A randomized, double-blind study on the safety and immunogenicity of rTSST-1 variant vaccine: phase 2 results

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Summary

Background Toxic shock syndrome toxin-1 (TSST-1) is a superantigen produced by *Staphylococcus aureus* that causes the life-threatening toxic shock syndrome. The development of a safe and immunogenic vaccine against TSST-1 remains an unmet medical need. We investigated the safety, tolerability and immunogenicity of a recombinant TSST-1 variant vaccine (rTSST-1v) after 1–3 injections in healthy volunteers.

Methods In this randomised, double-blind, adjuvant-controlled, parallel-group, phase 2 trial, healthy adults aged 18–64 were randomly allocated to undergo 1–3 injections of either 10 or 100 μg rTSST-1v or $Al(OH)_3$. The primary endpoint was safety and tolerability of rTSST-1v in the intention-to-treat population. The per-protocol population was used for the immunogenicity analysis. The trial is registered with EudraCT#: 2015-003714-24; ClinicalTrials.gov#: NCT02814708.

Findings Between April and November 2017,140 subjects were enrolled and 126 completed the trial. rTSST-1v showed a good safety and tolerability profile. A total of 855 systemic adverse events occurred, 280 of which were suspected related adverse events, without dose dependency. Two participants were discontinued early because of allergic reactions. Seroconversion occurred in >81% of subjects within 3 months of the first immunisation which was sustained until 18 months after the third immunisation in over 70% of subjects in the pooled low-dose group and in over 85% in the pooled high-dose group.

Interpretation rTSST-1v in cumulative doses of up to 300 μg was safe, well-tolerated and highly immunogenic. Two immunisations with 100 μg rTSST-1v provided the most persistent immune response and may be evaluated in future trials.

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Keywords: Staphylococcus aureus; Toxic shock syndrome; Superantigen; TSST-1; Vaccine

Introduction

Systemic infection with a *Staphylococcus aureus* strain capable of producing toxic shock syndrome toxin-1 (TSST-1) may lead to the development of staphylococcal toxic shock syndrome, a critical illness, characterised by fulminant onset of fever, rash, hypotension and multi-organ failure.[1] Driven by its superantigen characteristics, the exotoxin TSST-1 bypasses the conventional pathway of T-cell activation by interfering directly with the class II major histocompatibility complex on T-cells, causing subsequent release of interleukin (IL)-1, IL-6, TNF-α, TNF-β and IFN-γ, and

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Research in context

Evidence before this study

Staphylococcus aureus-associated toxic shock syndrome was first described as a menstruation-associated condition, which was especially linked with the use of absorbent tampons among girls and young women, although in 50% of cases this potentially lethal, rapidly progressing syndrome with rather unspecific initial symptoms such as fever, rash or hypotension is of non-menstrual origin. Several unsuccessful attempts at creating a safe and effective vaccine targeting selected staphylococcal surface antigens have been made in order to protect the population at risk that mostly consists of young individuals or immunocompromised patients lacking protective antibodies. rTSST-1v is the first superantigen-based vaccine, which targets the most prevalent *S. aureus* toxin TSST-1, yielding promising results regarding safety, tolerability, and immunogenicity in a previous first-in-man trial.

Added value of this study

This phase 2 trial was designed to test two dose groups of rTSST-1v (10 µg and 100 µg) for up to 3 immunisations in healthy volunteers and evaluate safety, tolerability, and immunogenicity. In 140 healthy subjects, the rTSST-1v vaccine was safe, well-tolerated and immunogenic with a seroconversion rate of more than 75% of subjects across all dose groups within 3 months after the first immunisation, which persisted towards the end of the study after 27 months. Neutralising antibodies inhibiting T-cell activation, and for the expression of T-cell-associated cytokines IFN-γ, IL-2, IL-6 and TNF-α were increased significantly across both dose groups, suggesting a robust anti-TSST-1 antibody activity.

Implications of all the available evidence

rTSST-1v was safe, well-tolerated and highly immunogenic. Serologic data from patients with toxic shock syndrome and controls together with results from preclinical animal models suggest that the achieved anti-TSST-1 antibody titres are sufficiently high and long lasting to procure a protective effect against TSST-1. The rTSST-1v vaccine is a promising vaccine candidate for the prevention of toxic shock syndrome and may be further evaluated in a phase 3 trial in the target population.

inducing a cytokine storm.[2] Toxic shock syndrome has been classified into two distinct entities: menstrual toxic shock syndrome and nonmenstrual toxic shock syndrome. Albeit an overall low incidence of 0.03–0.5 per 100,000 people,[3] menstrual toxic shock syndrome remains an important cause of morbidity among young women with a mortality of around 8%.[4] Prerequisites include vaginal colonisation by a TSST-1-producing *S. aureus* strain, absence of protective antibodies against TSST-1 and prolonged tampon use,[5] although TSST-1 may also be associated with use of menstrual cups. TSST-1 accounts for 85–100% of menstrual toxic shock cases, as opposed to 40–60% of nonmenstrual cases.[6] However, 50% of cases of toxic shock syndrome are of nonmenstrual origin, extending the population at risk to men and children.[7] While antibody titres against TSST1 increase with age, the absence of neutralising antibodies, especially in immunocompromised patients, children and young adults, predisposes them to an increased risk of severe disease.[8] Nonmenstrual toxic shock syndrome occurs frequently in conjunction with *S. aureus*-associated infections such as skin lesions, surgical-wound infections, postpartum infections, or osteomyelitis, amongst others,[9] and is associated with a higher mortality than menstrual toxic shock syndrome.[10]

Consequently, the development of a safe and effective vaccine targeting TSST-1 remains an unmet medical need. The rTSST-1v vaccine is a recombinant superantigen-based vaccine, containing a detoxified double-mutant rTSST-1 antigen.[11] In a previous first-in-man trial, the rTSST-1v vaccine in a dose of up to 30 µg was safe, well-tolerated and yielded a strong antibody response.[12] In this phase II trial, we aimed to further assess safety, tolerability and immunogenicity with increasing doses of rTSST-1v in healthy subjects.

Methods

Study design and participants

We performed a prospective, single-centre, randomised, double-blind, parallel-group, adjuvant-controlled, phase 2 trial of two doses of rTSST-1v in healthy adult volunteers at the Department of Clinical Pharmacology, Medical University of Vienna, Austria. Oral and written informed consent was obtained prior to any trial-related procedure. The trial lasted for 12–14 months for each participant, with the option of participating in a long-term follow-up study that lasted 27 months for the individual subject. Inclusion criteria furthermore comprised age 18–64 years, a negative pregnancy test in all women of childbearing potential unless they were menopausal for >1 year or childbearing potential was surgically terminated, and normal findings in the medical history and physical examination, as judged by the investigator. Baseline TSST-1 antibody titre was determined during a screening visit taking place 60–14 days before study enrolment. Subjects were excluded if the baseline TSST-1 antibody titre exceeded 1000. Further exclusion criteria were a history or recent signs of autoimmune disorders, positive HIV, hepatitis A virus, hepatitis B virus serology, pregnancy or inadequate contraception in women with childbearing potential. A

complete list of in- and exclusion criteria is provided in the Appendix (p 9).

The safety population included all participants who received at least one immunisation and is identical to the intention-to-treat analysis in this trial. The per-protocol population comprised only participants who adhered to the study protocol without any major protocol deviation and was used as the primary analysis population for immunogenicity.

The study was approved by the local ethics committee (Ethics Committee of the Medical University of Vienna, EK-number 1810/2015) and was conducted in compliance with the Declaration of Helsinki, the Good Clinical Practice guidelines and with the Note for Guidance on Clinical Evaluation of New Vaccines. The complete trial protocol is available in the Appendix (p 32).

Randomisation and masking

At visit 1, subjects were randomised to receive either 10 μg or 100 μg rTSST-1v vaccine or $Al(OH)_3$ control one to three times and thus, were allocated to one of seven groups. Participants of each group were planned to receive three injections, the first one at time 0, the second one 3 months ±4 weeks after the first, and the third one 6 months ±4 weeks after the second.

