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ORIGINAL

Safety and tolerability of a single administration of AR-301, a human monoclonal antibody, in ICU patients with severe pneumonia caused by *Staphylococcus aureus*: first-in-human trial

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Abstract

Purpose: Hospital-acquired bacterial pneumonia (HABP) is a critical concern in hospitals with ventilator-associated bacterial pneumonia (VABP) remaining the most common infection in the ICU, often due to *Staphylococcus aureus*, an increasingly difficult to treat pathogen. Anti-infective monoclonal antibodies (mAb) may provide new, promising treatment options. This randomized, double-blinded, placebo-controlled study aimed at assessing the safety and pharmacokinetics of AR-301, an *S. aureus* alpha toxin-neutralizing mAb, and exploring its clinical and microbiologic outcomes when used adjunctively with standard-of-care antibiotics.

Methods: Eligibility in this trial required microbiologically confirmed severe *S. aureus* pneumonia, including HABP, VABP or CABP, treated in the ICU and an APACHE II score ≤ 30. Standard-of-care antibiotics selected by the investigators were administered to all patients in the study following clinical and microbiologic confirmation of *S. aureus* pneumonia. Adjunctive treatment of AR-301 was to start < 36 h after onset of severe pneumonia. AR-301 was administered to four sequentially ascending dose cohorts. The placebo cohort received antibiotics and a placebo buffer. Clinical outcomes were adjudicated by a blinded committee. *S. aureus* eradication was declared based on a negative follow-up culture and presumed to be negative when no culture was obtained in the presence of clinical improvement.

Results: Thirteen ICUs enrolled 48 patients, with pneumonia attributable to MRSA in six subjects. The study drug displayed a favorable safety profile: Of 343 AEs reported, 8 (2.3%) were deemed related, none serious. In a post hoc subgroup analysis of VABP patients receiving AR-301, ventilation duration was shorter for AR-301-treated patients compared with the placebo group. Overall, there was a trend toward a better and faster microbiologic eradication at day 28. The PK profile of AR-301 is consistent with that of a human IgG1 mAb, with a plasma half-life of about 25 days.

Conclusions: Adjunctive treatment of severe *S. aureus* HABP with anti-staphylococcal mAbs appears feasible and suggests some clinical benefits, but larger randomized studies are needed to better define its safety and efficacy.

Keywords: *Staphylococcus aureus*, Monoclonal antibody, HAP/VAP, Adjunctive therapy

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Introduction

Hospital-acquired bacterial pneumonia (HABP) is the most common nosocomial infection in the intensive care unit (ICU), and while mechanical ventilation is an important risk factor, ventilator-associated bacterial pneumonia (VABP) represents up to 80% of HABP cases in ICUs. Even with first-line high-dose antibiotics, HABP remains the leading cause of mortality and morbidity in nosocomial infections [1]. *Staphylococcus aureus* is among the most frequent pathogens involved in ICU-HABP, which is also a common cause of bloodstream and skin/soft tissue infections [1–3]. Treatment of these infections has become more challenging because of the emergence of multi-drug-resistant strains globally. In developed countries such as the USA, methicillin-resistant *S. aureus* (MRSA) strains are a major problem in hospitals with up to one half of staphylococcal pneumonia isolates classified as MRSA, resulting in mortality as high as 56% [4, 5]. Given that the MICs of antibiotics are on the rise for both MSSA and MRSA clinical isolates, the declining effectiveness of available standard of care treatments represents increasing public health risks [6]. Thus, alternative treatments to current antibiotics must be found to address the growing occurrence of bacterial resistance.

Staphylococcus aureus has a broad arsenal of virulence factors, including toxins, that allows it to survive within the human host and thereby contribute its pathogenicity [7]. Among these factors, alpha toxin, also known as alpha-hemolysin (Hla), has been shown to have hemolytic, cytotoxic, dermo-necrotic properties and can also provoke cardiovascular collapse and pulmonary edema [8]. In a recent study, alpha toxin was also shown to modulate the activity of macrophages against *S. aureus* as well as co-infecting pathogens such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* [9]. Wardenburg has demonstrated that the severity of lung disease in mice correlates with the levels of alpha toxin produced by a particular *S. aureus* isolate [10]. Furthermore, these authors showed that immunization against a non-pore-forming alpha toxin variant induced immunity to pneumonia caused by *S. aureus*. In another setting, the authors demonstrated that antibodies against alpha toxin also protected human lung epithelial cells from *S. aureus*-induced lysis [11]. Treatment of *S. aureus*-infected mice or rabbits with anti-alpha toxin was necessary and sufficient to confer protection against morbidity and mortality, which suggests that among the many virulence factors that *S. aureus* produces, neutralizing alpha toxin alone is sufficient to confer protection [12, 13].

