RESEARCH PAPER

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Efficacy profile of a bivalent *Staphylococcus aureus* glycoconjugated vaccine in adults on hemodialysis: Phase III randomized study

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Abbreviations: T5/T8, *Staphylococcus aureus* type 5 and 8 capsular polysaccharides; CI, confidence interval; ClfA, *S. aureus* clumping factor A; ELISA, enzyme-linked immunosorbent assay; ESRD, end-stage renal disease; GMC, geometric mean concentration; OPK, opsonophagocytic killing; SAE, Serious adverse event; VE, vaccine efficacy.

In a previous study in end-stage renal disease (ESRD) hemodialysis patients, a single dose of *Staphylococcus aureus* type 5 and 8 capsular polysaccharides (T5/T8) conjugated to nontoxic recombinant *Pseudomonas aeruginosa* exotoxin A investigational vaccine showed no efficacy against *S. aureus* bacteremia 1 year post-vaccination, but a trend for efficacy was observed over the first 40 weeks post-vaccination. Vaccine efficacy (VE) of 2 vaccine doses was therefore evaluated. In a double-blind trial 3359 ESRD patients were randomized (1:1) to receive vaccine or placebo at week 0 and 35. VE in preventing *S. aureus* bacteremia was assessed between 3–35 weeks and 3–60 weeks post-dose-1. Anti-T5 and anti-T8 antibodies were measured. Serious adverse events (SAEs) were recorded for 42 days post-vaccination and deaths until study end. No significant difference in the incidence of *S. aureus* bacteremia was observed between vaccine and placebo groups between weeks 3–35 weeks post-dose 1 (VE -23%, 95%CI: -98;23, $p = 0.39$) or at 3–60 weeks post-dose-1 (VE -8%, 95%CI: -57;26, $p = 0.70$). Day 42 geometric mean antibody concentrations were 272.4 μg/ml and 242.0 μg/ml (T5 and T8, respectively) in vaccinees. SAEs were reported by 24%/25.3% of vaccinees/placebo recipients. These data do not show a protective effect of either 1 or 2 vaccine doses against *S. aureus* bacteremia in ESRD patients. The vaccine induced a robust immune response and had an acceptable safety profile. Further investigation suggested possible suboptimal vaccine quality (manufacturing) and a need to expand the antigen composition of the vaccine. This study is registered at www.clinicaltrials.gov NCT00071214.

Introduction

Staphylococcus aureus, a human commensal frequently carried in the nose and on the skin, is the most common nosocomial pathogen, responsible for approximately 16% of healthcare-associated infections.[1] Patients with end-stage renal disease (ESRD) who are receiving hemodialysis are at high long-term risk of *S. aureus* infection as a result of being immune compromised and the need for regular vascular access.[2,3] The incidence of culture-confirmed *S. aureus* bacteremia in more than 293000 patients receiving chronic hemodialysis in the United States was 4.0 per 100 outpatient-years.[4] In the United States, between one-third and one-half of *S. aureus* bacteremias in hemodialysis patients are due to multi-resistant *S. aureus* strains.[1,4-6] Healthcare costs associated with *S. aureus* bacteremia in hemodialysis patients are substantial.[5] Complications include endocarditis, osteomyelitis, discitis and soft tissue abscesses, and the case fatality rate is as high as 20%.[5,7] In hemodialysis patients the type of vascular access may predispose toward bacteremia, with higher rates of *S. aureus* bacteremia observed in patients with venous catheters *in situ* than in patients with arteriovenous fistulas.[8,9]

S. aureus produces a range of virulence factors and toxins that contribute to its invasive capacity and ability to evade host defense systems.[10] Several virulence factors have been investigated as possible candidates for active or passive vaccination against *S. aureus.* However as yet, none have been licensed for use. Several immunotherapy candidates failed to show efficacy in humans: *Altastaph* (Nabi Biopharmaceuticals) containing *S.aureus* capsular

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polysaccharides type 5 (T5) and type 8 (T8) antibodies purified from subjects vaccinated with *StaphVAX*™ (investigational vaccine originally developed by Nabi Biopharmaceuticals, Rockville, MD, USA); *Veronate* (Inhibitex), polyclonal antibodies targeting *S. aureus* clumping factor A (ClfA) and *S. epidermidis* adhesion SdrG; *Aurexis* (Tefibazumab, Inhibitex), monoclonal antibodies targeting ClfA; *Aurograb* (NeuTec Pharma), single chain antibodies against an ATP-binding cassette transporter; and Pagibaximab (Biosynexus), a monoclonal anti-lipoteichoic acid antibody.[11,12]

More recently, the V710 IsdB vaccine (Merck &Co) which contains an iron scavenging protein, failed to demonstrate efficacy against staphylococcal bacteremia and/or deep sternal wound infections in cardiothoracic surgery patients.[13] The vaccine was associated with increased mortality among patients who developed *S. aureus* infections.[13]

The *S. aureus* capsular polysaccharides prevent opsonophagocytotic killing by neutrophils, resulting in bacterial clearance by the host.[14] *S. aureus* capsular T5 and T8 account for over 85% of clinical isolates.[15-17] *StaphVAX*™ is an investigational bivalent *S. aureus* T5 and T8 capsular polysaccharide conjugate vaccine. In a phase III study in ESRD patients on hemodialysis (study 1356, www.clinicaltrials.gov NCT00071214), a single dose of *StaphVAX*™ showed partial efficacy (57%, 95% confidence interval [CI] 10; 81) against *S. aureus* bacteremia over the first 40 weeks of follow-up, although no efficacy was shown 1 year post-vaccination.[18] Rapid antibody decline during the study suggested that potential benefits in prolonging protection could be gained by administration of a second dose. We therefore evaluated the immunogenicity, safety and efficacy of *StaphVAX*™ in preventing *S. aureus* bacteremia in ESRD patients for up to 35 weeks after a single dose, and for up to 60 weeks after 1 or 2 doses (study 1371, www.clinicaltrials.gov NCT00071214). Evaluation of health economic outcomes of patients with *S. aureus* bacteremia enrolled in this study has been reported elsewhere.[5]

Results

There were 3359 patients who were randomized (1:1) to receive 2 doses of the investigational vaccine or placebo (phosphate-buffered saline) at weeks 0 and 35. Of these, 3358 were included in the modified-intention-to-treat-for-efficacy cohort (**Fig. 1**). One quarter of subjects (25.8% in the placebo group and 24.1% in the vaccine group) withdrew from the study, mostly due to death unrelated to vaccination (**Fig. 1**). The two groups were balanced with regard to age, sex and race (**Table 1**).