Group 1 received 10 μg of rTSST-1v vaccine followed by two administrations of adjuvant; Group 2 received two doses of 10 μg of rTSST-1v vaccine followed by one dose of adjuvant; and Group 3 received three doses of 10 μg of rTSST-1v vaccine. Groups 4 to 6 received 100 μg of rTSST-1v vaccine adhering to the same pattern explained for groups 1–3. Group 7 was to receive three doses of the adjuvant $Al(OH_3)$. This approach was chosen to establish a possible dose-immunogenicity effect and to investigate the ideal number of immunisations for maximal immunogenicity. A phase I trial of rTSST-1v successfully investigated two doses that ranged from 100 ng to 30 μg in 49 healthy volunteers.[12] All doses were safe and doses ≥3 μg increased antibody titres effectively. Therefore, the starting dose of this study, 10 μg rTSST-1v (1–3 doses), was chosen as a safe and effective dose. However, in the phase I study, a rather large variability in the antibody titers was observed for all doses. To overcome this issue, also higher doses were investigated (100 μg 1–3 times) in this study.

For safety reasons, block randomisation enrolling the first 35 subjects (blocks 1–5) was used in a staggered approach during the first five weeks, the first 3 randomization blocks each including one slot for each of the treatment groups 1, 2, 3, 6 and 7, the fourth block randomizing one subject each to the groups 1, 2, 3, 5, 6 and 7 and the fifth block randomizing one subject each to groups 1, 2, 3, 6 and 7, 5 subjects to group 4 and 4 subjects to group 5. Thereafter, subjects were enrolled by block randomisation in blocks of 14 subjects until the final number of 140 participants (20 in each group) was reached, resulting in an overall randomization ratio of 1:1:1:1:1:1:1. Randomization was not stratified. The randomization code list was created by the unblinded statistician using nQuery Advisor.

Randomisation was performed by a pharmacist, not otherwise involved in trial activities at the study site using opaque, sealed envelopes containing consecutive subject numbers, group allocation and vaccine volume. Likewise, study drugs were prepared by a study pharmacist and were delivered in ready-to-use syringes to the investigator as blinded medication for application. Active substance and placebo were not distinguishable based on their physicochemical properties and looks. Al(OH)3 was chosen as a placebo, because it provokes injection site reactions similar to other vaccinations, while it does not exert any immunogenic effects. Apart from screening values for TSST-1 antibody titers, when excessive pre-existing titers leading to exclusion were present, such data were not provided for treating physicians throughout the trial to maintain the double-blind character.

Procedures

Eligible participants had to undergo a screening visit that involved a complete medical history and physical examination, vital signs, and routine blood and urine tests. (Appendix pp 8–9) Prior to the first, second and third immunisation, a urine pregnancy test was done in participating women of child-bearing potential to rule out pregnancy. Furthermore, demographic information was collected during this screening visit including age, gender and ethnicity. Participants were vaccinated with rTSST-1v or $Al(OH)_3$ control intramuscularly (M. deltoideus) on day 0, at month 3 and at month 9. Blood samples were obtained on the day of immunisation (before injection) and after 24 h (Appendix p 8 and 9) In parallel, vital signs, monitoring of adverse events and concomitant medication were recorded at the day of immunisation and at 24 h. Safety evaluations were continued up to month 27. Adverse events and injection site reactions were specifically monitored using a study diary handed out to each study participant. Adverse events were recorded in the study diary and subsequently discussed with study physicians.

In this phase 2 trial, the safety and immunogenicity of three doses of rTSST-1v were evaluated in healthy adult volunteers. rTSST-1v contains a genetically modified rTSST-1 antigen, designed specifically to lose its property as a superantigen.[11] Implementation of the double mutation G31R-H135A precludes binding of rTSST-1v to both major histocompatibility complex II (MHC II)[13] and the T-cell receptor.[14] Production of the recombinant detoxified TSST-1 was in compliance with good manufacturing practice protocols. The final pharmaceutical product was presented in 2.0 mL single dose vials containing 10 μg or 100 μg of rTSST-1v vaccine and 1.0 mg $Al(OH)_3$ in 0.5 mL PBS with 0.02% polysorbate

80. To test for adverse events of the adjuvant alone, 1.0 mg of the vaccine adjuvant $Al(OH)_3$ in phosphate-buffered saline (PBS) was chosen as comparator.

The safety analysis (intention to treat population) included all patients who received at least one injection. Vital sign checks (including blood pressure, heart rate and oral body temperature), local injection–site reactions and blood samplings were performed for safety assessment at baseline and during all subsequent visits. Participants were to document any local or systemic adverse events in a study diary up until month 12, which was assessed by a study physician during the next follow-up visit.

The laboratory safety analysis included a complete blood cell count (including red blood cell counts, hemoglobin concentration, hematocrit, white blood cell count, differential leukocyte counts, and platelet counts), blood urea nitrogen and serum creatinine to monitor kidney function, alanine aminotransferase and total protein to monitor liver function, glucose to monitor metabolism and liver function, and C-reactive protein to monitor systemic inflammatory responses. All abnormal laboratory values were documented.

Adverse events were recorded using the Medical Dictionary for Regulatory Activities (MedDRA, version 22.1), categorised based on the System Organ Class (SOC) and preferred term (PT) and graded using a three-point scale (mild, moderate or severe). The definition of serious adverse event followed the International Council on Harmonisation guidelines and WHO Good Clinical Practice guidelines.

Local injection site reactions (ISR) were assessed separately from adverse events and included injection site swelling, injection site induration, injection site redness, injection site pain, algesia and injection site itching.

Immunogenicity was assessed at baseline, at each immunisation visit and at each subsequent follow-up visit. Antibody titres to rTSST-1 were determined by ELISA IgG, an assay specifically developed to detect rTSST-1 IgG antibodies in serum samples.[15] Results of the ELISA assay are reported as titre, given as the inverse value of the dilution factor that still elicits a positive signal. To determine the neutralisation of superantigenicity, neutralising antibodies inhibiting T-cell activation and neutralising antibodies inhibiting gene expression of T-cell associated cytokines IFN-γ and IL-2 were analysed.[12] A detailed description of laboratory assays is presented in the supplement.

Outcomes

The primary study endpoint was safety and tolerability of the rTSST-1v vaccine at 12 months after the first immunisation, assessed by the incidence of adverse advents, abnormal laboratory findings and local injection site reactions according to FDA guidelines. The secondary study endpoint was immunogenicity, as measured by TSST-1 binding and neutralising antibodies at predefined timepoints. Antibody response was defined as seroconversion from a TSST-1 binding antibody titre of <20 to ≥40 or a ≥4-fold increase as compared to the baseline titre.

Sample size considerations

A formal sample size calculation was not calculated due to the descriptive nature of the primary endpoint. However, inclusion of 140 subjects allows for the detection of an AE with a true underlying prevalence of 2.1% with a probability of 95%. In addition, with respect to immunogenicity comparisons, a sample size of 60 subjects (=analysis of pooled dose groups: low dose vs. high dose) suffices to show a statistically significant difference in the geometric mean titre (GMT) ratio of 2.73 (or higher), assuming a geometric mean SD of 7, a power of 80% and a significance level of 5%.

Statistical analysis

The primary safety endpoint was assessed descriptively. We used one-way analysis of variance (ANOVA) for statistical analysis of antibody titres. Group-wise comparison of GMTs was performed by ANOVA. This was done using $\log_{10}$ transformed data and taking the anti-log of the resulting point estimates for the least squares means, least squares mean differences and the corresponding 95% CIs. The anti-log of the mean difference estimate on the logarithmic scale corresponds to the geometric mean ratio (GMR) estimate. Levene's test was conducted to assess homogeneity. Tukey's HSD test was used for pairwise comparisons. For between group comparisons and antibody persistence, Mann-Whitney-U and Wilcoxon signed rank tests were applied. All performed tests were two-tailed and a p value of less than 0.05 was considered statistically significant. Data were analysed using SAS 9.3. The trial is registered with EudraCT, number 2015-003714-24 and Clinicaltrials.gov, NCT02814708.