Accordingly, monoclonal antibodies could be one option to treat severe HABP in the ICU. AR-301 (formerly known as KBSA301) is a fully human monoclonal antibody of the IgG1 lambda isotype that specifically neutralizes alpha toxin, thus providing passive immunotherapy in the context of *S. aureus* infections. AR-301 was discovered by screening the B cell repertoire of a *S. aureus* pneumonia patient for the monoclonal antibody with the highest alpha toxin neutralizing activity. Treatment of *S. aureus*-challenged mice with AR-301 either prophylactically or therapeutically, was also protective (unpublished data). The medical need targeted for AR-301 is hospital-acquired and ventilator-associated bacterial pneumonia (HABP and VABP) due to *S. aureus*.

Take-home message

Staphylococcal bacterial pneumonia in the ICU is increasingly difficult to treat in part because of resistant bacteria and, as a result, new treatment options such as a monoclonal antibody neutralizing *Staphylococcus aureus* alpha toxin used as adjunctive therapy is of interest to treat severe *S. aureus* pneumonia (HABP and VABP) in the ICU. Therefore, AR-301, a fully human mAb, is a new promising treatment that appears safe, with few adverse events, and efficacious as it seems to shorten duration of ventilation, time to microbiological eradication and length of stay in the hospital.

The present study is a first-in-human phase 1/2a clinical evaluation of AR-301 mAb as an adjunctive therapeutic treatment to standard-of-care antibiotics in Staphylococcal pneumonia patients. The primary endpoints are safety and pharmacokinetic measurements, with secondary exploratory endpoints that included efficacy-related outcomes such as mortality, ventilation time, microbiologic cure and health benefit parameters such as hospitalization and ICU days.

Materials and methods

This was a phase I/II, randomized, double-blind, placebo-controlled, single ascending dose study conducted between 2012 and 2016 at 13 ICUs in 5 countries (France, Belgium, UK, the USA and Spain). This study was conducted in compliance with current Good Clinical Practices and the Declaration of Helsinki. Informed consent was obtained from each patient or his/her legal authorized representative before any procedure related to the study was performed (Clinicaltrials.gov: NCT01589185).

The primary objective of this study was to assess the safety and tolerability of a single administration of AR-301 in patients with severe *S. aureus* pneumonia. Secondary objectives were to assess the pharmacokinetics (PK), immunogenicity, and microbiologic and clinical efficacy of AR-301.

Patients were eligible if they were older than 18 years, had severe HABP, VABP, or CABP (community-acquired bacterial pneumonia) caused by *S. aureus* (either MSSA or MRSA) for less than 36 h (defined with a *Staphylococcus* documentation within 36 h prior to enrollment and a pneumonia severity criterion pre-existing for no

longer than 36 h whatever the duration of antibiotic prior to enrollment), and an Acute Physiology and Chronic Health Evaluation II (APACHE II) score ≤ 30 at enrollment. Mechanical ventilation was not required for inclusion. The diagnosis of pneumonia additionally required clinical signs including fever (defined as body temperature >38 °C) or hypothermia (defined as body temperature <35 °C), presence of a new or progressive infiltrate on a chest X-ray, and either leukocytosis (white blood cells $>10{,}000/mm^3$) or leucopenia (white blood cells $<4500/mm^3$). Pneumonia was deemed severe in the presence of respiratory failure ($PaO_2/FiO_2 \leq 200$ for at least 1 h) or the need for vasopressor support. VABP was defined by the same clinical signs and a modified clinical pulmonary infectious score (CPIS) ≥ 6. Identification of *S. aureus* was performed on respiratory tract samples (bronchoalveolar lavage or endotracheal aspirate decided by treating physician) using any standard microbiology laboratory bacterial culture method or a rapid diagnostic system (GeneXpert, Cepheid, CA, USA). Exclusion criteria were: pregnancy, childbearing potential in the absence of proper contraception, hypersensitivity to one of the excipients or the antibody itself, cancer, long-term tracheostomy (>60 days), HIV, immunosuppressive treatment, liver function deficiency and moribund patients.

Upon confirmation of eligibility, patients were randomized in a blinded fashion using an interactive web response system to either AR-301 or placebo depending on the cohort with an unblinding functionality of the system in order to unblind a patient in case of medical emergency. The design is a first-in-human study with four increasing single doses of AR-301, which were administered to sequential cohorts (1, 3, 10 and 20 mg/kg) in 8, 12, 15 and 13 patients, respectively, each cohort including 2–5 patients receiving placebo. Dose escalation was approved by an independent Data Safety Monitoring Board (DSMB). Continuation at a given dose level was also approved by the DSMB upon review of the first two patients of each cohort (one active, one placebo). The study drug vials (active and placebo) were visually indistinguishable and packed in a blinded way. Both clinicians and pharmacist were blinded.