The primary efficacy study endpoint was the incidence of culture-proven first-time *S. aureus* bacteremia that occurred during the 3-35 week period following dose 1. Strains that were typed but were not T5 or T8 were excluded from the primary analyses. Strains not received by Nabi Pharmaceuticals for typing were classified as "not typed."

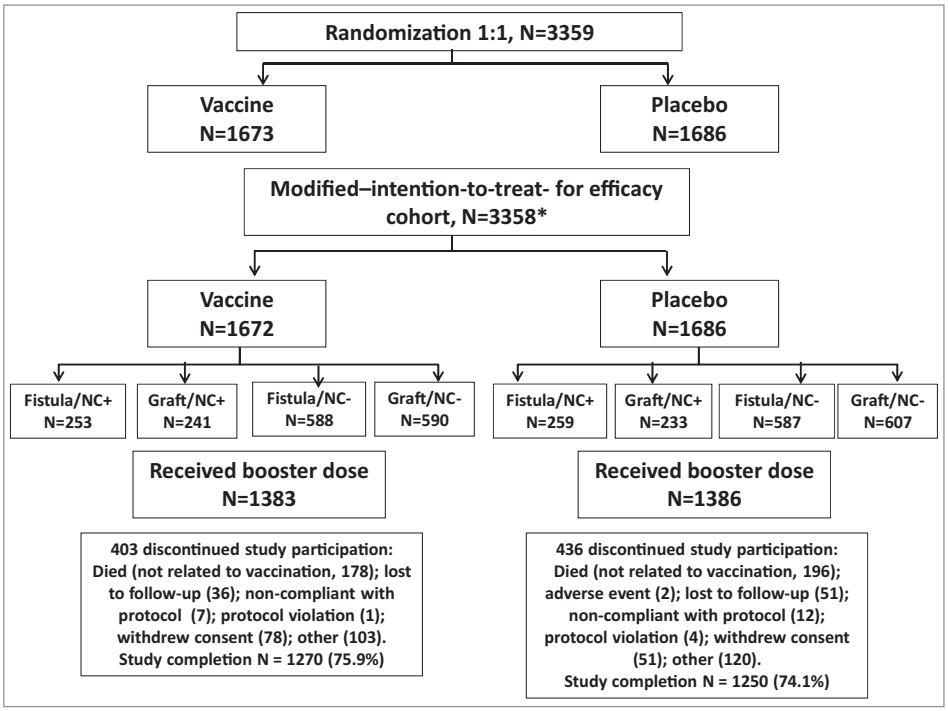

Figure 1. Study flow Randomization was stratified by (**1**) the use of a native-vessel fistula or synthetic/heterologous graft for vascular access, and (**2**) the presence (NC+) or absence (NC-) of *S. aureus* nasal carriage. N = total number of patients within treatment group *One patient was not included in the modified intent-to-treat-or-efficacy cohort due to known serious *S. aureus* infection within 3 months of injection. Vaccine = *S. aureus* polysaccharide conjugate vaccine Placebo = phosphate-buffered saline.

Efficacy

There was no protective effect of the vaccine on the incidence of first time *S. aureus* bacteremia between weeks 3-35 (primary endpoint), or between weeks 3-60 either overall, or considering intravenous access type (native-vessel fistula or synthetic/heterologous graft, but not catheter) or *S. aureus* nasopharyngeal carriage status (**Table 2**, **Fig. 2**). Between weeks 3–35, 12 (32%) T5, 12 (32%) T8, and 14 (37%) "not typed" *S. aureus* bacteremia cases were reported in the vaccine group. In the same period, 13 (42%) T5, 5 (16%) T8 and 13 (42%) "not typed" *S. aureus* bacteremia cases were reported in the placebo group. Between weeks 3-60, 19 (34%) T5, 17 (30%) T8 and 20 (36%) "not typed" *S. aureus* bacteremia cases were reported in the vaccine group, versus 21 (40%) T5, 12 (23%) T8, and 19 (37%) "not typed" *S. aureus* bacteremia cases reported in the placebo group. There was no significant difference between the vaccine and placebo groups in the distribution of time-to-

Table 1. Summary of demographic characteristics (all randomized subjects)

Characteristics	Parameter or category	Vaccine N=1673	Placebo N=1686
Age	Mean (SD)	58 (14)	58 (14)
	Range	18-99	18-96
Gender	Female, n (%)	679 (40.6)	677 (40.2)
	Male, n (%)	994 (59.4)	1009 (59.8)
Race	African-American, n (%)	801 (47.9)	740 (43.9)
	Caucasian, n (%)	553 (33.1)	595 (35.3)
	Hispanic, n (%)	249 (14.9)	265 (15.7)
	Asian, n (%)	62 (3.7)	69 (4.1)
	*Other, n (%)	8 (0.5)	17 (1.0)

*Other = American Indian /Alaskan Native, Black/Hispanic, Asian/Black, and a patient from Cape Verde
N = total number of patients within treatment group
SD = standard deviation
n (%) = number (percentage) of patients within the category

S. aureus bacteremia (2-sided stratified logrank test, p=0.39 for week 3–35, and p=0.7 for week 3–60 follow-up periods; **Fig. 2**).

None of the evaluated risk factors (age, gender, presence of diabetes or prior hemodialysis infection experience) influenced the effect (or lack thereof) of the study vaccine ($p > 0.05$ for all risk factors).