Role of the funding source

The funder of the study played no part in clinical data collection, data monitoring, safety monitoring, analysis of data, or generation of the clinical trial report. The analysis of serological endpoints was carried out by blinded employees of the funder in accordance with Good Laboratory Practices (GLP) standards. Data monitoring was performed by an independent monitor (CRETA [Clinical Research & Trial Agency GmbH]). Safety monitoring was the investigator's responsibility. The statistical analysis was done by the Assign Data Management and Biostatistics GmbH. GG, CS and BJ wrote the first draft of the manuscript. Further revisions were done in collaboration with the funder. The first author and the corresponding authors (BJ, AR) had full access to all study-related data and had final responsibility for the decision to submit for publication.

All authors had access to the study data and agreed to submit the final version for publication.

Results

Between Apr 27 2016 and Nov 13 2017, 184 subjects were screened for eligibility, of which 140 subjects (49 [35%] males, 138 [98.6%] Caucasian) with a mean age of 32 years (SD 11.2) and a mean BMI of 24.1 kg/m2 (SD 4.5) were enrolled (Table 1, Appendix p 11). The study was terminated after the last study visit of the last subject. Among the 91 female subjects, 82 were of childbearing potential. Each group (rTSST-1v 1 × 10 µg, rTSST-1v 2 × 10 µg, rTSST-1v 3 × 10 µg [summarised as low-dose groups], rTSST-1v 1 × 100 µg, rTSST-1v 2 × 100 µg, rTSST-1v 3 × 100 µg [summarised as high-dose groups], and adjuvant/control) included 20 subjects (Fig. 1). All 140 subjects received at least one dose of the rTSST-1v vaccine or placebo and were thus included in the safety analysis (safety population). Thirteen subjects discontinued the study prematurely (11 for personal reasons (1 in the placebo group), due to the duration of the trial, one subject withdrew consent, one subject discontinued the study because of an adverse event), one subject was excluded from the analysis because of a major protocol deviation (subject received the wrong dose at the second immunisation; he was included in the safety population but excluded from the per-protocol population), leaving 126 subjects for the per-protocol analysis. The 126 subjects who completed all three immunisations were asked to participate in the study's follow-up period, which aimed to evaluate long-term immunogenicity. 105 subjects from the safety population (and 102 subjects from the per-protocol population) underwent the extended follow-up period until 27 months after the first immunisation (rTSST-1v vaccine dose level 10 µg: 46 subjects; rTSST-1v vaccine dose level 100 µg: 43 subjects; placebo: 16 subjects).

Adverse events and injection site reactions per group are summarised in Table 2. A total of 1270 adverse events, consisting of 855 systemic adverse events and 415 injection site reactions, occurred among 133 subjects. 166 adverse events in 20 subjects were observed in the control group. The number of observed adverse events was similar between the treatment groups. Laboratory findings outside the normal range were found across all treatment groups, were transient and self-limiting, and occurred mostly during the follow-up period and were therefore considered unrelated to rTSST-1v.

Common systemic adverse events were infections (52% of subjects), headache (47% of subjects), general disorders such as fatigue or influenza-like illness (33% of subjects), gastrointestinal disorders (26% of subjects), myalgia (24% of subjects), skin disorders (17% of subjects), and respiratory disorders (13% of subjects) (Appendix p 13, 15 and 16).

Considering only suspected related adverse events (280), all groups showed similar numbers (range 26–53). (Appendix p 14) Five suspected related adverse events were classified as severe and occurred mostly in low-dose groups receiving 1 × 10 µg, 2 × 10 µg and 3 × 10 µg rTSST-1v. Participation of one subject was terminated early in the 2 × 10 µg rTSST-1v group because of severe allergic urticaria after receiving the second dose. The subject developed no further symptoms (detailed narrative in Appendix p 24).

The overall incidence of systemic adverse events during the treatment phase was similar between the treatment groups and the control group, which was also true for suspected related adverse events (ratio placebo: 2.5 adverse events per subject; dose groups 1–3: 1.6 adverse events per subject; dose groups 4–6: 2.2 adverse events per subject). In the extended follow-up period, the incidence of adverse events substantially decreased, with no adverse event being considered related to the study drug.

Eleven serious adverse events occurred in 8 subjects, ten of which were classified as not related to the study drug. (Appendix p 22) One was a suspected related

	rTSST-1v 1 × 10 µg n = 20	rTSST-1v 2 × 10 µg n = 20	rTSST-1v 3 × 10 µg n = 20	rTSST-1v 1 × 100 µg n = 20	rTSST-1v 2 × 100 µg n = 20	rTSST-1v 3 × 100 µg n = 20	Al(OH_3) (control group) n = 20	pooled low-dose n = 60	pooled high-dose n = 60
Sex									
Female	14 (70)	11 (55)	12 (60)	15 (75)	10 (50)	15 (75)	14 (70)	37 (62)	40 (67)
Male	6 (30)	9 (45)	8 (40)	5 (25)	10 (50)	5 (25)	6 (30)	23 (38)	20 (33)
Age [years]	32 (11)	31 (12)	29 (10)	30 (9)	31 (12)	37 (12)	34 (13)	31 (11)	33 (11)
BMI [kg/m^2]	24 (5)	25 (4)	24 (4)	24 (4)	25 (4)	25 (7)	23 (3)	24 (4)	24 (5)
Ethnic origin									
White	20	20	19	20	20	19	20	59 (98)	59 (98)
Asian	0	0	1	0	0	1	0	1 (2)	1 (2)
Other	0	0	0	0	0	0	0	0	0

Data are n (%) or mean (SD). rTSST-1v = recombinant detoxified toxic shock syndrome toxin-1 variant. Al(OH)3 = aluminium hydroxide. Pooled low-dose = 1 × 10 µg, 2 × 10 µg, 3 × 10 µg. Pooled high-dose = 1 × 100 µg, 2 × 100 µg, 3 × 100 µg.

Table 1: **Baseline characteristics of each treatment group and pooled study cohorts.**

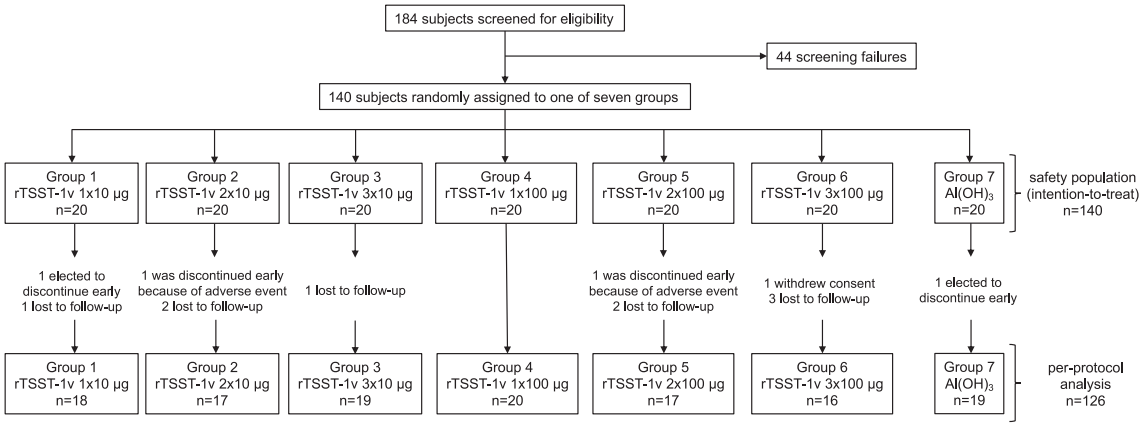


Fig. 1: Trial profile.

anaphylactic reaction occurring approximately 20 min after the second 100 µg dose of rTSST-1v, which prompted discontinuation of further study treatment. This subject developed transient hemodynamic instability, which did not require vasopressor support, erythema and urticaria, which were treated with corticosteroids, and mild gastrointestinal symptoms, which did not require any treatment (detailed narrative on p. 24 in Appendix).

