CLINICAL STUDY PROTOCOL

Study Name:

Phase 2 Clinical Trial of the BioMed rTSST-1 Variant Vaccine in Healthy Adults

Study identifying number:

BioMed 0515

Version 1.6 / March 20, 2020

Test drug (IMP) and **Pharmaceutical Company**	rTSST-1 Variant Vaccine Biomedizinische Forschung und Bio-Produkte AG Lazarettgasse 19, 1090 Vienna Austria
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2. PROTOCOL SYNOPSIS

<table>
<tr><td>TITLE</td><td>Phase 2 Clinical Trial of the BioMed r-TSST-1 Variant Vaccine in Healthy Adults</td></tr>
<tr><td>OBJECTIVES</td><td>Primary Objective
To demonstrate the safety and tolerability of two doses of the BioMed rTSST-1 Variant Vaccine after one to three vaccinations in healthy adults.

Secondary Objectives

Secondary objectives of the study are to assess the immunogenicity and persistence of TSST-1 binding and neutralizing antibodies after one, two, and three immunizations with two doses of the vaccine in healthy adults over a period of 12-14 or optionally 27-29 months.</td></tr>
<tr><td>DESIGN / PHASE</td><td>Prospective, single center, randomized, parallel group, double-blind, adjuvant controlled, phase 2 study.</td></tr>
<tr><td>STUDY PLANNED DURATION</td><td>First subject First visit Q1 2016 Last subject First visit Q1 2018 Last subject Last visit Q3 2020</td></tr>
<tr><td>CENTER(S) / COUNTRY(IES)</td><td>One
Austria</td></tr>
<tr><td>SUBJECTS / GROUPS</td><td>Each subject of a total of seven groups will receive three injections (first injection day 0; second injection 3 months ± 4 weeks after the first, third injection 6 months ± 4 weeks after the second) of one of two different doses of Vaccine or Adjuvant, each group comprising 20 subjects, as shown below:

<table>
<tr><th>Group</th><th>Sample Size</th><th>Vaccine Strength</th><th>Number of Immunizations</th><th>Adjuvant Al(OH)3</th><th>Number of Immunizations</th></tr>
<tr><td>1</td><td>20</td><td>10 µg</td><td>1</td><td>1 mg</td><td>2</td></tr>
<tr><td>2</td><td>20</td><td>10 µg</td><td>2</td><td>1 mg</td><td>1</td></tr>
<tr><td>3</td><td>20</td><td>10 µg</td><td>3</td><td>1 mg</td><td>0</td></tr>
<tr><td>4</td><td>20</td><td>100 µg</td><td>1</td><td>1 mg</td><td>2</td></tr>
<tr><td>5</td><td>20</td><td>100 µg</td><td>2</td><td>1 mg</td><td>1</td></tr>
<tr><td>6</td><td>20</td><td>100 µg</td><td>3</td><td>1 mg</td><td>0</td></tr>
<tr><td>7</td><td>20</td><td>---</td><td>---</td><td>1 mg</td><td>3</td></tr>
</table>

Subjects will be controlled 24 h post vaccination. Follow-up will last 3 or optionally 18 months on the average, with visits every three months (± 2 weeks).

Response to treatment is defined by seroconversion from a TSST-1 binding Ab titer of < 20 to > 40 or a 4-fold increase in TSST-1 binding Ab titer.</td></tr>
<tr><td>SCREENING</td><td>Over a period of sixty days prior to first vaccination, subjects will be screened for eligibility using the criteria below, plus HIV Ab, HCV Ab, HB_s Ag, and binding and neutralizing TSST-1 Ab.</td></tr>
<tr><td>INCLUSION CRITERIA</td><td>• males or females aged 18-64 years</td></tr>
</table>

	• signed informed consent • physical exam: no abnormal findings unless considered irrelevant by the investigator • uneventful medical history • Females with childbearing potential: adequate contraception
EXCLUSION CRITERIA	• females with childbearing potential: pregnancy, lactation or unreliable contraception • positive HIV Ab and/or positive HCV Ab and/or positive HBsAG • signs and symptoms of autoimmunity • TSST-1 Ab titer $\geq$ 1:1000 • current or recent (< 1 month) immunosuppressive therapy with corticosteroids or immunomodulators
STUDY PERIODS	• Screening: 60 days • Immunization: 9 months (± 8 weeks) • Follow-up: 3 months (± 2 weeks) or optionally 18 months (± 2 weeks) • Total: 12 – 14 months or optionally –27-29 months
INVESTIGATIONAL DRUG	rTSST-1 variant vaccine 10 µg and 100 µg
COMPARATIVE DRUG /CONTROL CONDITION	$Al(0H)_3$
CONCOMITANT MEDICATION	No concomitant medication allowed that would interfere with the assessment of trial specific results as assessed by the investigator.
TOLERABILITY / SAFETY (PRIMARY) ENDPOINTS	Adverse events Abnormal laboratory findings Local reactions
EFFICACY ENDPOINTS	TSST-1binding and neutralizing Ab Persistence of TSST-1 binding and neutralizing Ab
EXPLORATORY ENDPOINTS	 2nd test of neutralizing Ab
PARAMETERS	**Vital Signs:** BP, PR, body temperature **Hematology:** RBC WBC Leukocyte differential count (absolute values of neutrophils, basophils, eosinophils, monocytes, and lymphocytes) Platelet count Hemoglobin Hematocrit **Clinical Chemistry:** ALT BUN Creatinine Glucose Total protein **C-Reactive Protein** **TSST-1 Ab (binding and neutralizing)** **Local Reactions** **Adverse Events**

	Tests to be performed prior and 24 h (± 3 h) after each vaccination, and every three months (± 2 weeks) during the follow-up period. In the case of abnormal findings, the respective tests will be repeated in between. TSST-1 Ab tests will be performed prior to each vaccination and at the three-month follow-up visits .
STATISTICS	**Primary Endpoints:** Descriptive **Other Endpoints:** Antibody titers will be analyzed by one-way or repeated measures ANOVA. Subjects with antibody titers of 200 to 1000 at entry will be stratified. Mann-Whitney-U and Wilcoxon signed rank tests will be used post hoc for comparison among groups and Ab persistence, respectively, to determine statistical significance. A statistical difference, if any, in the rate of Ab response among treatment groups and between treatment and control groups will be tested by Chi square statistics.

3. LIST OF ABBREVATIONS

Ab	antibody
Ag	antigen
AE	adverse event
AGES	Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH
AMG	Arzneimittelgesetz
ALT	alanine aminotransferase
BASG	Bundesamt für Sicherheit im Gesundheitswesen
BP	blood pressure
BUN	blood urea nitrogen
b.w.	body weight
C	centigrade, Celsius
CA	competent authority
CBC	complete blood count
CRF	case report form
CSR	clinical study report
DOH	Declaration of Helsinki
d	day
EC	Ethics Committee
ECG	electrocardiogram
ELISA	enzyme-linked immunosorbent assay
EOS	End of Study
EudraCT	European Clinical Trial Database
GCP	Good Clinical Practice

h	hour (s)
HBsAg	hepatitis B virus surface antigen
HCV	hepatitis C virus
HIV	human immune deficiency virus
IB	Investigator's Brochure
IEC	Institutional Ethics Committee
ICH	International Conference on Harmonization
ID	identification
IgG	immunoglobulin G
i.m.	intramuscular(ly)
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
kg	kilogram
LLT	lower level term
n.a.	not applicable
ng	nanogram
µg	microgram
mg	milligram
MedRA	Medical Dictionary for Regulatory Activities
MHC	major histocompatibility complex
min	minute (s)
ml	milliliter
MNC	mononuclear cells
n.a.	not applicable
neg	negative
PBS	phosphate buffered saline
PI	principal investigator
PR	pulse rate
RBC	red blood count
RT	room temperature
rTSST-1	recombinant TSST-1
SAE	serious adverse event
S.aureus	Staphylococcus aureus
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SUSAR	suspected unexpected serious reaction
TCR	T cell receptor
TSS	toxic shock syndrome
TSST-1	toxic shock syndrome toxin –1
Vβ chain	Variable beta chain
WBC	white blood count
WMA	World Medical Association