Among the *S. aureus* bacteremias reported but not included in the primary efficacy analyses, 4 were episodes reported before week 3 (all in the vaccine group and all T5), and 18 were strain 336 episodes reported between weeks 3-60 (10 in the vaccine group, 8 in the placebo group).

Immunogenicity

Anti-*S. aureus* antibodies to capsular polysaccharides T5 ("anti-T5") and T8 ("anti-T8") geometric mean antibody concentrations (GMCs) peaked at day 42 following the first vaccine dose (**Fig. 3**). At day 42, the anti-T5 GMC was 272.4 μg/ml in the vaccine group and 8.9 μg/ml in the placebo group (**Fig. 3A**), and the anti-T8 GMC was 242.0 μg/ml in the vaccine group and 13.8 μg/ml in the placebo group (**Fig. 3B**). Antibody GMCs increased after dose 2 (GMC at day 266 in the vaccine group 149.1 μg/ml for anti-T5 and 130.1 μg/ml for anti-T8) but were lower than after dose 1. Thus, the observed booster effect was minimal.

Assessment of vaccine efficacy failure

Capsular expression of infection isolates collected during the study was assessed. The vast majority were either T5 or T8, or non-capsulated strains. Some of the T5 or T8 isolates reacted with both capsular-specific and 336 antibodies, indicating that these isolates were partially encapsulated.[19]

The antibody concentration against the capsular serotype of the infecting *S. aureus* strain measured at the closest time point prior to bacteremia was displayed for all vaccine failures (**Fig. 3**). No trend for lower responses in cases of vaccine failure was observed.

To assess the reasons for failure to demonstrate efficacy, the immune responses induced by vaccination were further characterized and compared with those induced by the vaccine lot used in the previous phase III study in patients with ESRD (study 1356).[18] Comparison of the individual sera from patients enrolled in the previous study 1356 with those from patients enrolled in the present study (1371) showed no significant difference in terms of antibodies when measured by enzyme-linked immunosorbent assay (ELISA) and opsonophagocytic killing assay (OPK) (**Fig. 4**). Affinity of antibodies to T8 but not T5 was lower in the present study compared to study 1356 (T8 mean 1.199M vs. 1.477M, p=0.02; T5 mean 1.873M versus 1.913M, p=0.72, unpaired t-test, **Fig. 5**). However, T8 antibody affinity was higher after vaccination in study 1371 compared to the pooled group of pre-vaccination sera and sera from

Table 2. Person-time rates of *S. aureus* bacteremia and vaccine efficacy by dialysis access mode and nasal carriage between weeks 3-60 (modified intention-to-treat-for-efficacy cohort).

Observation period	Strata	Vaccine (N=1672)	Placebo (N=1686)	Vaccine efficacy
(weeks)		n/(p-t)=r	n/(p-t)=r	(95% CI)
3–35	Fistula, Nasal carriage positive	7/147.8=0.0473	8/152.1=0.0526	9.96
	Graft, Nasal carriage positive	19/135.6=0.1401	15/133.7=0.1122	−24.89
	Fistula, Nasal carriage negative	3/350.3=0.0086	1/353.1=0.0028	−202.37
	Graft, Nasal carriage negative	9/350.7=0.0257	7/358.0=0.0196	−31.23
	Stratified efficacy	38/984.5=0.0386	31/996.9=0.0311	−23% (−98, 23)
p-value				0.39
3–60	Fistula, Nasal carriage positive	8/240.0=0.0333	13/240.0=0.0542	38.46
	Graft, Nasal carriage positive	22/212.1=0.1037	21/208.2=0.1009	−2.83
	Fistula, Nasal carriage negative	8/566.8=0.0141	5/565.2=0.0088	−59.54
	Graft, Nasal carriage negative	18/563.0=0.0320	13/575.9=0.0226	−41.64
	Stratified efficacy	56/1581.9=0.035	52/1589.3=0.032	−8% (−57, 26)
p-value				0.70

N = total number of patients within treatment group
n = number of *S. aureus* bacteremia episodes
p-t = accumulated person-time
r = person-time rate of *S. aureus* bacteremia episodes
95% CI = 95% confidence interval

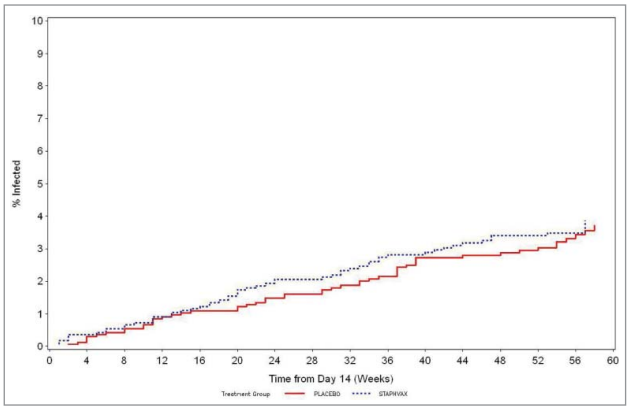

Figure 2. Kaplan-Meier estimate of time-to-*S. aureus* bacteremia in the vaccine and placebo groups (weeks 3–60, Modified–intention-to-treat-for-efficacy cohort).

unvaccinated healthy subjects (1.199M vs. 0.822M, $P < 0.0043$, unpaired t-test, **Fig. 5**).

In the mouse lethality model, animals injected with antibodies collected from patients in the present study (1371) exhibited sub-optimal protection (40% survival) against a T8 challenge as compared to antibodies derived from patients in study 1356 (91% survival) (**Table 3**). Protection against T5 lethal challenge was similar between lots.

Similarly, animals vaccinated with the same lots as those used in the present study (1371) and the previous study (study 1356) showed that the 1371 lot afforded sub-optimal protection (37% survival) against T8 challenge as compared to the lot used in study 1356 (83% survival) (**Table 4**). As for passive immunization experiments, protection against T5 lethal challenge following active immunization was similar between lots.