Five women became pregnant throughout the trial, one in group 4 (100 µg rTSST-1v), one in group 6 (3 × 100 µg rTSST-1v) and three in group 7 ($Al(OH)_3$, control group). All five women delivered healthy newborns, three without any complications, one was born prematurely by two weeks, and one was delivered by elective caesarean section due to an underlying condition.

A total of 415 injection site reactions were observed in 109 subjects, 152 resolved within 24 h (71 subjects) (Table 2). Among injection site reactions, algesia or injection site pain were most commonly reported (319 events) and were characterised as mild or moderate in nature. (Appendix p 23) Only one was considered severe (sensitivity to pain at the injection site observed after the second dose of 100 µg rTSST-1v, resolved within 48 h without medication). The number of injection site-related events was moderately higher in subjects receiving the rTSST-1v vaccine than in $Al(OH)_3$ recipients. All injection site reactions resolved on their own without the need for medication.

The per-protocol population (n = 126) was used for the immunogenicity analysis and consisted of 16–18 subjects per group.

Seroconversion occurred in 81.5% and 88.5% of participants after the first dose of 10 µg and 100 µg rTSST-1v, respectively, whereas no seroconversion was detected in the $Al(OH)_3$ group. Seroconversion rates slightly decreased by 10% in the low dose groups, but remained largely unchanged over time after high doses until the end of the trial (27 months; Table 3).

Fig. 2 shows binding titres of all participants (treatment groups vs. control group) before immunisation, and following first, second and third immunisation. rTSST-1v at a dose of 100 µg more than doubled the TSST-1 binding antibody titre compared with rTSST-1v at a dose of 10 µg at three months after the first immunisation (GMT 3148 vs. 1263, GMR with 95% CI: 0.4 [0.2, 1.0]). Titres in the pooled high-dose groups remained twice as high as in the pooled lower-dose groups until the end of the study (GMR ranges from 0.4 to 0.5 with 95% CI from 0.2 to 1.2). TSST-1 binding antibodies were most persistent in the 2 × 100 µg rTSST-1v group, reaching a GMT of 1696 at 27 months. In contrast to all actively treated groups, TSST-1 binding antibodies did not change in the control group. Fig. 3 shows *in vitro* inhibition of T-cell proliferation and IL-2 gene expression by 1000-fold and 3000-fold diluted sera in rTSST-1v recipients and the control group. Neutralising antibodies inhibiting T-cell activation were found to show the highest titres after two administrations of 100 µg rTSST-1v, while no change was observed in the control group over time. Fig. 4 shows neutralising antibody titres for the expression of T-cell-associated cytokines IFN-γ, IL-2, IL-6 and TNF-α in rTSST-1v recipients and the control group. Neutralising antibodies for the expression of T-cell-associated cytokines IFN-γ and IL-2 were detected as soon as at 3 months after the first immunisation and persisted until the end of the study. Likewise, the incidence and persistence of neutralising antibodies for the expression of the inflammatory cytokine TNF-α and IL-6 mirrored the dynamics of IL-2 and IFN-γ. Consistently, in the control group there were no obvious changes observable regarding T-cell-associated cytokines. Across all four investigated T-cell-associated cytokines and for the inhibition of T-cell activation, the treatment groups that received two or

	rTSST-1v 1 × 10 µg n = 20	rTSST-1v 2 × 10 µg n = 20	rTSST-1v 3 × 10 µg n = 20	rTSST-1v 1 × 100 µg n = 20	rTSST-1v 2 × 100 µg n = 20	rTSST-1v 3 × 100 µg n = 20	$Al(OH_3)$ (control group) n = 20
Any AE, n (%)	20 (100)	18 (90)	19 (95)	20 (100)	20 (100)	16 (80)	20 (100)
Obs	165	145	171	248	193	182	166
Any systemic AE, n (%)	19 (95)	17 (85)	16 (80)	20 (100)	18 (90)	15 (75)	18 (90)
Obs	103	109	99	190	126	108	120
Any local AE (injection site reaction), n (%)	17 (85)	13 (65)	17 (85)	17 (85)	17 (85)	15 (75)	13 (65)
Obs	62	36	72	58	67	74	46
Any severe systemic AE, n (%)	2 (10)	1 (5)	1 (5)	0 (0)	2 (10)	0 (0)	0 (0)
Obs	2	1	1	0	3	0	0
Any suspected related systemic AE, n (%)	9 (45)	11 (55)	11 (55)	12 (60)	10 (50)	13 (65)	10 (50)
Obs	41	30	26	53	40	40	50
Any unrelated systemic AE, n (%)	18 (90)	16 (80)	14 (70)	19 (95)	18 (90)	13 (65)	17 (85)
Obs	62	79	73	137	86	68	70
Any systemic SAE, n (%)	1 (5)	1 (5)	0 (0)	3 (15)	3 (15)	0 (0)	0 (0)
Obs	1	1	0	4	5	0	0
Any severe systemic SAE, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	1 (5)	0 (0)	0 (0)
Obs	0	0	0	0	2	0	0
Any suspected related systemic SAE, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	1 (5)	0 (0)	0 (0)
Obs	0	0	0	0	1	0	0
Injection site reactions							
Algesia (sensitivity to pain at the injection site), n (%)	15 (75)	12 (60)	16 (80)	15 (75)	16 (80)	14 (70)	13 (65)
Obs	33	19	33	33	30	29	23
Injection site induration, n (%)	3 (15)	4 (20)	6 (30)	2 (10)	3 (15)	3 (15)	2 (10)
Obs	4	4	8	2	7	3	4
Injection site pain (without touching), n (%)	12 (60)	6 (30)	13 (65)	10 (50)	9 (45)	11 (55)	10 (50)
Obs	23	8	23	15	14	19	17
Injection site redness, n (%)	0 (0)	3 (15)	3 (15)	4 (20)	4 (20)	6 (30)	1 (5)
Obs	0	3	3	6	5	7	1
Injection site swelling, n (%)	0 (0)	2 (10)	3 (15)	1 (5)	7 (35)	7 (35)	1 (5)
Obs	0	2	4	1	9	10	1
Itching, n (%)	1 (5)	0 (0)	0 (0)	1 (5)	1 (5)	4 (20)	0 (0)
Obs	1	0	0	1	1	6	0
Other, n (%)	1 (5)	0 (0)	1 (5)	0 (0)	1 (5)	0 (0)	0 (0)
Obs	1	0	1	0	1	0	0

n = number of subjects, Obs = number of events, AE = adverse event, SAE = serious adverse event.

Table 2: **Overview of adverse events and injection site reactions by treatment cohorts in the safety population (n = 140).**

three doses of 100 µg rTSST-1v consistently displayed the highest neutralising antibody titres (Appendix pp 24–28).

Discussion

The search for a preventative measure for *S. aureus*-associated conditions started decades ago with vaccine candidates primarily targeting staphylococcal surface antigens, but their preclinical success failed to translate into clinical trials.[16,17] As opposed to targeting surface antigens, the rTSST-1v vaccine is superantigen-based containing a detoxified double-mutant rTSST-1 antigen. Preclinical testing of safety, tolerability and immunogenicity were extensively performed *in vitro* in human mononuclear cells, and *in vivo* in rabbits and mice.[11,15,18] In a first-in-man trial, rTSST-1v was safe and well-tolerated up to 30 µg. Antibody titres against rTSST-1v increased 15-fold and 28-fold after the first and second vaccination.[12] This phase 2 trial examined the safety, tolerability, and long-term immunogenicity of up to 3 doses of 10 and 100 µg doses of the rTSST-1v vaccine.