The study drug was administered by intravenous infusion over 2 h starting within 36 h following the diagnosis of severe pneumonia, start of the 36-h time window being defined by onset of the first qualifying severity criteria above mentioned. Standard-of-care antibiotic therapy targeting *S. aureus* was left to investigator judgment. After treatment administration, the follow-up period lasted 107 days (approximately $4 \times$ plasma half-life of AR-301) with test-of-cure visits on day 8, 15 and 29. Demographic data, patient's medical history, concomitant medications, clinical outcome, adverse events (including mortality), APACHE II score, Sequential Organ Failure Assessment (SOFA) score, CPIS, microbiology and chest X-ray results and end of mechanical ventilation were recorded in an electronic data capture system (EDC, Micron Research). Frequency, duration, severity and outcome of adverse events (AEs) were carefully recorded from the first administration of study drug until the end of study (day 107 ± 7) or early discontinuation. In addition to clinical AEs, signs and symptoms of allergic reactions, such as anaphylaxis, as well as blood pressure, heart rate, skin reactions and lung resistance, were monitored on day 1 pre-dose, 0.5, 1, 2, 4, 12 and 24 h after the start of infusion.

Standard regulatory definitions for AE and serious AE (SAE) are hereafter provided.

AE: An adverse event is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

SAE: A serious adverse event (experience) or reaction is any untoward medical occurrence that at any dose results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect. SAE was determined primarily by the site investigator first and then validated in a blinded manner by the pharmacovigilance group, which was independent from the sponsor of the study.

A clinical evaluation committee (CEC) blinded to patients' treatment assignment reviewed the data of all patients enrolled. Microbiologic eradication was assessed on day 28. *S. aureus* was considered eradicated if a negative culture was obtained during the follow-up period and presumed eradicated in the presence of clinical resolution without documentation of microbiologic outcome. *S. aureus* was not eradicated if a positive culture was obtained during the follow-up period and presumed not eradicated in the presence of clinical failure without documentation of microbiologic outcome. Antibiotic therapy at enrollment was classified as adequate or not according to the antimicrobial susceptibility test at baseline. Duration of anti-*S. aureus* antibiotic therapy, number of days with anti-*S. aureus* therapy, average number of anti-*S. aureus* antibiotics and number of antibiotic-free days were assessed. Clinical cure was assessed at day 28 [late follow-up (LFU)] using a formal process with pre-defined rules. Clinical success was declared when all five of the following criteria were

met: (1) survival through the LFU visit, except if death was adjudicated as indisputably not related to the recent episode of lower respiratory tract infection, (2) antibiotics for pneumonia were stopped on or before day 14 ± 2, (3) antibiotics active against the initial *S. aureus* strain have not been restarted after day 14, (4) no lung abscess or empyema was reported through the LFU visit and (5) improvement of respiratory function evaluated on day 14 ± 2, either because the patient has been extubated or, if still on mechanical ventilation, based on improvement in the PaO_2/FIO_2 compared with baseline. If any of these criteria were not met, the patient's outcome was adjudicated as clinical failure.

Blood samples for the purpose of pharmacokinetic assessment of AR-301 were collected at baseline (pretreatment), then on day 1 at 0.5, 1, 2, 4, 12, 24 and 48 h post-dose, and day 5, 8, 15, 22, 29, 57, 85 and 107 or study discontinuation. Anti-AR-301 antibodies were assessed in serum at baseline and on day 15, 29, 57 ± 7 and 107 ± 7 or study discontinuation. Pulmonary samples were collected for screening and as medically required at least once after treatment.

Statistical analyses

This was a first-in-human study, and thus the sample size of each cohort was not based on a formal power calculation.

The safety population included all 48 patients who received the study treatment. The efficacy population included all but one patient in whom *S. aureus* was positive by the rapid diagnostic test but not confirmed by microbiologic culture. The PK analysis included all 32 patients who received AR-301.

Most comparisons between placebo and AR-301 were intended to be descriptive. Nonetheless, whenever appropriate, relevant statistical methods were applied, including the Fisher's exact test and analysis of variance (ANOVA). Pharmacodynamic data were to be subjected to statistical comparisons with a descriptive intention per time point if warranted. These measures of effect were to be analyzed on the basis of a one-way ANOVA to test for between-dose differences with a descriptive intention.