4. TABLE OF CONTENTS

TABLE 1. DOSES AND SAMPLE SIZES

GROUPS:	TREATMENT :			CONTROL :		
	DOSES Adjuvanted rTSST-1 Variant Vaccine containing antigen below	**VOLUME**	**Number of Administrations**	**Adjuvant containing $Al(0H)_3$ as shown below**	**VOLUME**	**Number of Administrations**
1	10 µg	0.5 ml	1	1.0 mg	0.5 ml	2
2	10 µg	0.5 ml	2	1.0 mg	0.5 ml	1
3	10 µg	0.5 ml	3	1.0 mg	0.5 ml	---
4	100 µg	0.5 ml	1	1.0 mg	0.5 ml	2
5	100 µg	0.5 ml	2	1.0 mg	0.5 ml	1
6	100µg	0.5 ml	3	1.0 mg	0.5 ml	---
7	---	--	--	1.0 mg	0.5 ml	3

Sample Size is 20 in each Group

TABLE 2 . VISIT AND ASSESSMENT SCHEDULE FOR Groups 1 - 7

PERIODS	**Name**	**SCREENING**	**TREATMENT**						**FOLLOW-UP**					
	Duration		9 months (± 8 weeks)						18 months (± 2 weeks)					
VISITS	Number	1	2	3	4	5	6	7	8	9*	10*	11*	12*	13*
	Name	Screening	Randomization / Immunization 1	Control 1	Immun 2	Control 2	Immun 3	Control 3	Fup 1	Fup 2*	Fup 3*	Fup 4*	Fup 5*	Fup 6*
	Time	- 60 days to Day 0	Day 0	Day 1 (24 h post Imm 1)	Mo° 3**	24 h post Imm 2	Mo°° 6**	24 h post Imm 3	Mo° 12**	Mo° 15**	Mo°* 18**	Mo° 21**	Mo°* 24**	Mo° 27**
Informed Consent		X												
Inclusion Exclusion Criteria		X	X											
Medical History		X	X											
Concomitant Medication		X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Examination		X												
Body Weight and Height		X												
Vital Signs (BP, PR, temperature)		X	X	X	X	X	X	X	X	X	X	X	X	X
Hematology		X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical Chemistry		X	X	X	X	X	X	X	X	X	X	X	X	X
C-reactive Protein		X	X	X	X	X	X	X	X	X	X	X	X	X
TSST-1 binding Ab		X	X		X		X		X	X	X	X	X	X
Neutralizing Ab: inhibition of cytokine gene expression		X	X		X		X		X	X	X	X	X	X
Neutralizing Ab: Inhibition of T-cell activation		X	X		X		X		X	X	X	X	X	X
Local Reactions				X	X	X	X	X	X					
Adverse Events				X	X	X	X	X	X	X	X	X	X	X
Pregnancy Test		X	X		X		X							

° months from first immunization °° months from second immunization *optional **idealized

5. BACKGROUND INFORMATION

5.1 Background

The incidence of community or hospital acquired infections is about 500 000 in each, Europe and the U.S.A. Some 20 % of the patients with such infections require intensive care either immediately or after some time. Of the patients in intensive care, about 50% - 70% can be saved, however, many of them suffer from late sequelae, persisting for years. About 10% to 20% of the hospital or community acquired infections are caused by S.aureus. Most strains of *S. aureus* produce toxins, such as superantigens, including the Toxic Shock Syndrome Toxin-1 (TSST-1). These toxins cause significant illnesses, including pneumonia, acute kidney injury, infective endocarditis, and toxic shock syndrome (TSS) (Salgado-Pabon 2013). TSST-1 alone or in combination with other S.aureus virulence factors (such as SEA, SEB, and SEC) leads to toxic shock sydrome (TSS).

TSST-1 belongs to the pyrogenic toxin class of superantigens and plays a pivotal role as a causative agent in TSS and septic shock (Dinges et al.2000; McCormick et al.2001). Superantigens are produced by both methicillin-resistant and sensitive S.aureus strains (Hu et al 2008). It has been estimated that TSST-1 is involved in 50 % of nonmenstrual-associated TSS cases due to S. aureus and essentially all cases of menstrual-associated TSS (Dinges et al.2000). Experimental application of superantigens leads to an overwhelming inflammatory response (Hopkins et al.2005; Krakauer 2003; Dauwalder et al.2006) with uncontrolled release of inflammatory cytokines resulting in shock and multi organ failure (Miethke et al.1993; Faulkner et al.2005; Krakauer et al.2010; Dinges et al.2001; Stich et al.2010).

Treatment of infections with antibiotics has become increasingly difficult due to the widespread occurrence of antibiotic-resistant S. aureus strains (Chambers 1997; McCormick et al. 2001; Foster et al.2005; Zetola et al., 2005). The prevalence of multiresistant organisms such as Methicillin-resistant *S. aureus* is strongly increasing (Zetola et al. 2005). This being the case, it is generally agreed upon that it is desirable to develop a vaccine and specific immunoglobulin for active and passive immunization which could be used also in combination with antibiotics to save a greater number of patients and/or shorten the persistence of severe sequelae shortening patients´ lives.

Therapies have included the application of intravenous immunoglobulin (IVIG) which have been useful also in clinical trials (Kaul et al. 1998). Numerous efforts have been undertaken to develop a specific therapy (Krakauer 2013).

In a rabbit model, animals were immunized with atoxicTSST-1 mutants three times. Immunization protected them when challenged one week after the last immunization with otherwise lethal doses of native TSST-1 in miniosmotic pumps (Spaulding et al., 2012). In another model, 83% of mice that were vaccinated three times with mutant TSST-1 survived when challenged with viable *S. aureus* (Hu et al., 2003). Other experiments in mice showed that immunizations three times with mutants of the superantigens SEA, SEB, SEC1, or TSST-1 led to protection of the mice upon subsequent challenge with native superantigens plus endotoxin (Bavari et al, 1999).

<u>The BioMed r-TSST-1 Variant and Vaccine:</u>

Based on information on the structure and functional relationship of S. aureus TSST-1, mutants have been established with the objective of eradicating the toxic and superantigenic properties, while

leaving specific immunogenicity and protectivity unimpaired (Roggiani et al.2000; Ulrich et al 1998; Gampfer et al.2002). After molecular characterization and thorough biological and immunological testing, a TSST-1 double mutant (G31R-H135A) has been chosen for the rTSST-1 Variant Vaccine (Gampfer et al.2002). Amino acid changes in position 31 have been shown to result in impaired interaction with MHC class II molecules (Kum et al.1996), whereas mutation of histidine 135 to alanine negatively affects the binding of TSST-1 to the T cell receptor (McCormick et al.2003). The double mutant has been expressed in E. coli BL21, purified by chromatography and formulated with $Al(OH)_3$ as a vaccine, this being referred to as the BioMed rTSST-1 Variant Vaccine.

Proof of concept:

A non-clinical, prospective, controlled, randomized open Immunogenicity and Protection Study (Study Bio&Bio 031114) was conducted in conformity with GLP to evaluate the immogenicity and protection of the BioMed rTSST-1 Variant Vaccine in a rabbit model.

TSST-1 antibody negative rabbits were randomized and immmunized with the test or the reference item four times at three-week intervals. Blood samples were drawn prior to entry (baseline), one day prior to the second, third, and fourth immunizations, and three weeks after the fourth immunization to determine TSST-1 antibody levels. Thereafter, animals in both groups were challenged with TSST-1 and four hours later with lipopolysaccharide (LPS) and observed for survival for five days.