In the current trial, similar numbers of adverse events were observed across all groups, suggesting no dose dependency of adverse events. Likewise, the incidence of suspected related adverse events was similar between rTSST-1v groups and the control group. Injection site reactions were common, mostly characterised as mild or moderate and were self-limiting within 24 h. Overall, safety results of this trial suggest that single doses of 100 µg rTSST-1v may not be the

	rTSST-1v 1 × 10 µg n = 18	rTSST-1v 2 × 10 µg n = 17	rTSST-1v 3 × 10 µg n = 19	rTSST-1v 1 × 100 µg n = 20[a]	rTSST-1v 2 × 100 µg n = 17	rTSST-1v 3 × 100 µg n = 16	$Al(OH_3)$ (control group) n = 19	pooled low-dose n = 54	pooled high-dose n = 53
TSST-1 binding antibody titre[b]									
Screening	58.2 (4.8)	38.5 (3.2)	82.4 (4.8)	61.3 (4.2)	93.0 (4.3)	49.8 (4.2)	76.3 (2.9)	57.8 (4.3)	65.9 (4.2)
Total	18	17	19	19	17	16	19	54	53
Day 0	60.6 (4.8)	41.0 (3.0)	78.1 (4.7)	58.0 (4.0)	92.0 (4.4)	48.0 (4.0)	71.1 (3.0)	58.6 (4.1)	63.6 (4.1)
Total (n)	18	17	19	19	17	16	19	54	52
3 months	1312.5 (9.1)	1274.3 (8.5)	1209.3 (9.1	2768.0 (5.8)	3665.0 (13.8)	3144.0 (11.2)	72.3 (2.9)	1263.4 (8.5)	3147.5 (9.1)
Total (n)	18	17	19	20	17	16	19	54	53
9 months	751.0 (8.5)	793.7 (6.5)	1011.3 (6.0)	1318.0 (4.8)	2144.4 (11.0)	1934.7 (9.1)	62.7 (2.8)	848.5 (6.8)	1730.0 (7.6)
Total (n)	18	17	19	20	17	16	19	54	53
12 months	612.4 (7.4)	727.6 (7.1)	1224.4 (5.2)	1135.9 (4.7)	2451.1 (6.9)	2479.1 (7.6)	60.3 (2.9)	825.0 (6.5)	1829.8 (6.2)
Total (n)	18	17	19	19	16	16	19	54	52
15 months	479.6 (8.9)	495.6 (6.3)	1236.7 (6.2)	915.6 (4.7)	2057.2 (6.9)	1756.7 (8.1)	58.8 (2.8)	673.9 (7.1)	1468.7 (6.5)
Total (n)	15	15	16	16	15	14	18	46	45
18 months	444.7 (8.3)	452.1 (6.2)	966.6 (5.5)	837.1 (4.9)	1908.2 (7.1)	1309.6 (8.7)	54.9 (2.6)	585.7 (6.6)	1266.3 (6.6)
Total (n)	15	15	16	16	15	14	18	46	45
21 months	444.9 (8.3)	455.3 (6.2)	786.0 (6.3)	739.7 (4.7)	1776.8 (6.9)	1239.6 (8.3)	57.6 (2.8)	546.4 (6.8)	1163.3 (6.5)
Total (n)	15	15	16	16	15	14	18	46	45
24 months	370.0 (8.3)	422.2 (6.2)	716.2 (5.6)	680.7 (4.7)	1786.6 (7.6)	1142.1 (8.0)	59.3 (2.8)	489.0 (6.5)	1103.0 (6.6)
Total (n)	14	15	16	15	14	14	17	45	43
27 months	368.5 (8.3)	485.9 (5.1)	664.3 (6.5)	744.1 (4.9)	1696.1 (7.9)	954.0 (7.9)	64.3 (2.8)	498.3 (6.3)	1045.5 (6.6)
Total (n)	14	15	16	15	13	13	16	45	41
Seroconversion[c]									
3 months	14 (77.8)	15 (88.2)	15 (78.9)	18 (94.7)	14 (82.4)	14 (87.5)	0 (0.0)	44 (81.5)	46 (88.5)
Total (n)	18	17	19	19	17	16	19	54	52
9 months	13 (72.2)	15 (88.2)	16 (84.2)	17 (89.5)	14 (82.4)	14 (87.5)	0 (0.0)	44 (81.5)	45 (86.5)
Total (n)	18	17	19	19	17	16	19	54	52
12 months	13 (72.2)	15 (88.2)	17 (89.5)	17 (89.5)	13 (81.3)	15 (93.8)	0 (0.0)	45 (83.3)	45 (88.2)
Total (n)	18	17	19	19	16	16	19	54	51
15 months	10 (66.7)	13 (86.7)	14 (87.5)	14 (87.5)	12 (80.0)	12 (85.7)	0 (0.0)	37 (80.4)	38 (84.4)
Total (n)	15	15	16	16	15	14	18	46	45
18 months	10 (66.7)	12 (80.0)	13 (81.3)	14 (87.5)	12 (80.0)	12 (85.7)	0 (0.0)	35 (76.1)	38 (84.4)
Total (n)	15	15	16	16	15	14	18	46	45
21 months	10 (66.7)	12 (80.0)	10 (62.5)	14 (87.5)	12 (80.0)	12 (85.7)	0 (0.0)	32 (69.6)	38 (84.4)
Total (n)	15	15	16	16	15	14	18	46	45
24 months	9 (64.3)	13 (86.7)	10 (62.5)	13 (86.7)	11 (78.6)	12 (85.7)	0 (0.0)	32 (71.1)	36 (83.7)
Total (n)	14	15	16	15	14	14	17	45	43
27 months	9 (64.3)	13 (86.7)	10 (62.5)	14 (93.3)	10 (76.9)	11 (84.6)	0 (0.0)	32 (71.1)	35 (85.4)
Total (n)	14	15	16	15	13	13	16	45	41

(18 months after the third immunisation equals to 27 months after the first immunisation). [a]Results for one subject were not included in the calculation (data base issue). [b]Geometric mean titre presented as geometric mean (SD). [c]Seroconversion rate presented as n (%).

Table 3: **Geometric mean titres and seroconversion rates by treatment cohorts in the per-protocol population (n = 126).**

maximum tolerated doses. The option for higher dosing may be relevant for immunocompromised patients who may need repeated doses to achieve a sufficient immune response.[19]

While tolerability and safety in the current trial were largely comparable with the phase 1 trial, two subjects (1.4%) in the phase 2 trial were discontinued early due to onset of severe allergic urticaria (2 × 10 µg rTSST-1v) and a moderate anaphylactic reaction (2 × 100 µg rTSST-1v). The observed incidence of hypersensitivity reactions in this trial may be higher than in other contemporary vaccine trials, such as human papillomavirus vaccine in Tanzanian girls,[20] or the BNT162b2 mRNA Covid-19 vaccine in persons 16 years of age or older.[21] Due to a lack of allergy testing, we cannot exclude that the trigger was an excipient such as polysorbate 80 rather than rTSST-1v itself. Whether the rTSST-1v vaccine truly confers an increased risk of allergic reactions remains to be seen in a larger safety population.

In the elimination of encapsulated bacteria, cooperation of cellular and humoral mediators of innate immunity, granulocytes and the complement system are

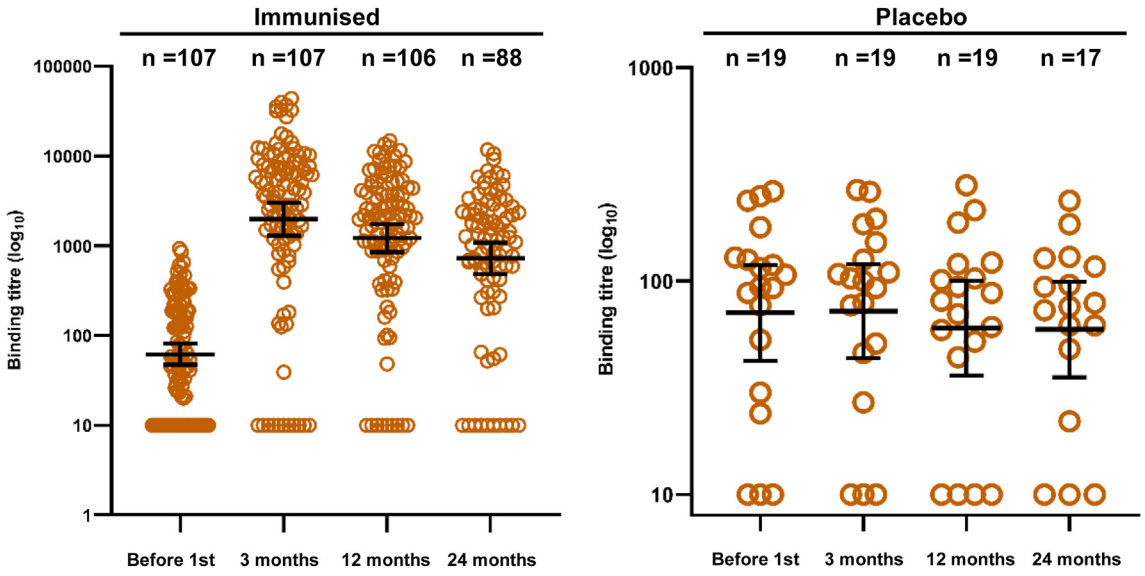


Fig. 2: Increase of TSST-1 binding antibody titres after the first immunisation and following the second and third immunisation in the per-protocol population divided by rTSST-1v recipients and the control group. Scatter dot blots are shown with GMTs and 95% CI.