Taken together, we hypothesized that the vaccine used in the present study may have been suboptimal, generating antibodies of lower quality and functionality than the vaccine used in the previous 1356 study, at least for T8. This hypothesis was substantiated by testing the re-formulated 1371-alum-adjuvanted vaccine against the same vaccine in PBS in the animal challenge model. There were 73% (11/15) of animals that received the alum-formulated vaccine who survived the T8 challenge compared to only 7% (1/15) in the group that received the vaccine in PBS, despite the induction of similar levels of anti-T8-specific IgG induced by both vaccines (**Fig. 6A**). Furthermore, the formulation in alum restored antibody affinity generated by the 1371-vaccine (**Fig. 6B**).

Safety

SAEs were reported by 829 of patients (24.7%) of whom 402 (24%) were in the vaccine group and 427 (25.3%) were in the placebo group. The SAEs reported encompassed 19 MedDRA System Organ Classes. Across both treatment groups, SAEs occurred most frequently under "Cardiac Disorders" (7.8% of vaccines and 8.7% of placebo recipients). The second most common was "Infections and Infestations" (6.0% and 5.7%, respectively). The four most frequent Preferred Terms were "cardiac arrest" (2.0% of vaccinees and 2.4% of placebo recipients),

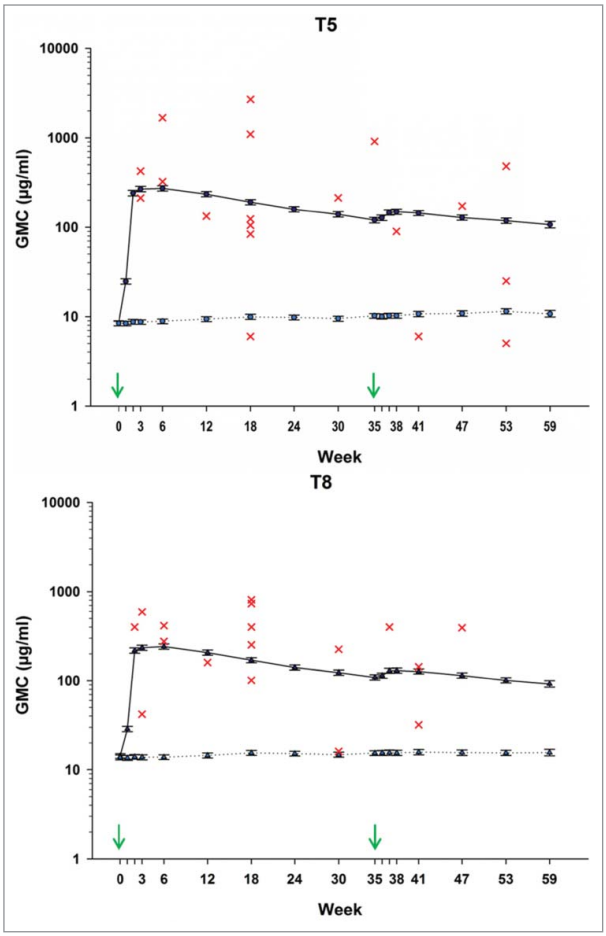

Figure 3. Anti-T5 (**A**) and anti-T8 (**B**) capsular polysaccharide geometric mean antibody concentrations (GMC) in vaccine and placebo groups (Modified–intention-to-treat-for-efficacy cohort). Individual antibody concentrations in patients reporting *S. aureus* T5 (**A**) or T8 (**B**) bacteremia are shown for the vaccine group. Red crosses represent individual anti-T5 or anti-T8 antibody concentrations of patients in the vaccine group reporting *S. aureus* Type 5 or Type 8, bacteremia, respectively at the closest time point prior to the onset of the disease. Black = GMC in the vaccine group, blue = GMC in the placebo group. Error bars: 95% confidence interval. Arrows indicate vaccination time points.

"congestive cardiac failure" (1.3% and 1.2%, respectively), "pneumonia" (1.1% and 0.9%, respectively) and acute myocardial infarction (1.0% in each group). Two SAEs in each treatment group were considered to be possibly related to study vaccine/placebo: one case of pyrexia in each treatment group, limb abscess in one vaccine recipient, and angioneurotic edema in one placebo recipient. There was one case of multi-organ failure (0.1%) in the placebo group and 4 cases (0.2%) in the vaccine group ($p > 0.05$). No cases of multi-organ failure occurred in patients with *S. aureus* bacteremia.

There were 374 deaths reported during the entire study, 178 (10.6%) in the vaccine group and 196 (11.6%) in the placebo group (**Table 5**). No deaths were considered related to the study vaccine/placebo. Among patients that reported at least one *S. aureus* bacteremia, 15/52 (29%) died in the placebo group and 14/59 (24%) died in the vaccine group.

Discussion

Despite robust antibody responses to primary vaccination, this study failed to demonstrate VE of the bivalent *S. aureus* T5 and T8 conjugate vaccine in preventing *S. aureus* bacteremia in ESRD patients receiving hemodialysis at any time interval studied, or in any strata considered. These results contrast with a previous clinical trial (1356) conducted in a similar population, which suggested short term (to 40 weeks) efficacy against *S. aureus* bacteremia after a single dose of *StaphVAX*™.[18] Of note, the positive results for short-term efficacy were not the primary objective of the 1356 trial and were observed after post-hoc analyses.