Results: 20 female rabbits weighing 1,672 g on average with TSST-1 antibody titers of < 20 were randomly assigned to Group 1 (adjuvanted vaccine) or Group 2 (adjuvant alone). TSST-1 antibody titers in the immunized group rose to a GM of 624 as early as three weeks after the 2nd immunization and reached a GM of 22,328 and 37,217 three weeks after the third and fourth, respectively. In Group 2, TSST-1 antibody titers remained < 20 throughout. All animals in Group 1 seroconverted (i.e. had > 4-fold increases in TSST-1 antibody titer with respect to baseline) after the third immunization. None did so in Group 2 ($p < 0.001$). All animals in Group 1 survived the challenge with TSST-1 and LPS, while all animals in Group 2 succumbed ($p < 0.001$).

No evidence of superantigenicity:

Non-clinical experiments *in vivo* have shown that rTSST-1 variant lacks super-antigen activity and does not lead to T-cell activation or induce an increase in IL-2 gene activation in the spleen of rabbits. Lack of super-antigen activity has also been demonstrated in mice. IL-2 concentrations remain at background level after injection of rTSST-1 variant.

Non-clinical experiments *in vitro* have shown r-TSST-1 variant not to induce IL-2 gene activation up to concentrations of 1000 ng/ml in human mononuclear cells proving rTSST-1 variant to be detoxified. Lack of activation and release of inflammatory cytokines could be proven. rTSST-1 variant lost its super-antigen property by the detoxifying mutations. rTSST-1 variant does not induce T-cell proliferation of human mononuclear cells as estimated by H_3thymidin incorporation in concentrations up to 01 to 1µg. It lacks the characteristics of a super-antigen and behaves as a normal antigen.

No evidence of toxicity:

rTSST-1 variant was tested in rabbits in doses up to 1 mg per rabbit , and doses this high were well tolerated

in vivo experiments in rabbits have shown that the rTSST-1 Variant Vaccine antigen does not induce fever, leukopenia or lympohopenia . Studies performed in mice and rabbits have demonstrated that super-antigen activity and toxicity with respect to activation and release of the inflammatory cytokines TNFα , INF-γ, and IL6 have been eliminated due to the mutations in rTSST-1 variant. Suprantigen toxins are known to increase the lethal effect of endotoxin (LPS) up to over 1000-fold. rTSST-1 variant has been extensively tested in rabbits and mice and did not increase LPS lethality.

A prospective, controlled, randomized, open repeated dose toxicity and local tolerance study of the BioMed rTSST-1 Variant Vaccine (Study Bio & Bio 101115) was conducted in conformity with GLP to evaluate the toxicity and local tolerance of the Biomed rTSST-1 Variant Vaccine after repeated applications in a rabbit model.

Ten male and ten female rabbits were randomly assigned to one of two groups: Group 1 received 1 ml 30 µg of rTSST-1 Variant Vaccine, Group 2 was given 1 ml of adjuvant ($Al(OH)_3$) four times at intervals of three weeks. Blood was drawn prior to the first immunization and at specified time points during the study for hematology, coagulation, clinical chemistry, electrolytes, C-reactive protein, antibody against antigenic substances that can cross react with human tissue, and immune complexes. TSST-1 antibody was determined prior to the fourth immunization. Vital signs, body weight, and intake of food and water were monitored. Clinical and physical observations were made on scheduled days. General health was assessed daily. Local tolerance was determined by visual inspection before and at several time points after each vaccination for indications of reactogenicity. Moribundity/mortality, clinical and physical observations and general health were monitored throughout the treatment and observation periods.

Two to three days after the last immunization, the animals were euthanized. Opthalmoscopy was performed prior to sacrifice. Post mortem examinations included gross pathology and histopathology of a number of organs, including the injection site tissue.

Results and Conclusion: This repeated dose toxicity study investigated a number of parameters including hematology, clinical chemistry,coagulation, induction of autoantibodies, CRP, local tolerance, vital signs, ophthamology, gross pathology, and histopathology. There were a few differences between groups, most of them small and gender specific and within the variability of the normal range. These variations are considered as clinically irrelevant. The study reveals that rTSST-1 Variant Vaccine does not have any toxicity at the human dose in this animal model after four immunizations.

in vitro experiments have shown that rTSST-1 variant does not induce the release of TNFα , INF-γ, and IL6 incultures of human mononuclear cells.

Summarizing, the safety of rTSST-1 variant has been shown in numerous state-of-the art experiments and non-clinical studies. Innocuity with respect to increasing LPS activity has also been demonstated up to dose levels of 10 times the intended dose to be used in humans.

Phase 1 study in healthy adults

A phase 1 dose-escalation study of 100 ng, 300 ng, 1 µg, 3 µg, 10 µg, and 30 µg the rTSST-1 Variant Vaccine in 49 healthy human subjects has been completed. There have been no product related serious expected or unexpected adverse events.

Secondary objective of the study was to assess the immunogenicity of the BioMed rTSST-1 Variant Vaccine in healthy adults.

In an investigatory study in this trial neutralizing antibodies were tested. The results demonstrated the induction of high titer neutralizing antibodies of uncontrolled T cell activation and cytokine induction (Roetzer et al. 2016; Schwameis et al. 2016).

With these results, the rTSST-1 Variant Vaccine is proposed to be tested in a phase 2 clinical trial using two doses of the vaccine in one to three immunizations to further demonstrate its safety and immunogenicity in healthy individuals and determine antibody persistence.

Residual risk

There were no safety concerns with any of the doses ranging from 100 ng to 30 µg in the phase 1 study, however, the 100 µg dose has not been tested in humans thus far. Any dose escalation is associated with a residual risk. Nevertheless, local tolerance and adverse reactions are expected to be no greater than with any other vaccine.

5.2 Study Rationale

The BioMed rTSST-1 Variant Vaccine has been developed by Biomedizinische Forschungund Bio-Produkte AG as one component of a polyvalent staphylococcal vaccine for the prevention of toxic shock and hyperimmunization of donors for the production of TSST-1 immunoglobulin.

6. STUDY OBJECTIVES (HYPOTHESIS)

6.1 Primary Objective

Primary objective of the study is to demonstrate the safety and tolerability of two doses of the BioMed rTSST-1 Variant Vaccine after one to three administrations in healthy adults.

6.2 Secondary Objectives (Hypothesis)

Secondary objectives of the study are to assess the immunogenicity and persistence of TSST-1 binding and neutralizing antibodies after one, two, and three immunizations with two doses of the vaccine in healthy adults over a period of 12-14 or optionally 27-29 months.

The hypothesis is that the higher dose and more frequent administrations of the vaccine will produce higher binding and neutralizing antibodies in vaccinated subjects and that these will persist for a longer period of time than in subjects vaccinated with the lower dose or in the control group.

7. STUDY DESIGN

This is a prospective, randomized, parallel control, phase 2 study of the safety , local tolerance, immunogenicity, and TSST-1 antibody persistence in healthy human subjects who have been

vaccinated with one of two doses of the BioMed rTSST1 Variant Vaccine or Adjuvant one, two, or three times.

Over a period of 60 days prior to entry into the study, 145 male and female subjects 18 - 64 years of age will be screened for eligibility. Screening criteria will include physical examination, medical history, pregnancy/ adequate contraception in females, HIV Ab, HCV Ab, HBs Ag and TSST-1 Ab. 140 qualifying subjects will be entered into the study.

The 140 qualifying subjects will be randomly assigned to one of seven groups of 20 each. Subjects in each group will receive three injections, the first at time 0, the second three months ± 4 weeks after the first, and the third 6 months ± 4 weeks after the second:

Group 1 will receive 10 µg of rTSST-1 Variant Vaccine and two administrations of Adjuvant ; Group 2 will receive the same dose of Vaccine twice and one dose of Adjuvant; and Group 3 will be injected the 10 µg of the Vaccine three times. Groups 4 to 6 will be given 100 µg of Vaccine following the same pattern. Group 7 will receive Al(OH3) adjuvant three times. See Table 1.