continuously present, but inefficient. Products of adaptive immunity, antibodies, memory, regulatory and effector T cells, amplify the response of adherence to receptors on phagocytes, engulfment, and killing, but need time to develop after first contact. The role of regulatory and effector T cells is important but incompletely understood. B cells have to be activated to produce opsonizing antibodies and this might take days and longer at first contact. Time for an optimal response is shortened and efficiency is increased in the second contact. Opsonizing antibodies efficiently activate the complement system and bind to Fc receptors on phagocytes. Memory T and B cells are crucial for getting longer lasting immunity by vaccination.

It is our hypothesis that superantigenic exotoxins are crucial for the outcome of systemic disease. We aim to induce anti-toxic immunity[22]; with the toxic shock syndrome toxin-1 (TSST-1) as the main mTSS causative agent as target. TSS is mediated by interaction of superantigen and host lymphocytes, resulting in a massive immune dysregulation.[2] T cell activation by superantigens is different from conventional T cell activation, they are able to simultaneously activate a large proportion of T cells in a variable beta chain-dependent manner. TSST-1 stimulates human T cells that express variable beta chain 2, thereby activating up to 20–30% of the total T cell population.[23] Upon activation of more T cells than conventional antigens, they induce an overwhelming production of cytokines by T cells characterized by a Th1/Th17 profile.[24]

Further, it was shown that TSST-1 could induce pro-inflammatory chemokine production from human vaginal epithelial cells via binding to the CD40 receptor. Chemokine production results in proinflammation.[25] A further disruption of the mucosal barrier due to harmful inflammation could be essential for the systemic effects of TSST-1.

Here, immunogenicity was demonstrated by the increase of TSST-1-binding as well as neutralising antibodies. The maximum seroconversion rate was 82% in the pooled low-dose group and 89% in the pooled high-dose group. Seroconversion rates remained consistently high and were 71% in the pooled low-dose group and 85% in the pooled high-dose group at 24 months after the first immunisation. Binding antibody titres increased to similar values as seen in the phase 1 trial after one dose of 10 µg rTSST-1v[12] demonstrating consistency between different vaccine batches. A single dose of 100 µg rTSST-1v enhanced GMT >2-fold compared with the lower dose, and these higher GMT values persisted at 27 months. Although, the sample size was somewhat limited to draw definite conclusions, the 2 × 100 µg rTSST-1v yielded numerically the best immune response after single and repeat injections. Overall, considering safety, tolerability and immunogenicity results, the use of single or repeat 100 µg doses (or possibly even higher doses) may be the most sensible choice for future studies. In that context, it may be worth mentioning that the theoretical target population of this vaccine differs from the included population, which, although consisting of young healthy adults, may still have been too old and lacks diversity. Immunologic responses must be investigated in an adolescent population in a future trial.

The rise in -and persistence of-neutralising antibodies followed a similar pattern as binding antibodies,

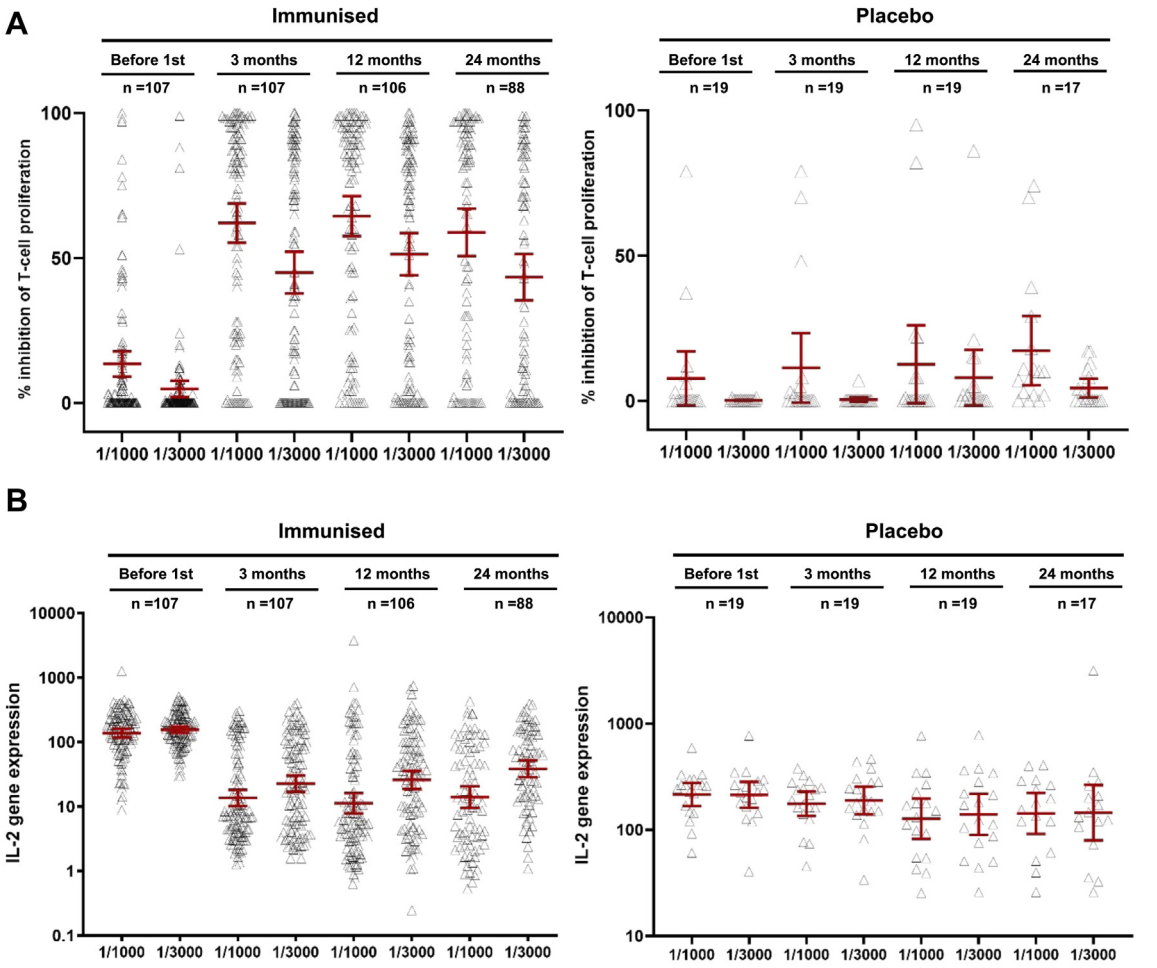
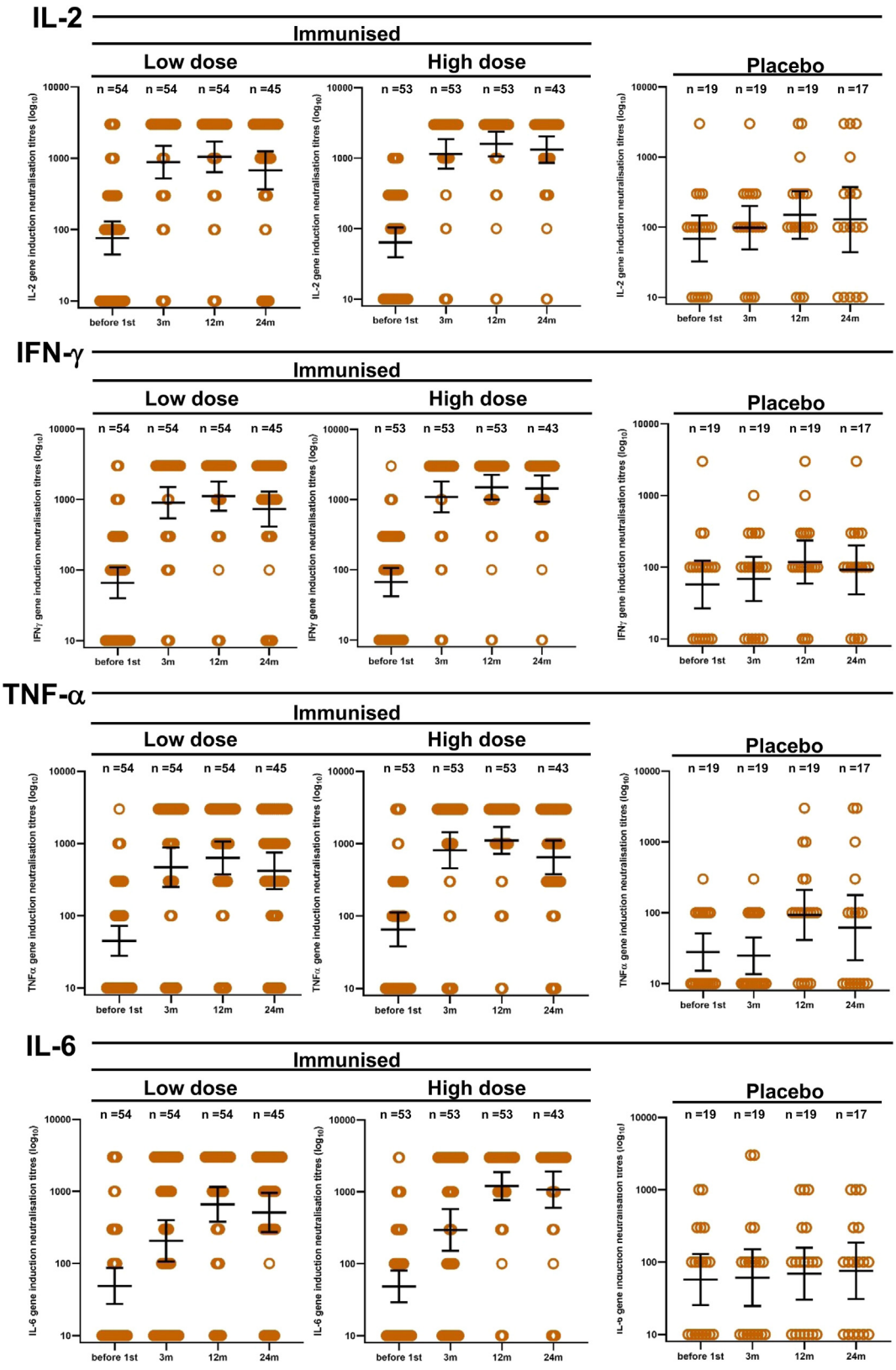