One possible contributing factor to the failure of the vaccine is the immunological impairment associated with ESRD and hemodialysis that may result from hyper-uremia syndrome, underlying disease such as diabetes, and complement depletion due to the dialysis process. Of importance, neutrophils of ESRD patients have reduced phagocytotic and killing ability. While vaccination successfully induced functional antibodies, patients with ESRD typically show deficiencies in antigen-presenting cells and T-lymphocytes,[3,20-22] which may also have been important for protection. Comparison of the population enrolled in this study to that of the population enrolled in study 1356 failed to identify differences in the health status, frequency of underlying disease, dialysis procedures, or routine treatment provided for these ESRD patients. Although *S. aureus* infections appeared to occur more frequently in patients with nasal *S. aureus* colonization, VE did not differ between colonized or non-colonized subjects. We did not assess the effect of vaccination on nasophayngeal carriage, but another study showed similar immune responses to *StaphVAX*™ vaccination in subjects with and without nasal colonization with *S. aureus*, and no impact of vaccination on nasal colonization.[23]

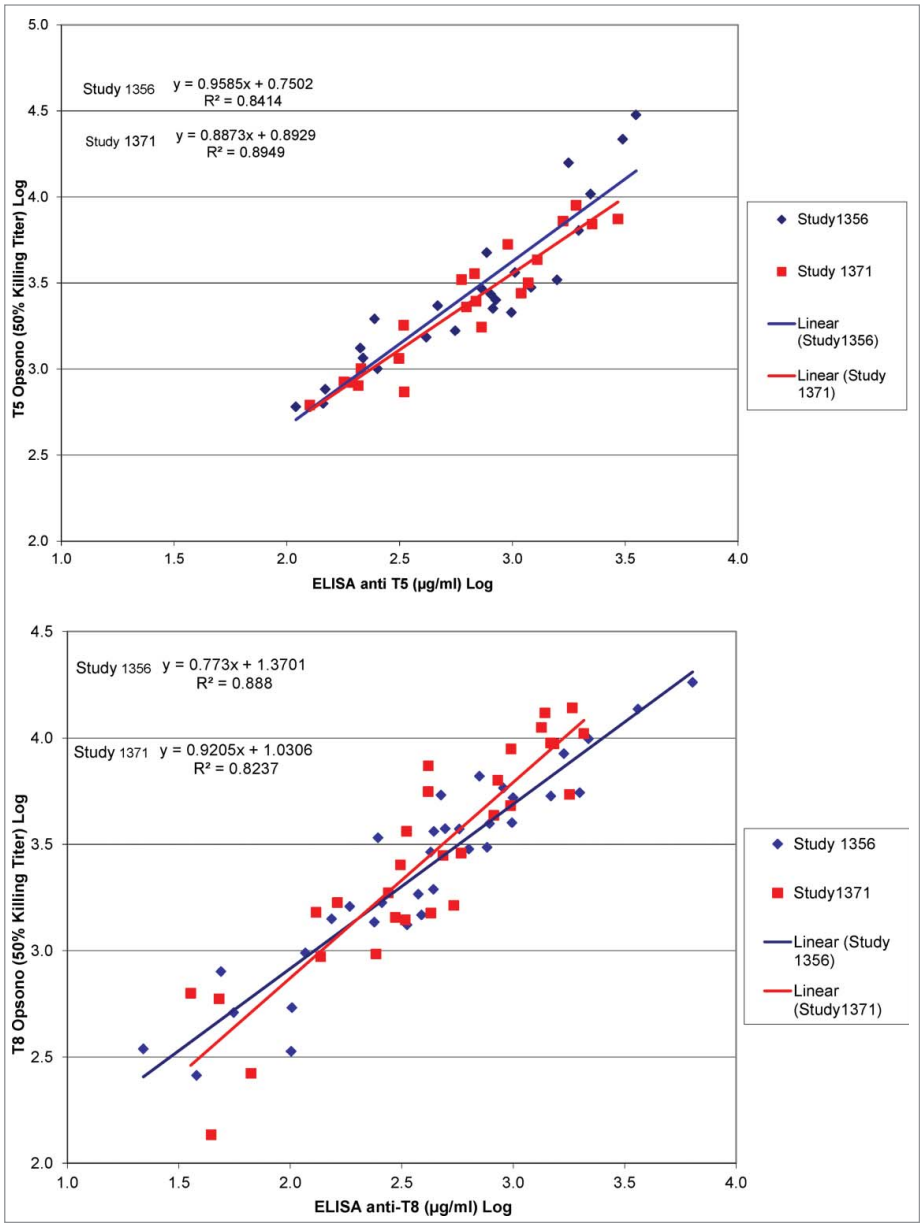

Figure 4. Opsonophagocytosis of *S. aureus* type 5 and type 8 mediated by antibodies from the present study and study 1356[18] (day 42 samples).

Polysaccharide capsule expression may vary according to *S aureus* strain, and strains not expressing a capsule may express a cell-wall polysaccharide 336.[19] About one third of bacteremia cases included in the primary analysis relate to strains that were unavailable for typing, raising questions on the capsulation of these strains. However as expected,[15] approximately 80% of all typed strains in our study were T5 or T8. We therefore extrapolated that the same proportion of T5 or T8 strains would apply to the bacteremia cases classified as "not typed." If we except US300 epidemic strain, the majority of human infection *S. aureus* strains express capsule in humans,[19] and we consider it unlikely that the lack of efficacy in our study can be attributed solely to uncapsulated *S. aureus* strains.

Studies of isogenic T5 and T8 *S. aureus* strains indicate that T5 strains are more virulent than T8 strains, inducing less opsonophagocytic killing and longer survival in infected mice.[24] The efficacy against *S. aureus* in study 1356 was mostly directed toward T8 strains[17] and we observed no VE against T5 in the present study. These data may indicate that patients with ESRD

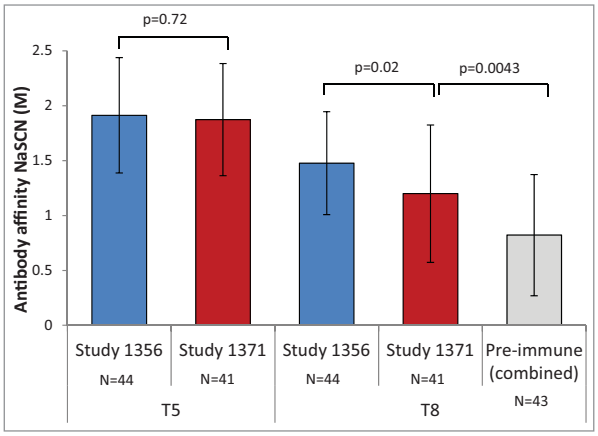

Figure 5. Geometric mean affinity of anti-T5 and anti-T8 antibodies from the present study (1371), study 1356[18] (day 42 samples), and compared to pooled pre-immune sera in ESRD patients and unvaccinated healthy individuals. Footnote to figure: NaSCN (M) = Sodium Thiocyanate concentration (Molar). Vertical lines represent standard deviation. N = number of samples.

are less able to clear more virulent (T5) infections than less virulent strains. We are unable to test this hypothesis in the framework of the present study as we did not assess the virulence of the T5 versus the T8 strains isolated from patients.