Results

Demographic and baseline characteristics

Recruitment proceeded from 16 May 2012 to 28 May 2016. For administrative reasons the study was effectively on hold from March 2013 to November 2014. A total of 48 VABP, CABP and HABP patients were enrolled in the study at 13 sites, randomized and treated. Sixteen were assigned to the placebo group, and the remaining 32 were all dosed per protocol. The type of pneumonia was VABP in 26 (55.3%) patients overall, with an imbalance between the placebo and the treated group: 5 (31.3%) in the placebo group versus 21 (65.6%) in treated patients were VABP [5 (83.3%) in cohort 1, 4 (50.0%) in cohort 2, 7 (70.0%) in cohort 3, 5 (62.5%) in cohort 4]. Nine (9) patients (19.1%) had CABP, with four (4) in the placebo group (25%), three (3) in cohort 2 (37.5%), and one each in cohort 1 (16.7%) and cohort 4 (12.5%). Patient' characteristics are presented in Table 1. At baseline, pneumonia was considered severe in all patients because of hypoxemia ($PaO_2/FiO_2 = 147.8 \pm 41.3$; placebo: 140.7 ± 40.1 versus treated group: 151.4 ± 42.1) and/or catecholamine requirement prior to the study drug treatment [16 patients out of

Table 1 Patient characteristics at baseline

	Placebo *n* = 16	Cohort 1 *n* = 6	Cohort 2 *n* = 8	Cohort 3 *n* = 10	Cohort 4 *n* = 8	All treated *n* = 32
Gender: male (%)	13 (81.3)	4 (66.7)	8 (100)	6 (60)	7 (87.5)	25 (78.1)
Age (years)	52 (25–80)	65 (50–78)	49 (21–69)	62 (47–74)	61 (39–76)	59 (21–78)
BMI (kg/m^2)	26.8 (18.8–35.6)	32.6 (23.9–46.5)	28.1 (17.9–39.8)	31.1 (22.8–43.5)	29.1 (23.9–37.2)	30.2 (17.9–46.5)
PaO_2/FiO_2 ratio at screening	140.7 ± 40.1	146.7 ± 30.7	173.7 ± 38.4	138.8 ± 53.6	148.4 ± 34.5	151.4 ± 42.1
SOFA score	6.8 (3–15)	6.8 (4–8)	8.8 (6–15)	5.6 (3–8)	6.8 (5–10)	6.9 (3–15)
APACHE score	17.5 (8–25)	18.3 (16–24)	21.5 (15–28)	17.9 (9–23)	19.3 (12–24)	19.3 (9–28)
VABP (%)	5 (31.3)	5 (83.3)	4 (50.0)	7 (77.8)	5 (62.5%)	21 (65.6)

Safety and ITT populations coincide in this study

BMI body mass index, *SOFA* Sequential Organ Failure Assessment, *APACHE* Acute Physiology and Chronic Health Evaluation, *VABP* ventilator-associated bacterial pneumonia

48 (33.3%) for a mean duration of 1.7 days in AR-301 patients and 2.0 days in the placebo group]. *S. aureus* was documented in all but one patient and found to be MRSA in six patients [two in the placebo group (12.5%), two in cohort 1 (33.3%), two in cohort 4 (25%)]. Antibiotic therapy at baseline was found adequate in all but three (6.4%) patients (three patients documented with MRSA were initially treated respectively with imipenem, piperacillin/tazobactam and cefepime for at least 72 h before modification). All patients were included in the analyses of safety and pharmacokinetics. Microbiologically confirmed evidence of *S. aureus* was not certain in one patient (positive PCR but negative culture), who was excluded from the analyses of efficacy, but not of safety. The diagnosis of severe pneumonia was made 1.0 ± 1.0 days prior to treatment with the study drug. Overall, treatment was administered 13.4 ± 11.3 h after pneumonia severity was documented.

Safety

The safety population included all 48 patients who were treated. Among them, 46 experienced at least one AE. A total of 343 AEs were reported, of which 8 (2.3%) were deemed treatment-related by the investigator [placebo: 2 (2.4%), cohort 1: 1 (3.3%), cohort 2: 0, cohort 3: 1 (1.3%), cohort 4: 4 (6.8%), all treated: 6 (2.3%); Table 2]. Two patients were withdrawn from the study because of an SAE (one each in placebo and cohort 1). Most adverse events were mild or moderate. There were 36 (10.5% of AEs) serious AEs (SAEs) affecting 18 patients; none of the SAEs were deemed treatment related (Table 3). AEs and SAEs were similar across all study groups (Table 3). Six (12.5%) patients died during the study [placebo: 1 (6.3%), cohort 1: 1 (16.7%), cohort 2: 2 (25.0%), cohort 3: 2 (20.0%), cohort 4: 0]. All deaths were attributed to the underlying condition of the patients by the treating physician, and none was thought to be treatment-related by the CEC. Safety laboratory tests did not suggest a

Table 2 Patients with treatment-related adverse events (ITT population)

