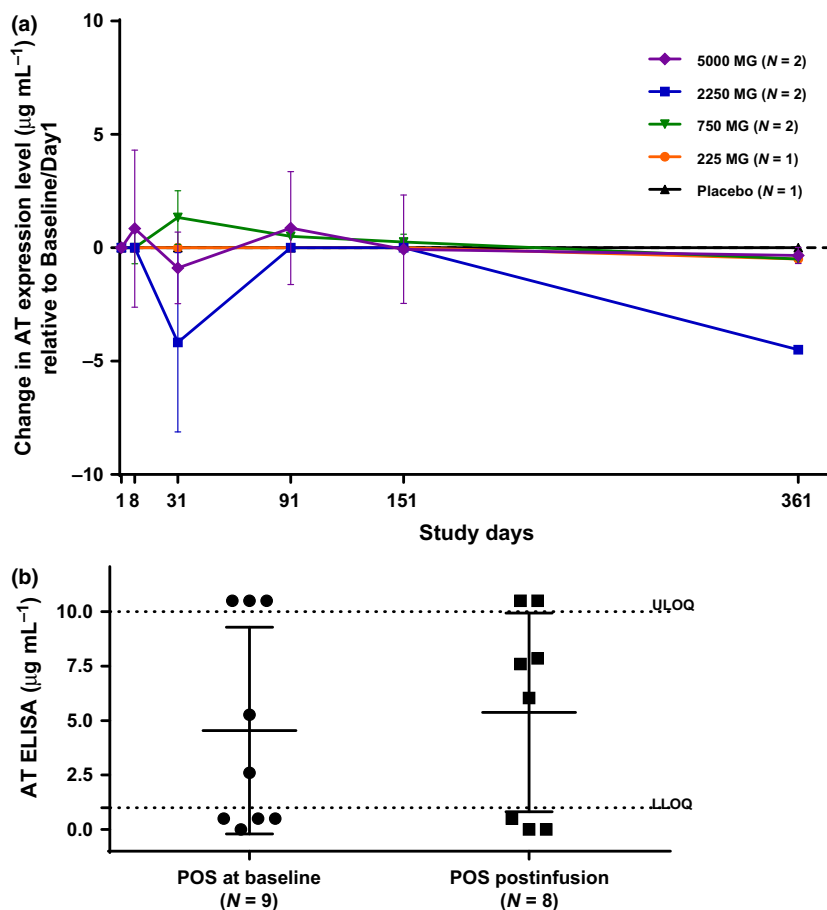


Figure 4. Alpha toxin protein expression in *Staphylococcus aureus* nasal isolates. *In vitro* AT expression was measured in the supernatants of overnight *S. aureus* cultures grown in TSB. Each sample was tested in duplicate. **(a)** For subjects who were *S. aureus* positive at baseline, changes of AT expression levels from the baseline were summarised in mean and standard deviation for each treatment group at each visit time point. The dashed line at 0 represents no change. **(b)** For subjects who were *S. aureus* negative at baseline but tested positive at postinfusion visits, the AT expression levels were compared with isolates from baseline. The dashed lines are the LLOQ ($1 \mu\text{g mL}^{-1}$) and the upper limit of quantitation (ULOQ) ($10 \mu\text{g mL}^{-1}$), where 10.5, 0.5 and $0 \mu\text{g mL}^{-1}$ were used for measurements greater than the ULOQ, positive (POS), or below the LLOQ, and negative, respectively.

Because MEDI4893 targets the secreted toxin (AT) and not the pathogen (*S. aureus*) itself, any emergence of resistance would be likely to manifest as amino acid substitutions in AT that would compromise binding and neutralisation by MEDI4893. In the present study, five different AT subtypes were observed in *S. aureus* isolates, none of which possessed AT variants with amino acid substitutions in MEDI4893 binding region despite prolonged exposure of *S. aureus* strains to MEDI4893 due to its extended half-life. However, it is possible that amino acid substitutions outside of the defined MEDI4893 binding region may change the conformation of the toxin and affect the MEDI4893–AT interaction as a consequence. Four of the *S. aureus* subtypes (subtypes 1, 4, 11

and 45) in this study were also detected in a recent global surveillance study of a large collection ($n = 994$) of *S. aureus* isolates; those AT variants possessed lytic activity that was fully neutralised by MEDI4893.²⁸ Subtype 58 detected in this study is novel and, upon testing, demonstrated lytic activity that was fully neutralised by MEDI4893. The aforementioned global surveillance study showed that only 19 of 994 isolates (1.8%) harboured AT with substitutions in MEDI4893 binding region, of which only a single AT variant (G218V) had lytic activity not neutralised by MEDI4893. Because lytic activity from that particular isolate (subtype 10) was neutralised by polyclonal IgG directed against γ -haemolysin and not neutralised by a polyclonal

anti-AT IgG, the lytic activity from subtype 10 was probably derived from γ -haemolysin.²⁸ Thus, the MEDI4893 molecular target is well conserved, and the potential for emergence of MEDI4893 resistance appears to be low.