Fig. 3: In vitro inhibition of T-cell proliferation (A) and IL-2 gene expression (B) by 1000-fold and 3000-fold diluted sera in rTSST-1v recipients and the control group obtained before the first immunisation (day 0), 3 months after day 0 (idealized; = 3 months after the first immunisation), 12 months after day 0 (idealized; = 3 months after the third immunisation), and 24 months after day 0 (idealized; = 15 months after the third immunisation). Horizontal lines correspond to geometric means and 95% confidence intervals. For detailed methodology, please refer to the appendix.

but the fewer dilutions tested provide somewhat less granularity. The slow decline with half-lives of more than 15 months suggests that booster doses may not be needed soon after a 1–2 dose regimen.

Antibody responses to toxoid vaccines against tetanus and diphtheria seem to exhibit biphasic kinetics, showing a 90% decline of antibody titres within the first 12 months after the third immunisation[26] followed by a steady decline with an estimated half-life of >10 years.[27] If the elimination of antibodies against rTSST-1v follows a similar pattern, this could mean a long-lived immune response against rTSST-1v and booster intervals may follow those regimens for tetanus and diphtheria.

The low incidence of toxic shock syndrome[3] renders an efficacy trial unfeasible similar to other toxoid vaccines, targeting *Clostridium tetani* or *Corynebacterium diphtheriae*, where vaccine efficacy trials have never been conducted. This leaves the open question of thresholds for protective antibody titres. However, efficacy of the rTSST-1v vaccine might be inferred from serologic data of patients and controls, and preclinical animal models as follows. Serologic data of patients with toxic shock syndrome and healthy controls[28,29] indicate that the protective level of anti-TSST-1 antibodies is probably a titre of >100.[8] In lethal rabbit models, all rTSST-1v-vaccinated rabbits survived a subsequent challenge with TSST-1 and endotoxin (lipopolysaccharide), whereas all animals in the control group died (Supplementary Materials). Rabbits that received only two immunisations of rTSST-1v achieved anti-TSST-1 antibody titres similar to humans, and it was reassuring to see protective immunogenicity also applied


IL-2
Immunised
Low dose
High dose
Placebo
n =54 n =54 n =54 n =45
n =53 n =53 n =53 n =43
n =19 n =19 n =19 n =17
IL-2 gene induction neutralisation titres (log10)
10000
1000
100
10
before 1st
3m
12m
24m
IFN-γ
IFNγ gene induction neutralisation titres (log10)
TNF-α
TNFα gene induction neutralisation titres (log10)
IL-6
IL-6 gene induction neutralisation titres (log10)
IL-∞ gene inoculation neutralisation titres (log10)

to those two animals with the lowest antibody titres ranging from 102 to 140. However, further research is necessary to confirm these observations and establish the relationship between antibody titres and protection against TSST-1.

Aside from rTSST-1v, there are currently no other TSST-1-targeting vaccines in clinical development. Targeting another superantigen, the STEBVax vaccine, containing recombinant staphylococcal enterotoxin B, was shown to be safe and immunogenic in a phase 1 trial.[30]

While the strengths of this trial involve its randomised, double-blind, adjuvant-controlled, parallel-group design, the inclusion of more women than men and its extended follow-up period, it shows several limitations including its sample size of 140 subjects and inclusion of almost only Caucasians. A larger pivotal, multinational, randomised-controlled, pivotal, phase 3 trial is needed to confirm safety and immunogenicity of the eventual rTSST-1v dose regimen. Based on the well-known risk-factors, a lack of protective antibody levels and the use of tampons or menstrual cups, the target population of such a trial should include healthy adolescents, especially girls, aged ≥11 years and a more diverse study population. Thus, one obvious limitation of this study is its limited generalizability, which needs to be addressed in future trials. There are some missing data due to subjects, who have terminated their study participation early, and it is impossible to exclude some degree of selection bias. The per-protocol analysis is prone to such bias. Furthermore, analysis of the ITT introduces measurement bias, because it assesses assigned and not actually received treatments. Furthermore, the follow-up period of 27 months is somewhat limited. In vaccine development, longer term immunogenicity is usually addressed as a post-marketing authorisation commitment. Inclusion of a real placebo control group (e.g. receiving saline) could allow a more robust analysis of safety and tolerability."

In summary, the results of this trial represent a critical step towards a successful vaccine against *S. aureus*-associated toxic shock syndrome and support its continued development and testing in young adolescents.

Contributors

BJ and MME designed the trial. CF, CS, GG, and BJ recruited participants. GG, CS, MMS, NB, UD, AT, CF and BJ conducted the study at the site. DH, LS, and AR did the binding and neutralising antibody testing. NM performed animal experiments. MD and JL performed the statistical analysis. GG and AR prepared figures and tables. AR, GG, MME, and BJ interpreted the data. GG, CS and BJ wrote the first version of the report. All authors approved the final submitted report.