	Total *n* = 48 (%)	Placebo *n* = 16	Cohort 1 *n* = 6	Cohort 2 *n* = 8	Cohort 3 *n* = 10	Cohort 4 *n* = 8	All treated *n* = 32
Any AE	6 (12.5)	1 (6.3%)	1 (16.7%)	0	1 (10.0%)	3 (37.5%)	5 (15.6%)
LDH increase[a]	1 (2.1)	0	0	0	0	1 (12.5%)	1 (3.1%)
Eosinophil count increase[a]	1 (2.1)	1 (6.3%)	0	0	0	0	0
Hepatic enzyme increase[a]	1 (2.1)	0	0	0	0	1 (12.5%)	1 (3.1%)
Vomiting	1 (2.1)	0	0	0	0	1 (12.5%)	1 (3.1%)
Fever	1 (2.1)	0	0	0	1 (10.0%)	0	1 (3.1%)
Hepatocellular injury	1 (2.1)	0	1 (16.7%)	0	0	0	1 (3.1%)
Arthritis	1 (2.1)	0	0	0	0	1 (12.5%)	1 (3.1%)
Plasma cell myeloma	1 (2.1)	1 (6.3%)	0	0	0	0	0

AE adverse event, *LDH* lactate dehydrogenase

[a] Screening values of above AEs have been used as the baseline to measure change. If there was no screening value the infusion day value was used

Table 3 Overview of AE frequency

	Total *n* = 343	Placebo *n* = 82	Cohort 1 *n* = 30	Cohort 2 *n* = 92	Cohort 3 *n* = 80	Cohort 4 *n* = 59	All treated *n* = 261
Treatment-related AEs	8 (2.3%)	2 (2.4%)	1 (3.3%)	0 (0%)	1 (1.3%)	4 (6.8%)	6 (2.3%)
SAEs	36 (10.5%)	8 (9.8%)	4 (13.3%)	10 (10.9%)	13 (16.3%)	1 (1.7%)	28 (10.7%)
Treatment-related SAEs	0	0	0	0	0	0	0
Deaths	6	1	1	2	2	0	5

AE adverse event, *SAE* serious adverse event

potential safety issue. One patient [cohort 3 (3 mg/kg)] was found positive for anti-drug antibodies on day 15 and day 29 post-treatment and negative at baseline and day 107. Additionally, this subject's PK profile was similar relative to the cohort as a whole. No AE in this patient was found related to immunogenicity, and none was found to be treatment-related. All these AEs resolved by the end of the study.

Clinical outcome

Staphylococcus aureus infection was not confirmed in 1 patient, thus reducing the efficacy population to 47 patients. Following blinded adjudication, the rate of clinical cure on day 28 was not statistically different between the AR-301 group and placebo [22 (71.0%) versus 14 (87.5%) patients; $p=0.3892$].

The duration of ventilation overall tended to be a little shorter for VABP, HABP, and CABP patients administered the active study drug compared with those receiving placebo (active: 9.7 ± 7.87 day; placebo: 11.0 ± 7.81 day; $p=0.4132$). In a post hoc analysis of the subset of 25 patients with VABP, there was a numeric difference in mean days on ventilation of 16.8 ± 8.44 days in the placebo groups versus 9.5 ± 7.57 day in the pooled AR-301-treated VABP patient (Fig. 1). While the result is of an exploratory nature, there appears to be a possible benefit in this very sick group of patients that needs to be confirmed in a larger clinical trial.

Six patients died during the 107-day follow-up period (12.5% mortality), [placebo 1/16 (6.3%); treatment 5/34 (14.7%); $p=0.3962$, including 5 before day 28]. The causes of death were worsened coma ($n=1$), alveolar hypoventilation ($n=1$), refractory septic shock ($n=2$), worsened acute respiratory failure ($n=1$), and multiple organ failure ($n=1$). No death was attributable to AR-301 according to both the investigators and the DSMB.

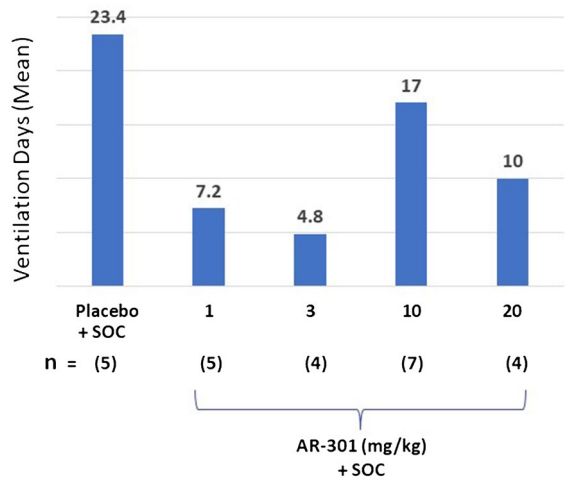

Fig. 1 Ventilation duration over cohorts for the subgroup of VABP patients. The observed ventilation days for each cohort and number of patients per VABP cohort are shown on top of each bar

Oxygenation improved in all study groups, as did clinical assessments using a variety of standard scoring methods (APACHE II, CPIS, SOFA), with no obvious difference between groups, owing to the small sample size and the high variability (Table 1).