Prior to, and 24 h (±3 h) after each vaccination, the subjects will be examined for vital signs. Blood will be drawn for hematology, clinical chemistry tests, and C-reactive protein. Local reactions and adverse events will be assessed in all post vaccination visits.

The subjects will be followed up for a period of 3 months (± 2 weeks) or optionally 18 months (± 2 weeks) if they decide to take part in the long-term follow-up, during which they will return to the clinic every three months (± 2 weeks). Tests performed will include vital signs, local reactions, clinical chemistry, C-reactive protein. Adverse events will be noted. See Table 2.

Binding and neutralizing TSST-1 antibodies will be determined prior to each vaccination and every three months during the treatment and follow-up periods.

7.1 Study Population

7.1.1 Study Population

healthy male or female subjects

7.1.2 Inclusion Criteria

males or females aged 18-64 years
signed informed consent
physical exam: no abnormal findings unless considered irrelevant by the investigator
uneventful medical history
females with childbearing potential: adequate contraception (see 7.1.4 below)

7.1.3 Exclusion Criteria

females with childbearing potential: pregnancy, lactation or unreliable contraception

positive HIV Ab and/or positive HCV Ab and/or positive HBsAG

signs and symptoms of autoimmunity
TSST-1 Ab titer $\geq$ 1:1000
current or recent (< 1 month) immunosuppressive therapy with corticosteroids or immunomodulators

7.1.4 Females with Childbearing Potential

Females of childbearing potential will be required to perform pregnancy tests at screening and prior to each immunization and to be practicing an acceptable method of birth control during the study. Acceptable methods of birth control include barrier methods (including male and female condoms), diaphragms (cervical caps) with intravaginal spermicide (including jellies, foams, and suppositories), intra-uterine devices, hormonal contraceptives, or same sex relationships.

7.1.5 Study Duration

Each participant will be in the study for 12 to 14 or optionally 27 to 29 months, if they decide to take part in the long-term follow-up.

7.1.6 Withdrawal and Replacement of Subjects

Criteria for Withdrawal

Subjects may prematurely discontinue from the study at any time. Premature discontinuation from the study is to be understood when the subject did not undergo End of Study (EOS) examination and / or all pivotal assessments during the study.

Subjects must be withdrawn under the following circumstances:

- at their own request
- if the investigator feels it would not be in the best interest of the subject to continue
- if the subject violates conditions laid out in the consent form / information sheet or disregards instructions by the study personal
- if a female subject becomes pregnant
- in the case of intolerable adverse reactions

In all cases, the reason why subjects are withdrawn will be recorded in detail in the CRF and in the subject's medical records. Should the study be discontinued prematurely, all study materials (complete, partially completed and empty CRFs) will be retained.

Follow-up of Subjects Withdrawn from the Study

In case of premature discontinuation of the study, the investigations scheduled for the EOS visit will be performed at least one week after the last immunization. The subjects will be advised that participation in these investigations is voluntary. Furthermore, they may request that from the time point of withdrawal no more data will be recorded and that all biological samples collected in the course of the study will be destroyed.

Replacement Policy

Drop outs may be replaced by a subject next in line for recruitment. The replacement subject will be allocated to the same group the drop-out was in. All available data on the drop-out will be evaluated.

7.1.7 Premature Termination of the Study

The sponsor has the right to close this study at any time. The IEC and the competent regulatory authority must be informed within 15 days of early termination.

The trial or single dose steps will be terminated prematurely in the following cases:

- If adverse events occur which are so severe that the benefit-risk ratio is not acceptable.
- If the number of dropouts is so high that proper completion of the trial cannot realistically be expected.

8. METHODOLOGY

8.1 Study Medication

<u>Test Preparation</u>

Active Agent and Characteristics:

TSST-1 is a staphylococcus toxin with the characteristics of superantigens. Toxicity of TSST-1 is brought about by cross-linking of MHC class II molecules on the antigen presenting cell with the Vβ chain and the T-cell receptor on the T-cell. This way, 5 to 30% of the entire T-cell population may be activated.

The Toxin was cloned from S. aureus and is genetically modified to lose its toxic properties. Specific amino acids that determine the binding sites for the MHC and the T-cell receptor have been changed so that TSST-1 cannot bind to either receptor, and the toxic characteristics are lost.

Mutagenesis of the TCR binding site is performed by exchange of histidine 135 to alanine. The MHC class II binding site is mutated by exchanging amino acid 31 from glycine to arginine.

While the application of TSST-1 is followed by fever, leukopenia and lymphopenia in rabbits, and an increase of inflammatory cytokines in human mononuclear cells as well as in the serum of mice, these toxic characteristics are lost in the rTSST-1 variant vaccine antigen.

The recombinant detoxified toxin is used as the vaccine antigen.

Trade Name of the Agent: BioMed rTSST-1 Variant Vaccine

Manufacturer: Biomedizinische Forschung und Bio-Produkte AG
Lazarettgasse 19
A 1090 Vienna Austria

Drug Supply:

The final pharmaceutical product is presented in 2.0 ml single dose vials containing the following quantities of rTSST-1 variant and 1.0 mg $Al(OH)_3$ in 0.5 ml PBS with 0.02 % polysorbate 80 (with overfill) :

10 µg rTSST-1 Variant

100 µg rTSST-1 Variant

Dosage Form: Suspension for i.m. injection.

Storage Instructions: 2 - 8°C protected from light

Route of Administration: i.m.

<u>Reference Preparation</u>:

Aluminum hydroxide suspension in phosphate buffered saline (vaccine adjuvant).

Trade Name: BioMed Aluminum Hydroxide Suspension 1 mg

Manufacturer: Biomedizinische Forschung und Bio-Produkte AG
Lazarettgasse 19
A 1090 Vienna
Austria

Drug Supply:

The reference preparation is presented in 2.0 ml single dose vials containing 1.0 mg $Al(OH)_3$ in 0.5 ml PBS and 0.02 % and polysorbate 80 (with overfill) .

Dosage Form: Suspension for i.m. injection

Storage Conditions: 2 - 8° C

Route of Administration: i.m.

8.1.1 Dosage and Administration

Test Preparation (Treatment Groups)

Preparation of doses for administration:

rTSST-1 Variant Vaccine containing 10 µg rTSST-1:
draw 0.5 ml from a vial containing Vaccine with 10 µg rTSST-1 Variant into a disposable syringe.

rTSST-1 Variant Vaccine containing 100 µg:
draw 0.5 ml from a vial containing Vaccine with 100 µg rTSST-1 Variant into a disposable syringe

Route of administration: i.m.

Administer 0, 1, 2, or 3 times depending on Group assignment. See Table 1.

Reference Preparation (Control Group)

Preparation for administration:

Adjuvant containing 1 mg $Al(0H)_3$:

Draw 0.5 ml from a vial containing Adjuvant into a disposable syringe.

Route of administration: i.m.

Administer 0, 1, 2 or 3 times depending on Group assignment. See Table 1.

8.1.2 Study-Drug up- and down Titration

n.a.

8.1.3 Study Drug Interruption or Discontinuation

The investigator must temporarily interrupt or permanently discontinue the study drug if continued administration of the study drug is believed to be contrary to the best interests of the subject.
The interruption or premature discontinuation of study drug might be triggered by an AE, a diagnostic or therapeutic procedure, an abnormal assessment (e.g., laboratory abnormalities), or for administrative reasons, in particular withdrawal of the subject's consent.
The reason for study drug interruption or premature permanent discontinuation must be documented in the CRF.

8.1.4 Study Drug Interruption

See 8.1.3. above.

8.1.5 Study Drug Premature Permanent Discontinuation

See 8.1.3. above

Study drug premature permanent discontinuation due to an adverse event

If the reason for premature permanent discontinuation of study treatment is an AE, the subject will have a "Premature End of Study (EOS)" visit with all the assessments performed before the study drug discontinuation, whenever possible.