Limitations of this study include a small subject sample size and lack of geographical diversity arising from recruitment at a single site in the United States. A larger and more geographically diverse phase 2 study is currently underway in Europe to evaluate the safety and efficacy of MEDI4893 in reducing the incidence of *S. aureus* pneumonia in mechanically ventilated patients. Data from this phase 2 study will extend the findings reported here, and we will continue to evaluate the impact of MEDI4893 on both the *S. aureus* pathogen and the human immune system.

METHODS

Study design

This first-in-human, double-blind, randomised, placebo-controlled, dose-escalation, phase 1 study was conducted at a single-study centre in the United States (www.clinicaltrials.gov: NCT01769417). Thirty-three healthy subjects were enrolled and randomly assigned to receive either MEDI4893, at 225 mg ($n = 3$); 750 mg ($n = 3$); 2250 mg ($n = 8$), or 5000 mg ($n = 12$), or placebo ($n = 7$). The subjects were followed for 360 days after the dosing.

Written informed consent was obtained from subjects before study procedures began. The institutional review board approved the study protocol and informed consent documents. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and the International Council for Harmonisation guidelines on Good Clinical Practice.

Characterisation of *Staphylococcus aureus* isolates

Nasal swabs, one from each nostril, were collected before nasal wash collection and transported in Universal Transport Medium tubes to International Health Management Associates, Inc, within 2 days of collection. Upon receipt, one nasal swab was used to inoculate a blood agar plate (tryptic soy agar with 5% sheep blood; Remel Microbiology Products, Lenexa, KS, USA)

and streaked into four quadrants for *S. aureus* identification and enumeration.³² Plates were incubated at 37°C in an atmosphere of 5% CO₂ for 18–24 h. Colonies with a staphylococcal morphology (smooth, butyrous colonies with a low convex edge and yellow to off-white colour) were identified to the species level by matrix-assisted laser desorption–ionisation time-of-flight mass spectrometry and evaluated for production of coagulase (BactiStaph Latex Agglutination Test Kit; Thermo Scientific, Waltham, MA, USA) and catalase (Gibson Biosciences, Lexington, KY, USA). In addition, isolates were screened for susceptibility to methicillin using the cefoxitin disc method (cefoxitin, 30 μ g; BBL Sensi-Disc; BD, Franklin Lakes, NJ, USA) according to Clinical Laboratory and Standards Institute guidelines.³³ DNA was extracted from isolates identified as *S. aureus* for PCR amplification and sequencing of the *hla* gene as previously described.³⁴ The AT protein sequences were obtained by translation of the consensus DNA sequences. Amino acid substitutions were identified using USA300 FPR3757 as a reference sequence.³⁵ AT subtypes were assigned, and a novel AT variant (subtype 58) was tested for lytic activity and neutralisation by MEDI4893 as described previously.²⁸

The second nasal swab was tested using the Xpert SA Nasal Complete PCR assay (Cepheid, Sunnyvale, CA, USA) according to the manufacturer's protocol.

In vitro AT expression was determined in the supernatants obtained from *S. aureus* cultures grown overnight in tryptic soy broth, as described previously.³⁴ Purified AT was used as a standard (0.01–10 ng mL⁻¹). An overnight culture supernatant prepared from a Δhla strain and diluted 1:100 000 into tryptic soy broth was used as a negative control (blank) for AT detection and also as the diluent for purified control AT protein used for quality control and protein quantitation. The sample was positive if the optical density readout was at least three standard deviations above the negative controls. The quantification range for a positive sample was between 1 and 10 μ g mL⁻¹.

Anti-AT IgG and neutralising antibody levels in human serum and upper respiratory tract (nasal wash)

Blood samples were collected on days 1, 2, 4, 6, 8, 15, 22, 31, 61, 91, 121, 151, 211, 271 and 361.

Quantification of anti-AT IgG and neutralising antibodies in serum samples was performed as described previously using an enzyme-linked immunosorbent assay (ELISA) and a red blood cell-based AT neutralisation assay, respectively.³⁴ Nasal wash samples were collected on the same dates as blood samples, except for day 6. Bioanalytical methods to quantify anti-AT IgG and neutralising antibodies in human serum samples were adapted and modified to quantify anti-AT IgG and neutralising antibodies in the nasal wash samples.

The standard and quality control samples used in these assays were calibrated to the National Institute for Biological Standards and Control reference standard,³⁶ and antibody levels for samples were reported in IU per millilitre. The lower limit of quantitation (LLOQ) for the ELISA assay was 0.0001 IU mL⁻¹. LLOQ for the AT neutralisation assay was 0.0007 IU mL⁻¹.

Statistical methods

The changes from baseline screening of *S. aureus* colonisation in PCR cycle threshold values and culture scores and the changes in AT expression level for *S. aureus*-positive subjects at screening were summarised in descriptive statistics of mean and standard deviation in log scale by treatment group and visits. The AT expression levels for *S. aureus* isolates from subjects who were negative at baseline but tested positive during postinfusion visits were compared with isolates from screening, and a two-sample *t*-test was used for comparison.

Anti-AT neutralising antibodies and IgG levels in serum and upper respiratory tract, in IU per millilitre and fold change from baseline, were summarised by using the geometric mean by study day and by treatment group.

SAS System Version 9.3 was used for summary statistics, and Prism 6.05 was used to generate figures.

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DISCLOSURES

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