The total duration of hospital stay was not statistically different between the AR-301 and placebo group; nor was the duration of ICU stay. However, there was a trend to a shorter duration of hospitalization by day 28 (placebo: 23.9 ± 6.4 versus all-treated: 21.2 ± 7.6, $p=0.42,647$, and median durations were 28.0 and 23.0 days respectively). A similar observation was made in regard to the duration of ICU stay (placebo: 16.5 ± 8.3 versus all-treated: 14.8 ± 8.8, $p=0.59,471$; median duration was 17.5 and 14.0, respectively). These differences were not observed by day 107 (end of study), as a number of patients remained hospitalized or in the ICU for a long time, up to 114 days, because of their underlying clinical condition.

Microbiology

Initial antibiotic management was adequate in 44 (93.6%) patients with no difference between the groups receiving placebo or active treatment. Inadequate initial antibiotic therapy was adjudicated by the CEC in three patients (two MRSA, one MSSA). The number of antibiotic-free days (placebo: 14.9 ± 6.4, all-treated: 14.1 ± 7.0, $p=0.68,304$) and of anti-*S. aureus* antibiotic-free days (placebo: 14.9 ± 6.4, all-treated: 15.5 ± 6.0, $p=0.76905$) did not differ between the two groups.

The rate of microbiologic eradication or presumed eradication on day 28 was accessed by an adjudication committee, which trended higher in the AR-301 treatment cohorts compared with placebo, 10 (62.5%) placebo patients as compared to 25 (80.6%) in those treated with active product. However, true confirmed microbiologic eradication (i.e., excluding the presumed eradicated population) was similar in both groups. The time duration of *S. aureus* eradication was numerically shorter in the AR-301 treatment group (Table 4); however, the difference was not statistically significant.

Pharmacology

Plots of the plasma concentration of AR-301 over time are shown in Fig. 2. The observed $t½$ of AR-301 was 22.7–31.0 days. The characteristics of the product were not altered by the dose given, although some values could not be computed for the lower dose level because of the lack of a measurable concentration in the corresponding samples. The mean concentration (C_{max}) values were 13.4, 56.1, 197, and 471 μg/ml for the 1-, 3-, 10-, and 20-mg/

Table 4 Time to eradication (mITT population)

	Placebo *n* = 16	Cohort 1 *n* = 6	Cohort 2 *n* = 8	Cohort 3 *n* = 9	Cohort 4 *n* = 8	All treated *n* = 31
Eradicated	7 (43.8%)	1 (16.7%)	5 (62.5%)	4 (44.4%)	4 (50.0%)	14 (45.2%)
Day to eradicate	10.9 ± 4.4	8.0	9.4 ± 3.1	9.8 ± 3.5	8.8 ± 1.0	9.2 ± 2.5
Presumed eradicated	3 (18.8%)	4 (66.7%)	2 (25.0%)	3 (33.3%)	2 (25.0%)	11 (35.5%)
Eradicated or presumed eradicated	62.5%	83.3%	87.5%	77.8%	75.0%	80.6%

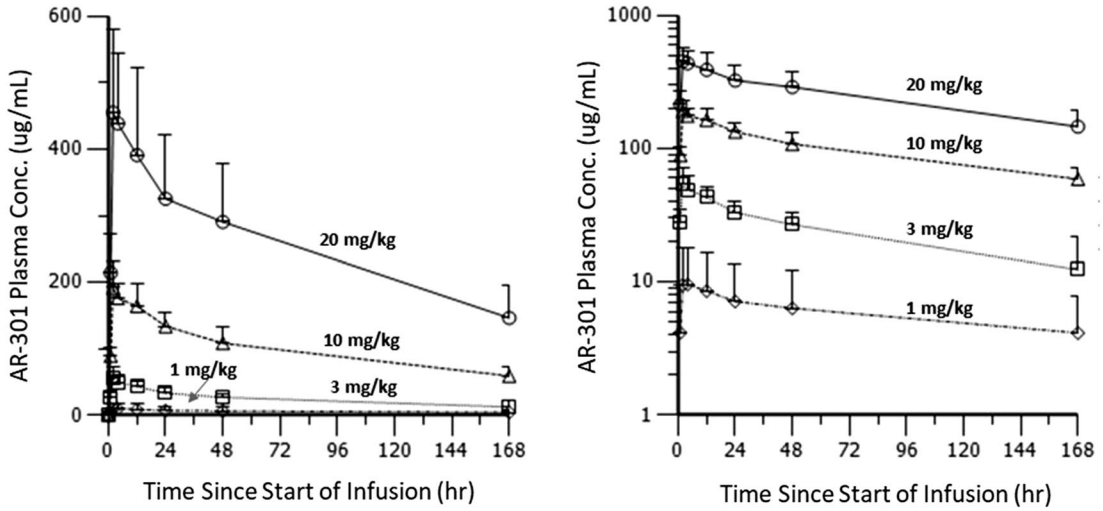

Fig. 2 Pharmacokinetic profiles over groups. Evolution of AR-301 (KBSA 301) concentration over time by dose level. Right side is a logarithmic concentration scale and left side is a linear scale