Study drug premature permanent discontinuation due to another reason than adverse event

The investigator may discontinue a subject if he feels it is in his best interest.

If the reason for premature permanent discontinuation of study treatment is not an AE, the subject will be withdrawn from the study (withdrawal of consent) and have the end of study (EOS) visit with all the assessments performed before the study drug discontinuation, whenever possible.

8.1.6 Study Drug Delivery & Drug Storage Conditions

Test and reference preparations will be delivered in 2 ml vials containing 0.5 ml of Vaccine or adjuvant. Store at 2 - 8° C.

8.1.7 Study Drug Packaging and Labeling

Study drug and reference preparations will be delivered to the study pharmacist labeled as shown below:

Inner label:

See Appendix 1.

Outer label:

See Appendix 1.

8.1.8 IMP Administration & Handling

Bring to RT before injection. Invert before use. Draw 0.5 ml from a vial containing Vaccine with 10 or 100 µg rTSST-1 Variant or Adjuvant into a 1 ml graduated syringe. See Section 8.1.1. above.

8.1.9 Drug Accountability

Drug accountability will be recorded on an ongoing basis in paper form.

8.1.10 Procedures to Assess Subjects Compliance

A diary will be used for the subject to record any adverse events between visits to the clinic.

8.1.11 Concomitant Medication

No concomitant medication will be allowed that would interfere with the assessment of trial specific results as assessed by the investigator.

8.2 Randomization

Subjects will be randomized to receive either 10 µg or 100 µg of Vaccine or $Al(OH)_3$ control one to three times. Block randomization will be used.

Since the 100 µg dose will be administered for the first time, the first five blocks will provide for an only gradually increasing number of subjects to receive that dose as shown below.

TABLE 3. RANDOMIZATION DURING FIRST FIVE STUDY WEEKS

Group	Block 1 n=5 Week 1	Block 2 n=5 Week 2	Block 3 n=5 Week 3	Block 4 n= 6 Week 4	Block 5 n=14 Week 5
1	1	1	1	1	1
2	1	1	1	1	1
3	1	1	1	1	1
4					5
5				1	4
6	1	1	1	1	1
7	1	1	1	1	1

All subsequent blocks will ensure equal distribution of subjects among groups.

Envelopes will be prepared with consecutive subject numbers containing the group allocation.

Subjects vaccinated in blocks 1 – 4 will be followed by scripted telephone calls one and two weeks after vaccination for adverse events, if any.

8.3 Blinding

Study drug and reference preparations will be delivered to the study pharmacist open label. Since the study is to be conducted in a double blinded manner, they will be prepared by a pharmacist or other member of the study team not otherwise involved in the trial and be delivered in ready-to use syinges to the investigator blinded.

8.3.1 Emergency Procedure for Unblinding

In the case of serious adverse events, the investigator will have access to the individual code.

Sealed envelopes containing true treatment assigned will be stored in the investigator's study file. The premature breaking of the code should be confined to emergency cases in which knowledge of the administered drug is necessary for adequate treatment. Breaking of the code must be recorded in the subject file, the CRF and on the envelope (date and reason). The CRA and the sponsor will be notified of each unblinding event by PI. Should any code be broken by accident, the respective

subject will be withdrawn from further participation in the study and a written explanation must be given by the PI. Open and sealed envelopes must be returned to the monitor at the close out visit.

8.3.2 Unblinding at the End of the Study.

Unblinding will take place after data base lock and the BDRM (Blind Data Review Meeting).

8.4 Benefit and Risk Assessment

At present, immediate benefits cannot be expected for study participants. The aim of the research project is to develop a vaccine against a potentially fatal disease, and the study will help to gain knowledge and contribute toward that end.

Extensive studies in vitro, in experimental animals and a phase 1 study in man have shown the vaccine to be safe and tolerable. Nevertheless, risks may be related to the administration of a higher dose of the investigational product and are expected to be comparable to the ones associated with the administration of any vaccine, such as local reactions and/or fever and/or severe adverse events that might make immediate medical intervention necessary. For that reason, study subjects will be monitored during the study by the investigator and his team.

8.5 Study Procedures

8.5.1 General Rules for Trial Procedures

- All study measures like blood sampling and measurements will be documented with date (dd:mm:yyyy).
- In case several study procedures are scheduled at the same time point, there is no specific sequence that should be followed.
- The dates of all procedures will be according to the protocol. The time margins will be $\pm$ 4 weeks for the second immunization, and $\pm$ 4 weeks for the third. For the controls at 24 h of any immunization, the window will be $\pm$3 hours. Follow-up visits must be made $\pm$ two weeks of the time points given in Table 2. If for any reason, a study procedure is not performed within scheduled margins, a protocol deviation will be noted, and the procedure will be performed as soon as possible or as adequate.

8.5.2 Screening Investigation

Over a period of 60 days prior to entry into the study, subjects will be screened for the parameters shown in Table 2., i.e.

Inclusion criteria: males or females aged 18-64 years

physical exam: no abnormal findings unless considered irrelevant by the investigator

uneventful medical history
females with childbearing potential: adequate contraception

Exclusion criteria:

females with childbearing potential: pregnancy, lactation or unreliable contraception

positive HIV Ab and/or positive HCV ab and/or positive HBsAG

TSST-1 Ab ≥ 1:1000

current or recent (< 1 month) immunosuppressive therapy with corticosteroids or immunomodulators

Medical history
Concomitant medication
Body weight and height

Vital signs (BP, PR, temperature)
Hematology (see 8.5.3. below)
Clinical chemistry (see 8.5.3. below)
C-reactive protein
TSST-1 Ab (binding and neutralizing)
HIV Ab
HCV Ab
HB_sAg
Pregnancy test

Informed consent will be invited.

8.5.3 Laboratory Tests

The following laboratory tests will be performed on blood taken at screening, prior to each immunization, 24 h thereafter, and at the follow-up visits shown in Table 2. In the case of abnormal findings, the respective tests will be repeated in between.

Hematology: RBC
WBC
Leukocyte differential count (absolute values of neutrophils, basophils,eosinophils, monocytes, and lymphocytes)

Platelet count
Hemoglobulin
Hematocrit

Clinical Chemistry: ALT
BUN
Creatinine
Glucose
Total Protein

C-reactive Protein

TSST-1 Ab (binding and neutralizing) will be performed prior to each vaccination and at the the three-month follow-up visits.

Tests will require 3 ml of EDTA blood and 9 ml of serum or plasma on each occasion.

Tests for TSST-1 Ab will be performed by Biomedizinische Forschungsgesellschaft m.b.H.

8.5.4 Other Tests

At the times indicated under 8.5.3., vital signs (BP, PR and temperature) will be monitored at all visits. Pregnancy tests will be performed prior to each immunization. Local reactions will be monitored after each immunization and at the first three-month follow-up visit (with the windows described under 8.5.1.) Subjects will be required to keep a diary for adverse events after leaving the clinic and present it to the investigator for review at each visit.

8.5.5 Endpoints

Primary (Safety) Endpoints: Adverse events
Abnormal laboratory findings
Local reactions

Secondary (Efficacy) Endpoints:
TSST-1 binding and neutralizing Ab

Persistence of TSST-1 binding and neutralizing Ab

Exploratory Endpoint: 2nd test of neutralizing Ab

9. SAFETY DEFINITIONS AND REPORTING REQUIREMENTS

9.1 Averse Events (AEs)

9.1.1 Summary of Known and Potential Risks of the Study Drug

The expected risks will be comparable with those accompanying the administration of any vaccine, such as local reactions and fever.

9.1.2 Definition of Adverse Events

An AE is defined as any untoward medical occurrence in a subject administered vaccine or reference preparation that does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the administration of the vaccine or adjuvant. An AE includes any event, regardless of the presumed causality between the event and the preparation administered.