We undertook additional investigations to explore possible reasons for the lack of efficacy in the present study. ELISA antibody responses induced by vaccination were robust and of a similar magnitude to those observed in study 1356. In the majority of vaccinated patients who experienced T5 or T8 *S. aureus* bacteremia, the antibody concentration measured at the time point just prior to the onset of the disease was substantially higher than in the placebo group. After the second dose given at week 40, the booster effect was only minimal, possibly due to the short period between dose 1 and dose 2 and high levels of antibodies persisting at the time of the second dose.

Comparison of the individual sera from patients enrolled in each study showed no significant difference in terms of OPK. However, affinity of antibodies to T8 (but not T5) tended to be lower than those generated by the lot used in the previous study, and passive or active immunization of mice with the present vaccine lot resulted in suboptimal protection against a lethal T8 challenge. When the vaccine used in this study was adjuvanted with alum, it generated significantly higher affinity antibodies in

Table 3. Passive protection in mice afforded by antibodies from patients given bivalent *S. aureus* conjugate vaccine against lethal challenge with *S. aureus* T5 or T8

S. aureus	Clinical trial	Survivors	p-value (vs PBS placebo)
T8 challenge	1356	32/35 (91%)	<0.0001
	1371	18/45 (40%)	0.024
	AltaStaph IVIG	54/55 (98%)	<0.0001
	MEP IVIG	10/55 (18%)	NA
	PBS	0/35*	NA
T5 challenge	1356	15/15 (100%)	<0.0001
	1371	12/15 (80%)	<0.0001
	AltaStaph IVIG	34/35 (97%)	<0.000
	MEP IVIG	11/35 (31%)	NA
	PBS	1/35	NA

AltaStaph IVIG: a hyperimmune IgG prepared from plasma donors who received Nabi StaphVAX lots as positive control
MEP IVIG: IgG prepared from plasma donors immunized with the *Pseudomonas aeruginosa* mucoexopolysaccharide (MEP) as negative control
*Survival of PBS-vaccinated mice in the active immunization study (see **Table 4**)
p-values based on survival from combined experiments for each vaccine lot in mice compared to PBS-vaccinated animals

mice and restored protection of vaccinated mice against T8 challenge. Altogether, the data suggest that the quality of the vaccine lot (for T8) used in the present study may have been suboptimal. Nevertheless, the lack of efficacy against both T5 and T8 strains argues against this being the only factor responsible for the failure of the vaccine.

Another explanation could be that anti-capsular antibodies do not protect against *S. aureus* infections. In view of the results in animals and in the previous clinical trial, we consider this unlikely, especially for T8. However, although opsonophagocytosis is important in preventing gram positive infections, anti-capsular polysaccharide opsonic antibodies alone may not be sufficient for protection against invasive *S. aureus* isolates.[24] Failure to protect study subjects could not be correlated to a lack of vaccine-induced antibodies in either study 1356,[18] or the present study. It was recently suggested that although opsonization of *S aureus* enhanced phagocytosis by neutrophils in suspension, there was a weak correlation between uptake of *S aureus* and subsequent killing of the bacteria by neutrophils.[25] In view of the importance of toxins in *S. aureus* infections, toxin neutralizing antibodies may be needed to protect against infection.[26] Additionally, the role of T-cell mediated immunity may be important

Table 4. Protection in mice afforded by vaccination by vaccine lots used in studies 1356 and 1371 against lethal challenge with *S. aureus* T5 or T8 isolates

S. aureus isolate	Clinical trial lot	Survivors	p-value vs placebo	p-value 1371 lot vs 1356 lot
T8 (K17654)	1371	13/35 (37%)	<0.0001	p = <0.0001
	1356	29/35 (83%)	<0.0001	
	PBS placebo	0/35 (0%)	NA	
T5 (ST021)	1371	35/40 (88%)	<0.0001	p=1.00
	1356	36/40 (90%)	<0.0001	
	PBS Placebo	0/40 (0%)	NA	

p-values based on survival from combined experiments for each vaccine lot in mice compared to PBS immunized animals.
NA = not applicable

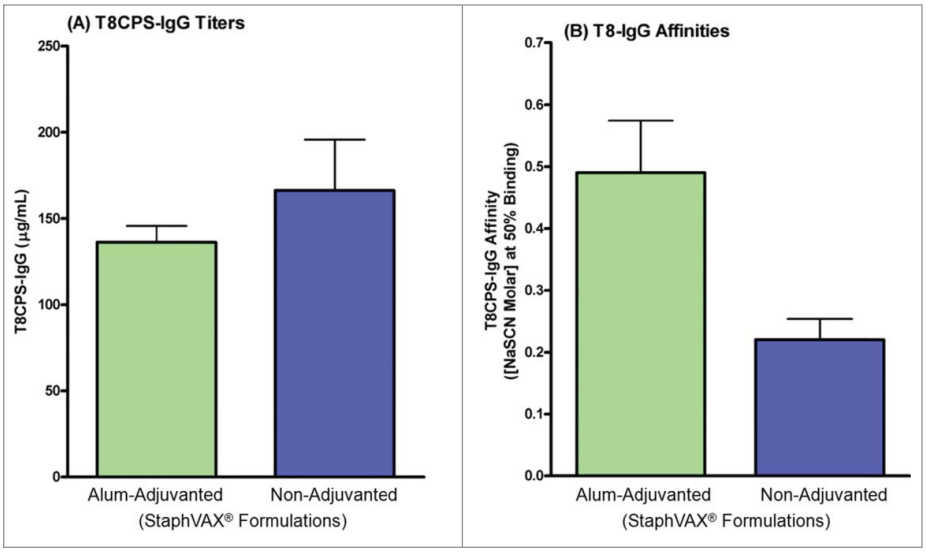

Figure 6. Comparison of mean (+ standard error) T8 antibody titers and affinity in mice vaccinated with study 1371 vaccine either adjuvanted to alum or administered with PBS (Number of serum samples evaluated per group = 15).

in protection against *S.aureus* bacteremia.[27-30] The ability of *S. aureus* to evade immune-protective mechanisms and induce immune-tolerance needs also to be considered when designing new vaccines.[31,32]

Consistent with the previous study (1356), the vaccine was well-tolerated, with no significant differences between vaccine or placebo groups in terms of nature or incidence of SAEs or deaths.