kg dose groups, respectively, with %CV ranging from 17.7 to 45.7%. Mean AUC_{0-t} values were 8530, 10,500, 41,900, and 95,700 μg × h/ml for the 1-, 3-, 10-, and 20-mg/kg dose groups, respectively, with %CV ranging from 38.3 to 101.8%. Mean clearance values were 0.238 and 0.185 ml/h/kg, and mean V_{ss} values were 92.7 and 104 ml/kg for the 10 and 20 mg/k dose groups, respectively. Box plots comparing the C_{max}, AUC_{0-t}, and AUC_{0-inf} over the range of doses are presented in Fig. 3.

Discussion

Experimental data suggest that antibody-mediated antagonism of toxin activity may protect animals from disease [10, 12, 13]. MAbs have been in development for nearly 30 years, and many have been tested on animal models, including mice [14]. Traditional monoclonal antibodies were murine. However, a human anti-mouse antibody reaction could occur when they were used to treat human diseases. Therefore, fully human antibodies are a preferable alternative to reduce the rejection reaction when treating human diseases [15]. AR-301 is a fully human mAb derived by screening the B-cell repertoire of an *S. aureus* pneumonia patient for mAbs with the highest alpha toxin-neutralizing activity, thus are naturally occurring, and in theory are functionally optimized by the human immune system. Soluble toxins greatly contribute to the pathogenesis of some bacterial infections, in particular those caused by *S. aureus*. Previous studies using mAb failed to show efficacy during phase II or III trials [16, 17]. These failures did not provide much insight into the design of future mAb therapies as they were due to multiple factors, including epitope selection [18, 19]. Furthermore, the agents that were assessed aimed at capsular targets and not exotoxins such as alpha toxin.

During infection, *S. aureus* releases a number of toxins, and *S. aureus* alpha toxin is expressed early in the infection cycle by most *S. aureus* strains and is among the most prevalent virulence factors causing tissue invasion and necrosis [20]. The pivotal role of alpha toxin in *S. aureus* pathogenesis is supported by numerous animal models [12, 13, 21, 22] and by observational studies in humans in which the presence of anti-alpha toxin antibodies during severe infections was associated with improved outcome [23].

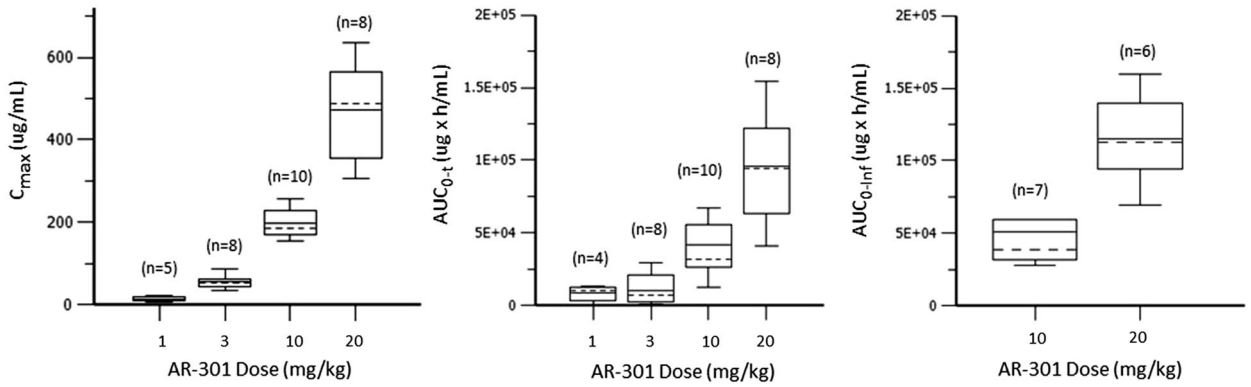

Fig. 3 Box plots of KBSA301 Cmax, AUC_{0-t}, and AUC_{0-inf} versus dose group. AUC_{0-inf}, area under the plasma concentration-time curve from time 0 extrapolated to infinity; AUC_{0-t}, area under the plasma concentration-time curve from time 0 to the time of the last measurable plasma concentration after drug administration; Cmax, maximum observed plasma drug concentration. The dashed and solid lines represent the median and arithmetic means, respectively. The box represents the 25th and 75th percentiles with the whiskers showing the lowest and highest data values within 1.5 times the interquartile range (the difference between the third and first quartiles, the middle 50%) of the lower and upper quartiles, respectively. Individual points are considered statistical outliers

This first-in-human study supports the feasibility of AR-301 mAb for the treatment of infection in clinical practice. It is also the first study to assess the potential of fully human monoclonal antibodies for the treatment of pneumonia caused by *S. aureus*.