9.2 Serious Adverse Events (SAEs)

A Serious Adverse Event (SAE) is defined by the International Conference on Harmonization (ICH) guidelines and WHO GCP guidelines as any AE fulfilling at least one of the following criteria:

- Results in deaths.
- Life-threatening – defined as an event in which the subject was, in the judgment of the investigator, at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death had it been more severe.
- Requiring subject's hospitalization or prolongation of existing hospitalization – inpatient hospitalization refers to any inpatient admission, regardless of length of stay.
- Resulting in persistent or significant disability or incapacity (i.e., a substantial disruption of a person’s ability to conduct normal life functions).
- Congenital anomaly or birth defect.
- Is medically significant or requires intervention to prevent at least one of the outcomes listed above.

Life-threatening refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe. Important medical events that may not immediately result in death, be life-threatening, or require hospitalization may be considered as SAEs when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definitions above.

9.2.1 Hospitalization – Prolongation of Existing Hospitalization

Hospitalization is defined as an overnight stay in a hospital unit and/or emergency room.

An additional overnight stay defines a prolongation of existing hospitalization.

The following reasons for hospitalizations are not considered AEs, and therefore not SAEs:

- Hospitalizations for cosmetic elective surgery, social and/or convenience reasons.

9.2.2 SAEs Related to Study-Mandated Procedures

Such SAEs are defined as SAEs that appear to have a reasonable possibility of causal relationship (i.e., a relationship cannot be ruled out) to study-mandated procedures (excluding administration of study drug) such as discontinuation of subject's previous treatment during a washout period, or complication of a mandated invasive procedure (e.g., blood sampling), or car accident on the way to the hospital for a study visit, etc.

9.2.3 Suspected Unexpected Serious Adverse Reactions (SUSARs)

SUSARs are all serious adverse reactions with suspect causal relationship to the study drug that is unexpected (not previously described in the Investigator's brochure) and serious.

9.2.4 Pregnancy

Any pregnancy that occurs during study participation must be reported to the investigator/sponsor. To ensure subject safety, each pregnancy must be reported to the investigator/sponsor immediately. The pregnancy must be followed up to determine outcome (including premature termination) and status of mother and child. Pregnancy complications and elective terminations for medical reasons must be reported as an AE or SAE. Spontaneous abortions must be reported as an SAE.
Any SAE occurring in association with a pregnancy brought to the investigator's attention after the subject has completed the study and considered by the investigator as possibly related to the investigational product, must be promptly reported to the principal investigator/sponsor.
In addition, the investigator must attempt to collect pregnancy information on any female partners of male study subjects who become pregnant while the subject is enrolled in the study. Pregnancy information must be reported to the investigator/sponsor as described above.

9.3 Severity of Adverse Events

The severity of clinical AEs is graded on a three-point scale: mild, moderate, severe, and reported on specific AE pages of the CRF.
If the severity of an AE worsens during study drug administration, only the worst intensity should be reported on the AE page. If the AE lessens in intensity, no change in the severity is required.
If an AE occurs during a washout or placebo run-in phase and afterwards worsens during the treatment phase, a new AE page must be filled in with the intensity observed during study drug administration.

Mild
Event may be noticeable to subject; does not influence daily activities; the AE resolves spontaneously or may require minimal therapeutic intervention;

Moderate
Event may make subject uncomfortable; performance of daily activities may be influenced; intervention may be needed; the AE produces no sequelae.

Severe
Event may cause noticeable discomfort; usually interferes with daily activities; subject may not be able to continue in the study; the AE produces sequelae, which require prolonged therapeutic intervention.

A mild, moderate or severe AE may or may not be serious. These terms are used to describe the intensity of a specific event (as in mild, moderate, or severe myocardial infarction). However, a severe event may be of relatively minor medical significance (such as severe headache) and is not necessarily serious. For example, nausea lasting several hours may be rated as severe, but may not be clinically serious. Fever of 39°C that is not considered severe may become serious if it prolongs hospital discharge by a day. Seriousness rather than severity serves as a guide for defining regulatory reporting obligations.

9.4 Relationship to Study Drug

For all AEs, the investigator will assess the causal relationship between the study test or reference preparation and the AE using his/her clinical expertise and judgment according to the following algorithm that best fits the circumstances of the AE:

Definitely: Temporal relationship to the administration of the study test or reference preparation and course following a known reaction pattern;

Probably: Good reasons and sufficient documentation to assume a causal relationship;

Possibly: A causal relationship is conceivable and cannot be dismissed;

Unlikely: The event is most likely related to an etiology other than the trial treatment;

Not Related: No temporal relationship to the administration of the drug or other factors have caused the event;

For reporting purposes, the categories “Definitely”, “Probably” and “Possibly” will be summarized as “Suspected” Adverse Reactions.

9.5 Reporting Procedures

All events meeting the definition of an adverse event must be collected and reported from the first administration of the test or reference preparation until the end of the post-treatment follow-up period. All adverse events, either observed by the investigator or reported by the subject, must be recorded by the investigator and evaluated.
A special section is designated to adverse events in the case report form. The following details must thereby be entered:

- Type of adverse event (The investigator should record the diagnosis, if available. If no diagnosis is available the investigator should record each sign and symptom as individual Adverse Events.)
- Start (date and time)
- End (date and time)
- Severity (mild, moderate, severe)
- Serious (no / yes)
- Unexpected (no / yes)
- Outcome (recovering/resoving, resolved, resolved with sequelae, ongoing at final examination, fatal)
- Relation to study drug (definitely, probably, posssible, unlikely, unrelated).

Adverse events are to be documented in the case report form in accordance with the above mentioned criteria.

9.5.1 Reporting Procedures for SAEs

In the event of serious AEs, the investigator has to use all supportive measures for best subject treatment. If there is any doubt about whether or not an adverse event has to be considered serious, the sponsor should be contacted.

All serious adverse events must be reported to Biomedizinische Forschungs GmbH irrespective of causality or expectedness. The reporting time period for serious adverse events begins with the day the test or reference preparation have been administered and ends within 14 days after discontinuing the study medication. However, if the terminationvisit takes place more than 14 days after the subject
has discontinued study medication, the reporting time has to be extended until the termination visit.

Serious adverse events have to be documented on the Serious Adverse Reporting Form and reported by fax or e-mail to

Biomedizinische Forschung und Bio-Produkte AG
Attention: Prof. Martha Eibl, MD

Fax: 01/408 10 91 -13
Telephone: 01/408 10 91
Email: " office@biomed-research.at"
with copy to

" martha.eibl@meduniwien.ac.at"

The following details should at least be available:

- Subject initials and number
- Subject: date of birth, sex, ethical origin
- The suspected investigational medical product (IMP)
- The adverse event assessed as serious
- Short description of the event and outcome

If applicable, the initial report should be followed by the Follow up report, indicating the outcome of the SAE.

Reporting time-frame for the Investigator is 24 h of learning of its occurrence, even if it is not felt to be treatment-related or at the latest the following working day. The Monitor must be informed accordingly. The original copy of the SAE form and the fax confirmation sheet must be kept with the documentation at the study site.

Please note: Each SAE has also be documented in the CRF as an adverse event. Follow-up information about previously reported SAE must also be reported within 24 hours of the investigator receiving it. A new SAE form is sent, stating that this is a follow-up to the previously reported SAE and giving the date of the original report. The follow-up report should describe whether the event has resolved or continues, if and how it was treated, and whether the subject continued or discontinued study participation. The original of the SAE form with the follow-up information and the fax confirmation sheet must be kept with the documentation at the study site.