In conclusion, the bivalent conjugate vaccine did not protect against T5 or T8 bacteremia even though vaccination induced a robust antibody response to both T5 and T8 *S. aureus* capsular polysaccharides. No differences in the study populations were identified. Factors that may have contributed to the results include aspects of vaccine quality (for the T8 component) and the target of only one virulence factor (the polysaccharide capsule) by the vaccine. Given the multi-factorial nature of the pathogenesis of staphylococcal infections, a successful staphylococcal vaccine should include in addition to the polysaccharide conjugates generating opsonic antibodies, a component that would generate toxin neutralizing antibodies. Alpha toxin, the most prominent and widespread toxin, was selected as a first choice. Assessment of a bivalent α toxin and PVL (panton valentine leukocidin) vaccine induced antibody responses with neutralizing activity in healthy adults.[33,34] Finally, T-cell-mediated immunity may also play a role in protection against disease.

Methods

Study objectives

The primary study objective was to demonstrate vaccine efficacy (VE) in reducing the incidence of *S. aureus* bacteremia for up to 8 months (i.e., from weeks 3-35 post-vaccination) following a first dose of vaccine in patients with ESRD receiving hemodialysis. Secondary objectives included assessment of cumulative efficacy after 1 or 2 vaccine doses from week 3–60 post-dose 1 (the second dose was administered at week 35); assessment of efficacy in sub-strata defined by fistula/graft status, nasopharyngeal carriage of *S. aureus,* and immunogenicity after one and 2 vaccine doses. Assessment of vaccine safety was also a secondary objective. In view of the recent termination of the Merck & Co study of V710,[13] a post-hoc analysis of safety in terms of episodes of multi-organ failure and death was performed.

Study subjects and design

The phase III study was prospective, randomized, placebo-controlled and double-blind, conducted at 163 dialysis centers in the United States between 29 September 2003 and 23 September 2005. The study was conducted according to the Declaration of Helsinki (1996). The protocol and associated documents were

Table 5. Summary table of deaths

	Vaccine (N=1673) n (%)	Placebo (N=1686) n (%)
All deaths*	178 (10.6)	196 (11.6)
Body System:		
Cardiac disorders	80 (4.8)	91 (5.4)
Infections and infestations	26 (1.6)	30 (1.8)
Nervous system disorders	18 (1.1)	11 (0.7)
General disorders and administration site conditions	16 (1.0)	11 (0.7)
Renal and urinary disorders	6 (0.4)	16 (1.0)
Respiratory, thoracic and mediastinal disorders	6 (0.4)	14 (0.8)
Vascular disorders	5 (0.3)	9 (0.5)
Neoplasms (benign and malignant)	7 (0.4)	2 (0.1)

N = total number of patients within treatment group
n (%) = number (percentage) of deaths within the category
*4 subjects in the vaccine group (0.2%) and 1 subject in the placebo group (0.1%) died due to multi-organ failure

reviewed and approved by the Schulman Associates Institutional Review Board, Cincinnati, Ohio, as well as local institutional review boards when required. All patients gave written informed consent prior to enrolment.

Patients were to be at least 18 years of age with a diagnosis of chronic ESRD receiving maintenance hemodialysis for at least 8 weeks prior to enrollment. Hemodialysis access using native vessel fistula or synthetic/heterologous graft was allowed. Women of childbearing potential were to have a negative serum pregnancy test within 7 days prior to vaccination.

Patients were excluded from participation if they had known serious *S. aureus* infection within 3 months prior to study entry, or known recurrent *S. aureus* infection of the current graft. Patients were also excluded if they had active viral or bacterial infection or symptoms/signs consistent with infection within 2 weeks prior to vaccination. Subjects known to be positive for human immunodeficiency virus, subjects who had known hypersensitivity or previous anaphylaxis to polysaccharides or polysaccharide-conjugate vaccines, or to components of such vaccines, and subjects with known/suspected drug abuse in the past year were also excluded. Current use of immunosuppressive or immunomodulatory drugs (10 mg of prednisone or equivalent per day was allowed), known malignancy or treatment for malignancy within the past 6 months, and previous receipt of the study vaccine were exclusion criteria.

Randomization was stratified by the type of venous access (native-vessel fistula or synthetic/heterologous graft, but not catheter), and by the presence or absence of *S. aureus* nasal carriage, defined by the recovery of *S. aureus* in at least one of 2 consecutive nasal swab cultures obtained at least 4 days apart.

Vaccine

The investigational bivalent *S. aureus* T5 and T8 capsular polysaccharide conjugate vaccine contained 100μg of each capsular polysaccharide conjugated to a total of 200μg recombinant non-toxic variant of *Pseudomonas aeruginosa* exotoxin A (rEPA). Vaccine and placebo were administered intramuscularly into the deltoid.

Assessment of efficacy

All episodes of bacteremia were recorded. An episode of bacteremia was considered new when at least one blood culture was positive for a bacterium and if the species had not been isolated from blood within the previous 10 days; if the isolate was obtained after antibiotic therapy had been terminated; or if a previously recovered species was detected after at least 10 days of systemic antibiotic therapy (to which the original isolate was sensitive), and for which at least 2 blood cultures obtained at least 24 hours apart had been negative for that same organism.