Based on the results presented above, AR-301 appears to be safe: there were relatively few treatment-related AEs, and none was serious. Regarding the AEs related to treatment, none were recurrent and they were different from those reported in previous studies on mAb [19]. No dose-related trend in the incidence of AEs or in other safety data (clinical or laboratory) was observed. Immunogenicity was observed in one case, but did not result in a safety risk and did not seem to affect clinical outcomes negatively.

The observed $t½$ of AR-301 is 22.7–31.0 days. At day 7, the concentration of AR-301 in cohort 4 (20 mg/kg) was close to 200 ng/ml. The long plasma half-life is consistent with the nature of the product, a fully human IgG1 lambda immunoglobulin [19]. Such characteristics allow for a single administration to provide high concentrations of antibodies for > 28 days and, if proven effective, may help treat infections as well as protect against reoccurrences.

Owing to the small sample size and the fact that all the deaths were thought to be attributable to the underlying condition of the study patients, this study does not allow an assessment of the effect of AR-301 on mortality. It should be noted, however, that the mortality observed in this study, overall (12.5%) as well as in the placebo group, was very low compared with the literature and that there was no death in cohort 4, the highest dose tested (20 mg/kg). To assess the effect of AR-301 treatment on mortality, a significantly larger sample size will be required.

There were no statistically significant differences in total ventilation days overall (CABP, HABP and VABP patients) in a pairwise comparison between each treated cohort versus placebo. However, the duration of ventilation was numerically shorter in study patients with VABP who received AR-301 compared with those who received the placebo. A post hoc exploratory statistical analysis comparing the pooled AR-301 treated VABP patients versus the placebo treated patients showed a p value <0.01. However, it should be noted that because the sample size of the VABP groups is small, observation of statistical significance could be attributed to chance, and this will need to be confirmed in a larger trial. Since the incidence of bacterial pneumonia increases with duration of ventilation [24], reducing the mechanical ventilation time is a critical challenge in the ICU and may result in significant benefits.

This study used a rapid diagnostic test in the form of the RT-PCR to enroll patients, which is validated for specificity and sensitivity by the classical culture test [25]. These new tools could represent a revolution, especially in the ICU setting, because they allow rapid, up-to-the-hour information on the etiologic agent infecting the patient and should be considered for future pathogen-specific trials.

Microbiologic eradication on day 28, which comprised patients with eradication or presumed eradication as determined by an adjudication committee, was numerically higher in the treatment groups and the time to *S. aureus* eradication was numerically lower in the AR-301 groups. However, as AR-301 is directed against alpha toxin, it does not trigger opsonization or eradication of the bacteria. If verified, this finding would be consistent with the hypothesis that by neutralizing alpha

toxin, AR-301 helps prevent the ablation of the immune response by *S. aureus* alpha toxin, thus preserving the effectiveness of the immune system sufficiently to reduce the bacteria burden. As noted above, previous studies failed to show the efficacy of anti-infective antibodies versus placebo [13, 16]. However, these studies were using anti-staphylococcal capsular polysaccharide IgG and a humanized mAb that binds to surface-expressed adhesion protein clumping factor A. Accounting for such differences in the characteristics of the investigational product, these earlier failures are not indicative of the potential of AR-301, a mAb with a totally different target and mode of action.

Several factors must be considered when assessing the results of this study. First, this first-in-human safety and pharmacokinetic study was not powered to assess the efficacy of AR-301. Thus, although supportive, the results reported here remain to be confirmed in properly sized pivotal trials. Moreover, there were differences in the profile of the placebo and treated groups at baseline; this may have biased the results in favor of the placebo group, which was overall younger, was less often obese, and had a much lower proportion of patients with VABP. Finally, while the small sample size in the current study precludes reaching firm conclusions on the clinical benefits from drug treatment, the positive trends observed for the ventilation time for VABP suggest that a larger study focusing on a VABP population is warranted.

Conclusion

Adjunctive treatment of severe *S. aureus* HABP in the ICU with anti-staphylococcal mAb appears feasible and safe. Although the sample size is limited and precludes firm conclusions about clinical benefits, trends toward shorter ventilation time for VABP patients and higher and faster microbiologic eradication were observed. This innovative therapy may represent a breakthrough approach to the treatment of critically ill patients diagnosed with pneumonia due to *S. aureus*. Larger studies are warranted to better assess this clinically important medical need and to further support this hypothesis.

Electronic supplementary material

The online version of this article (https://doi.org/10.1007/s00134-018-5229-2) contains supplementary material, which is available to authorized users.

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Compliance with ethical standards

Conflicts of interest

BF was the coordinating PI for the trial and was appointed as a member of both the study advisory board and the steering committee. PAdL was a consultant to Aridis Pharmaceuticals, Inc. PFL was a member of the advisory board and the steering committee. EM, CG, KA, SN, MF, AD, GP, FM: no conflict of interest to declare.

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