9.5.2 Reporting Procedures for SUSARs

It must be remembered that the regulatory authorities, and in case of SUSARs which could possibly concern the safety of the study participants, also the Institutional Review Board / Independent Ethics Committee (IRB / IEC) are to be informed. Such reports shall be made by the sponsor and the following details should be at least available:

- Subject initials and number
- Subject: date of birth, sex, ethical origin
- Name of investigator and investigating site
- Period of administration
- The suspected investigational medical product (IMP)
- The adverse event assessed as serious and unexpected, and for which there is a reasonable suspected causal relationship to the IMP
- Concomitant disease and medication
- Short description of the event:
    - Description
    - Onset and if applicable, end
    - Therapeutic intervention
    - Causal relationship

- Hospitalization of prolongation of hospitalization
- Death, life-threatening, persistent or significant disability or incapacity

Electronic reporting should be th
e expected method for reporting of SUSARs to the competent authority. In that case, the format and content as defined by Guidance (28) should be adhered to. The latest version of MedDRA should be applied. Lower level terms (LLT) should be used.

9.5.3 Annual Safety Report

The Annual Safety Report will be provided by the principal investigator at least once a year.
This report will also be presented annually to the Independent Ethics (IEC) and to the competent authorities by the sponsor.

10. FOLLOW-UP

10.1 Follow-up of Study Participants Including Follow-up of Adverse Events

Subjects will be immunized at the outpatient clinic of the Clinical Pharmacology Department of the University of Vienna Medical School. Thereafter, they will be released and allowed to return home with a subject diary. They will be expected to return to the clinic at 24 h hours, and at 3-month intervals (with the windows described under 8.5.1.). At those time points, the examinations shown in Table 2 will be performed and adverse events will be noted. Between visits, they will be expected to enter adverse events in the subject diary. Later occurring adverse events will be noted by study team during follow up visits.

10.2 Treatment After End of Study

There will not be any treatment after the end of the study.

11. STATISTICAL METHODOLOGY AND ANALYSIS

11.1 Analysis Sets

Per-protocol anaysis will be used.

The safety population will be the primary population for the safety analysis. The per protocol population will be the primary population for the immunogenicity analyses.

11.2 Sample Size Considerations

Sample sizes in this study are arbitrary

11.3 Relevant Protocol Deviations

Non-compliance in this study will be limited to clinic visits and the keeping of a diary.

All protocol deviations will be listed in the study report.

11.4 Statistical Analysis Plan

Analyses of the primary endpoints will be limited to descriptive statistics and narratives.

Antibody titers will be analyzed by one-way or repeated measures ANOVA . Subjects with antibody titers of 200 to 1000 at entry will be stratified. Mann-Whitney-U and Wilcoxon signed rank tests will be used post hoc for comparison among groups and Ab persistence, respectively, to determine statistical significance.

A statistical difference, if any, in the rate of binding Ab response among treatment groups and between treatment and control groups will be tested by Chi square statistics.

Antibody response is defined by seroconversion from a TSST-1 binding Ab titer of < 20 to >40 or a 4-fold increase in TSST-1 binding Ab titer.

Neutralizing Ab will be monitored.

11.5 Missing, Unused and Spurious Data

No data imputation is planned for missing and spurious data.

11.6 Endpoints Analyses

See 11.4.

11.6.1 Primary Endpoint Analysis

See 11.4.

11.6.2 Secondary Endpoint Analysis

See 11.4.

11.6.3 Exploratory Endpoint

See 11.4.

11.6.4 Baseline Parameters and Concomitant Medications

At baseline, antibody titers will be assessed for comparison with the efficacy endpoint.

11.7 Interim Analysis

One blinded interim analysis for safety and immunogenicity may be performed.

Criteria for the termination of the trial

n.a.

11.8 Software Program(s)

to be determined

12. DOCUMENTATION AND DATA MANAGEMENT

12.1 Study Documents and Case Report Forms

The investigator will maintain complete and accurate study documentation in a separate file (i.e., Investigator Site File). Documentation includes the clinical protocol as well any amendments, the agreement(s) between the Sponsor and the Investigator, and all other documents related to the study (e.g., medical records, records detailing the progress of the study for each subject, signed informed consent forms, drug disposition records, correspondence with the EC and the study monitor/sponsor, enrolment and screening information, CRFs, SAE-Reports, laboratory reports (if applicable), and data clarifications requested by the sponsor).

The investigator will comply with the procedures for data recording and reporting. Any corrections to study documentation must be performed as follows: 1) the first entry will be crossed out entirely, remaining legible; and 2) each correction must be dated and initialed by the person correcting the entry; the use of correction fluid and erasing are prohibited.

12.1.1 Case Report Form (CRF)

For each subject enrolled, regardless of study test or reference preparation initiation, a CRF will be completed and signed by the investigator or a designated sub-investigator. This also applies to those subjects who fail to complete the study. If a subject withdraws from the study, the reason will be noted on the CRF. CRFs will be provided in electronic form.

Since electronic format CRFs are provided by the Sponsor, only authorized study site personnel will record or change data on the CRFs. All data must be recorded in the source documents and should be transcribed on the eCRFs in a timely manner after the study visit. Changes to handwritten record or

eCRF will require documentation of the reason for each change. An identical (electronic/paper) version of the complete set of CRFs for each subject will remain in the investigator site file at the study site and in the trial master file at the sponsor in accordance with the data retention policy (see section 12.3).

The data will be checked for completeness and correctness by the responsible CRA who will subsequently flag the pages with a special "*mark"* ("*SDV done*"). Potential queries ("*discrepancies*") will be generated web-based by the CRA or Data Management. Immediately after the queries were authored, they become visible to the Investigator ("*raised discrepancies*").
Those eForms which were verified as correct and complete by the Data Management will be locked. As a consequence, no additional entries or changes are possible.

12.2 Direct Access to Source Data/Documents

The investigator/study site will cooperate and provide direct access to study documents and data, including source documentation for monitoring by the study monitor, audits by the sponsor or sponsor's representatives, review by the EC, and inspections by competent regulatory authorities, as described in the Clinical Study Agreement. If contacted by an applicable regulatory authority, the investigator will notify the sponsor of contact, cooperate with the authority, provide the sponsor with copies of all documents received from the authority, and allow the sponsor to comment on any responses.

12.3 Retention of Clinical Trial Documentation

Subject notes must be kept for the maximum time period as permitted by the hospital, institution or private practice. Other source documents and the investigator's site file must be retained for at least 15 years or longer in accordance with local regulation. However, the Subject Identification Codes must be kept for at least 15 years. The investigator must agree to archive the documentation pertaining to the trial in an archive after completion or discontinuation of the trial, if not otherwise notified. If any modifications become necessary or desirable, these will be documented in writing; major changes require the approval of all investigators and the ethics committee.

12.4 Quality Control and Quality Assurance

12.4.1 Periodic Monitoring

The study monitor is responsible for ensuring and verifying that each study site conducts the study according to the protocol, standard operating procedures, other written instructions/agreements, ICH GCP, and applicable regulatory guidelines/requirements.

The designated monitor will contact and visit the investigator regularly and will be allowed to have access to all source documents needed to verify the entries in the CRFs and other protocol-related

documents provided that subject confidentiality is maintained in agreement with local regulations. It will be the monitor's responsibility to inspect the CRFs at regular intervals according to the SOP throughout the study, to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered on them. The monitoring standards require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs and the recording of the main efficacy, safety, and tolerability endpoints.

The monitor will be working according to SOPs and will provide a monitoring report after each visit for the Sponsor. The investigator will resolve discrepancies of data.

Source data verification will be 100%

12.4.2 Unblinded Monitoring and IMP Handling

As complete blinding of the IMP in use is not possible for all parties involved in the study and to ensure that blinding is kept during ongoing study an unblinded person for study coordination at site and an unblinded monitor will be nominated for this study.

The unblinded study coordinator at site is responsible for all the administration/handling/accountability and storage procedures and preparation of the IMP.

Unblinded Monitoring visits will be done to ensure that all handling/storage/accountability and dispensing of IMP has been done according protocol and to do Source Data Verification to ensure that all subjects are treated according their randomization arm.

12.4.3 Audit and Inspections

Upon request, the investigator will make all study-related source data and records available to a qualified quality assurance auditor mandated by the sponsor or to competent authority inspectors. The main purposes of an audit or inspection are to confirm that the rights and welfare of the subjects have been adequately protected, and that all data relevant for assessment of safety and efficacy of the investigational product have appropriately been reported to the sponsor.