Data sharing statement

Data can be made available upon reasonable request by a qualified researcher to the corresponding author.

The trial protocol is provided in the Supplementary Appendix.

Declaration of interests

GG, CS, CF, MMS, NB, UD, AT, and BJ declare no competing interests. Martha M. Eibl was the owner of Biomedizinische Forschung & Bio-Produkte AG. DH, LS, NM, and AR are employees of the study funder Biomedizinische Forschung & Bio-Produkte AG, a biotechnology company engaged in the development of BioMed rTSST-1v.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2023.102404.

References

1 Davis JP, Chesney PJ, Wand PJ, LaVenture M. Toxic-shock syndrome: epidemiologic features, recurrence, risk factors, and prevention. *N Engl J Med*. 1980;303(25):1429–1435.
2 Burnham JP, Kollef MH. Understanding toxic shock syndrome. *Intensive Care Med*. 2015;41(9):1707–1710.
3 Berger S, Kunerl A, Wasmuth S, Tierno P, Wagner K, Brugger J. Menstrual toxic shock syndrome: case report and systematic review of the literature. *Lancet Infect Dis*. 2019;19(9):e313–e321.
4 DeVries AS, Lesher L, Schlievert PM, et al. Staphylococcal toxic shock syndrome 2000-2006: epidemiology, clinical features, and molecular characteristics. *PLoS One*. 2011;6(8):e22997.
5 Billon A, Gustin MP, Tristan A, et al. Association of characteristics of tampon use with menstrual toxic shock syndrome in France. *eClinicalMedicine*. 2020;21:100308.
6 Contou D, Colin G, Travert B, et al. Menstrual toxic shock syndrome: a French nationwide multicenter retrospective study. *Clin Infect Dis*. 2022;74(2):246–253.
7 Gaventa S, Reingold AL, Hightower AW, et al. Active surveillance for toxic shock syndrome in the United States, 1986. *Rev Infect Dis*. 1989;11(Suppl 1):S28–S34.
8 Vergeront JM, Stolz SJ, Crass BA, Nelson DB, Davis JP, Bergdoll MS. Prevalence of serum antibody to staphylococcal enterotoxin F among Wisconsin residents: implications for toxic-shock syndrome. *J Infect Dis*. 1983;148(4):692–698.
9 Reingold AL, Hargrett NT, Dan BB, Shands KN, Strickland BY, Broome CV. Nonmenstrual toxic shock syndrome: a review of 130 cases. *Ann Intern Med*. 1982;96(6 Pt 2):871–874.
10 Descloux E, Perpoint T, Ferry T, et al. One in five mortality in non-menstrual toxic shock syndrome versus no mortality in menstrual cases in a balanced French series of 55 cases. *Eur J Clin Microbiol Infect Dis*. 2008;27(1):37–43.

Fig. 4: Neutralising antibody titres for the expression of T cell-associated cytokines IFN-γ, IL-2, IL-6, and TNF-α in rTSST-1v recipients and the control group obtained before the first immunisation (day 0), 3 months after day 0 (idealized; = 3 months after the first immunisation), 12 months after day 0 (idealized; = 3 months after the third immunisation), and 24 months after day 0 (idealized; = 15 months after the third immunisation). For detailed methodology, please refer to the appendix. Horizontal lines correspond to geometric means and 95% confidence intervals.

11 Gampfer J, Thon V, Gulle H, Wolf HM, Eibl MM. Double mutant and formaldehyde inactivated TSST-1 as vaccine candidates for TSST-1-induced toxic shock syndrome. *Vaccine*. 2002;20(9-10):1354–1364.
12 Schwameis M, Roppenser B, Firbas C, et al. Safety, tolerability, and immunogenicity of a recombinant toxic shock syndrome toxin (rTSST)-1 variant vaccine: a randomised, double-blind, adjuvant-controlled, dose escalation first-in-man trial. *Lancet Infect Dis*. 2016;16(9):1036–1044.
13 Kum WW, Wood JA, Chow AW. A mutation at glycine residue 31 of toxic shock syndrome toxin-1 defines a functional site critical for major histocompatibility complex class II binding and superantigenic activity. *J Infect Dis*. 1996;174(6):1261–1270.
14 McCormick JK, Tripp TJ, Llera AS, et al. Functional analysis of the TCR binding domain of toxic shock syndrome toxin-1 predicts further diversity in MHC class II/superantigen/TCR ternary complexes. *J Immunol*. 2003;171(3):1385–1392.
15 Stich N, Model N, Samstag A, Gruener CS, Wolf HM, Eibl MM. Toxic shock syndrome toxin-1-mediated toxicity inhibited by neutralizing antibodies late in the course of continual in vivo and in vitro exposure. *Toxins (Basel)*. 2014;6(6):1724–1741.
16 Botelho-Nevers E, Verhoeven P, Paul S, et al. Staphylococcal vaccine development: review of past failures and plea for a future evaluation of vaccine efficacy not only on staphylococcal infections but also on mucosal carriage. *Expert Rev Vaccines*. 2013;12(11):1249–1259.
17 Salgado-Pabon W, Schlievert PM. Models matter: the search for an effective staphylococcus aureus vaccine. *Nat Rev Microbiol*. 2014;12(8):585–591.
18 Waclavicek M, Stich N, Rappan I, Bergmeister H, Eibl MM. Analysis of the early response to TSST-1 reveals Vbeta-unrestricted extravasation, compartmentalization of the response, and unresponsiveness but not anergy to TSST-1. *J Leukoc Biol*. 2009;85(1):44–54.
19 Speich B, Chammartin F, Abela IA, et al. Antibody response in immunocompromised patients after the administration of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine BNT162b2 or mRNA-1273: a randomized controlled trial. *Clin Infect Dis*. 2022;75(1):e585–e593.
20 Watson-Jones D, Changalucha J, Whitworth H, et al. Immunogenicity and safety of one-dose human papillomavirus vaccine compared with two or three doses in Tanzanian girls (DoRIS): an open-label, randomised, non-inferiority trial. *Lancet Glob Health*. 2022;10(10):e1473–e1484.
21 Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N Engl J Med*. 2020;383(27):2603–2615.
22 Proctor RA. Immunity to Staphylococcus aureus: implications for vaccine development. *Microbiol Spectr*. 2019;7(4).
23 Marrack P, Kappler J. The staphylococcal enterotoxins and their relatives. *Science*. 1990;248(4956):705–711.
24 Tuffs SW, Haeryfar SMM, McCormick JK. Manipulation of innate and adaptive immunity by staphylococcal superantigens. *Pathogens*. 2018;7(2):53.
25 Schlievert PM, Cahill MP, Hostager BS, et al. Staphylococcal superantigens stimulate epithelial cells through CD40 to produce chemokines. *mBio*. 2019;10(2):e00214–e00219.
26 Hong HA, Ke NT, Nhon TN, et al. Validation of the combined toxin-binding inhibition test for determination of neutralizing antibodies against tetanus and diphtheria toxins in a vaccine field study in Viet Nam. *Bull World Health Organ*. 1996;74(3):275–282.
27 Amanna IJ, Carlson NE, Slifka MK. Duration of humoral immunity to common viral and vaccine antigens. *N Engl J Med*. 2007;357(19):1903–1915.
28 Bergdoll MS, Crass BA, Reiser RF, Robbins RN, Davis JP. A new staphylococcal enterotoxin, enterotoxin F, associated with toxic-shock-syndrome Staphylococcus aureus isolates. *Lancet*. 1981;1(8228):1017–1021.
29 Bergdoll MS, Crass BA, Reiser RF, et al. An enterotoxin-like protein in Staphylococcus aureus strains from patients with toxic shock syndrome. *Ann Intern Med*. 1982;96(6 Pt 2):969–971.
30 Chen WH, Pasetti MF, Adhikari RP, et al. Safety and immunogenicity of a parenterally administered, structure-based rationally modified recombinant staphylococcal enterotoxin B protein vaccine, STEBVax. *Clin Vaccine Immunol*. 2016;23(12):918–925.