S. aureus isolates were to be sent to Nabi Biopharmaceuticals, Rockville, USA for serotyping.

Assessment of immunogenicity

Blood samples were tested for *S. aureus* serology on the day of each vaccination (day 0 and 245) and 7, 14, 21, 42, 84 126, 168 days after each dose. An additional blood sample was taken on day 210 after dose 1. Anti- T5 and anti-T8 were measured by ELISA (lower limit of detection is 0.1 μg/ml) .[35,36]

Post-hoc assessments of immunogenicity

Additional assessments of immunogenicity were done after unblinding to investigate possible reasons for failure to demonstrate efficacy.

OPK antibodies from serum samples from study subjects who had participated in the present study (1371) and the previous study (1356) reported by Shinefield et al,[18] were determined using an HL60 cell line and guinea pig complement as previously described.[35,37] The OPK titer was determined as the reciprocal of the highest dilution yielding 50% killing of bacteria. OPK assay was done on a subset of equally representative day 42 samples from the whole population that were randomly chosen to include high, medium and low responders.

Antibody affinity was measured by an ELISA method in which sodium thiocyanate (NaSCN) was used to dissociate immune complexes, assuming that the higher the affinity, the more NaSCN is needed to dissociate the antibody-antigen complex.[38] Affinity assays were done on subsets of Day 42 samples from subjects in both studies (1356 and 1371), selected to equally represent the 4 quartiles of IgG levels. Pre-vaccination sera from study 1371 and sera from unvaccinated healthy adults were also assessed for T8 antibody affinity at baseline.

A murine lethal challenge model assessed passive and active protection against lethal *S. aureus* infection. Passive protection was assessed from purified and quantified IgG obtained from serum samples from study subjects who had participated in the present study (1371) and the previous study reported by Shinefield et al,[18] (study 1356). Balb/c mice received 400μg of capsular polysaccharide specific IgG 48 hours prior to challenge. Controls received *AltaStaph*™ (hyperimmune IgG prepared from plasma donors who had received the vaccine) and MEP-IVIG (IgG prepared from plasma donors immunized with *P. aeruginosa* mucoexopolysaccharide). A separate control group consisted of mice who received PBS. Protective efficacy of active vaccination was assessed from lots used in study 1356 and 1371 (or placebo). Mice received 3 vaccine doses (subcutaneously) at 2 week intervals. Seven days after the last vaccination mice received a standardized aliquot (1-2.5x10^5 CFU/ml administered intraperitoneally), containing *S. aureus* T5, (ST021) or T8 (K17654) (strains isolated from clinical trial participants). Post-challenge morbidity and survival was recorded until study termination on day 5-7 post-challenge.

Finally, to assess the possible role of poor antibody affinity on lack of protection, we re-formulated the 1371 vaccine in alum and compared it to the non-adjuvanted vaccine in PBS in the mouse lethal challenge model. Affinity of antibodies generated by the 2 formulations was evaluated.

Nasopharyngeal carriage

Nasal swabs (CultureSwab, Becton Dickinson) were collected on 2 occasions during screening visits prior to vaccination for culture and identification of *S. aureus.* Nasal swabs were advanced

upward and backward toward the vertex of the head until meeting gentle resistance. The swab was rotated 360°, removed and placed back into the sleeve containing culture medium.

Assessment of safety

Serious adverse events (SAEs) were captured for 42 days following both injections by history and physical examination. An assessment of severity and potential relationship to the study vaccine was made by the site Investigator. Deaths were captured throughout the entire study duration.

Statistical analysis and sample size

The primary cohort for the efficacy and immunogenicity analyses was the modified intent-to-treat-for-efficacy cohort, which included all injected subjects except those with known serious *S. aureus* infection within 3 months prior to injection, or known recurrent *S. aureus* infection of their current graft. VE was assessed for 3–35 weeks post-dose 1. The cumulative efficacy of 2 doses was assessed for the 3 to 60 week period post-dose 1.

The incidence rate of first-time *S. aureus* bacteremia (type 5, type 8 or not typed) was compared between the vaccine and placebo groups by an exact, 2-sided stratified person-time incidence calculation.[35,36] Four strata were defined by baseline nasal carriage status (positive/negative) and vascular access modality (graft or fistula). Person-time infection rate was equal to the number of infections divided by the accumulated person-time.

VE (with 95% CI) was computed as 1-ψ, where ψ is the common ratio of *S. aureus* bacteremia incidence in vaccine group relative to control group. The time-to-first *S. aureus* bacteremia distributions in the vaccine and placebo groups were displayed by Kaplan-Meier curves and compared by a 2-sided stratified log-rank test.

A stratified Cox model for time-to-first *S. aureus* bacteremia was performed as secondary analysis. This model included treatment, age, gender, diabetes and prior hemodialysis infection experience as covariates, with strata used as a stratification variable.

Anti-T5 and anti-T8 GMCs at each pre-defined time point were calculated in all patients.

Safety analyses were performed on all randomized patients who had received at least 1 dose of vaccine or placebo. A post-hoc exploratory analysis was done to compare the rate of multi-organ failures between groups by Fisher's exact test.

For all tests, differences were considered statistically significant if p-values were <0.05.

Based on data from the previous efficacy study,[18] 3600 patients (3240 evaluable after allowing a 10% dropout rate) were considered sufficient to yield the necessary number of events to detect a difference in person-time incidence rates of *S. aureus* bacteremia between the 2 study groups with 80% power and an efficacy of at least 50% (2-sided α of 5%).

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Disclosure of Potential Conflicts of Interest

AF and KT were full-time employees of Nabi Biopharmaceuticals at time of study conduct. AF is currently employed by NanoBio Corporation. KT is currently employed by National Institute of Allergy and Infectious Diseases (NIAID). AM and JB received a study grant paid to their institution from Nabi Biopharmaceuticals for participation in this study, but declare no other conflicts of interest. SD and DB are full-time employees of GlaxoSmithKline Vaccines. DB declares ownership of GSK Vaccines stocks. DB is also an inventor of certain GSK patents.

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