12.5 Reporting and Publication

12.5.1 Publication of Study Results

The findings of this study will be published jointly by the investigator and the sponsor in a scientific journal and presented at scientific meetings. The manuscript will be circulated to all co-investigators before submission. Confidentiality of subjects in reports/publications will be guaranteed.

13. ETHICAL AND LEGAL ASPECTS

13.1 Informed Consent of Subjects

Following comprehensive instruction regarding the nature, significance, impact and risks of this clinical trial, the subject must give written consent to participation in the study.
During the instruction the trial participants are to be made aware of the fact that they can withdraw their consent – without giving reasons – at any time without their further medical care being influenced in any way.

In addition to the comprehensive instructions given to the trial participants by the investigator, the trial participants also receive a written subject information sheet in comprehensible language, explaining the nature and purpose of the study and its progress.

The subjects must agree to the possibility of study-related data being passed on to relevant authorities.

The subjects must be informed in detail of their obligations in relation to the trial participants insurance in order not to jeopardize insurance cover.

13.2 Acknowledgement / Approval of the Study

The principal investigator will submit this protocol and any related document provided to the subject (such as subject information used to obtain informed consent) to the Ethics Committee (EC) . Approval from the committee must be obtained before starting the study.

The clinical trial shall be performed in full compliance with the legal regulations according to the Drug Law (AMG - Arzneimittelgesetz) of the Republic of Austria.

An application must also be submitted to the Austrian Competent Authorities (Bundesamt für Sicherheit im Gesundheitswesen (BASG) represented by the Agency for Health and Food Safety (AGES PharmMed) and registered to the European Clinical Trial Database (EudraCT) using the required forms. The timelines for (silent) approval set by national law must be followed before starting the study.

13.2.1 Changes in the Conduct of the Study

Protocol Amendments

Proposed (substantial) amendments must be submitted to the appropriate CA and ECs. Substantial amendments may be implemented only after CA/EC approval has been obtained. Amendments that are intended to eliminate an apparent immediate hazard to subjects may be implemented prior to receiving CA/EC approval. However, in this case, approval must be obtained as soon as possible after implementation.

Study Termination

If the sponsor or the investigator decides to terminate the study before it is completed, they will notify each other in writing stating the reasons of early termination. In terminating the study, the sponsor and the investigator will ensure the adequate consideration is given to the protection of the subject interests. The investigator, sponsor or (designated CRO on behalf of the sponsor) will notify the relevant CA and EC. Documentation will be filed in the Trial Master and Investigator Files.

Clinical Study Report (CSR)

Within one year after the final completion of the study, a full CSR will be prepared by the sponsor and submitted to the EC and the competent authority.
The Investigator will be asked to review and sign the final study report.

13.3 Insurance

During their participation in the clinical trial, the subjects will be insured as defined by legal requirements. The investigator of the clinical trial will receive a copy of the insurance conditions of the 'subjects insurance'. The sponsor is providing insurance in order to indemnify (legal and financial coverage) the investigator/center against claims arising from the study, except for claims that arise from malpractice and/or negligence. The compensation of the subject in the event of study-related injuries will comply with the applicable regulations.
Details on the existing subjects insurance are given in the subject information sheet.

13.4 Confidentiality

The information contained in this document, especially unpublished data, is the property of the Sponsor. It is therefore provided to you in confidence as an investigator, potential investigator, or consultant, for review by you, your staff, and an Ethics Committee or Institutional Review Board. It is understood that this information will not be disclosed to others without written authorization from the Sponsor.

13.5 Ethics and Good Clinical Practice (GCP)

The investigator will ensure that this study is conducted in full conformance with the principles of the "Declaration of Helsinki" (as amended at the 56th WMA General Assembly, Tokyo, Japan, 2008) and with the laws and regulations of the country in which the clinical research is conducted.
The investigator of the clinical trial shall guarantee that only appropriately trained personnel will be involved in the study. All studies must follow the ICH GCP Guidelines (June 1996) and the EU Directive embedded in the Austrian drug act.

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15. APPENDICES

Appendix 1

Labels for Test (Treatment) and Reference (Control) Preparations

TEST PREPARATION (Treatment Groups 1-3):

10.0 µg rTSST-1 Variant Vaccine

INNER LABEL

Prüfer: Prof. Dr. Bernd Jilma
Prüfplan Code: BioMed 0515
Sponsor: Biomedizinische Forschungund Bio-Produkte AG
Lazarettgasse 19, A 1090 Wien

rTSST-1 Variant Vakzine
10 µg rTSST-1 Variant in 0,5 ml, adjuvantiert mit 1 mg $Al(OH)_3$

eine Einzeldosis (0,5 ml) enthält 10 µg rTSST-1 Variant adjuvantiert mit 1 mg $Al(OH)_3$

Suspension zur i.m. Injektion
Nur zur einmaligen Entnahme
Ch. Bez.: 01XYYYZ
Lagertemperatur: 2-8°C

Proband #: _____

OUTER LABEL

Prüfer: Prof. Dr. Bernd Jilma
Prüfplan Code: BioMed 0515
Eudra CT: 2015-003714-24

Sponsor: Biomedizinische Forschungund Bio-Produkte AG
Lazarettgasse 19, A 1090 Wien

rTSST-1 Variant Vakzine
10 µg rTSST-1 Variant in 0,5 ml, adjuvantiert mit 1 mg $Al(OH)_3$

eine Einzeldosis (0,5 ml) enthält 10 µg rTSST-1 Variant adjuvantiert mit 1 mg $Al(OH)_3$

Suspension zur i.m. Injektion
Nur zur einmaligen Entnahme

Ch. Bez.: 01XYYYZ

verwendbar bis: XX.YY.ZZZZ

Lagertemperatur: 2-8°C
NICHT EINFRIEREN
VOR GEBRAUCH SCHÜTTELN

Nur zur klinischen Prüfung

TEST PREPARATION (Treatment Groups 4-6):

100.0 µg rTSST-1 Variant Vaccine

INNER LABEL

Prüfer: Prof. Dr. Bernd Jilma
Prüfplan Code: BioMed 0515
Sponsor: Biomedizinische Forschungund Bio-Produkte AG
Lazarettgasse 19, A 1090 Wien

rTSST-1 Variant Vakzine
100 µg rTSST-1 Variant in 0,5 ml, adjuvantiert mit 1 mg $Al(OH)_3$

eine Einzeldosis (0,5 ml) enthält 100 µg rTSST-1 Variant adjuvantiert mit 1 mg $Al(OH)_3$

Suspension zur i.m. Injektion
Nur zur einmaligen Entnahme
Ch. Bez.: 01XYYYZ
Lagertemperatur: 2-8°C

Proband #: _____

OUTER LABEL

Prüfer: Prof. Dr. Bernd Jilma
Prüfplan Code: BioMed 0515
Eudra CT: 2015-003714-24

Sponsor: Biomedizinische Forschungund Bio-Produkte AG
Lazarettgasse 19, A 1090 Wien

rTSST-1 Variant Vakzine
100 µg rTSST-1 Variant in 0,5 ml, adjuvantiert mit 1 mg $Al(OH)_3$

eine Einzeldosis (0,5 ml) enthält 100 µg rTSST-1 Variant adjuvantiert mit 1 mg $Al(OH)_3$

Suspension zur i.m. Injektion
Nur zur einmaligen Entnahme

Ch. Bez.: 01XYYYZ
verwendbar bis: XX.YY.ZZZZ
Lagertemperatur: 2-8°C
NICHT EINFRIEREN
VOR GEBRAUCH SCHÜTTELN

Nur zur klinischen Prüfung

REFERENCE PREPARATION (Control Group 7):

1.0 mg Aluminium Hydroxide

Prüfer: Prof. Dr. Bernd Jilma
Prüfplan Code: BioMed 0515
Sponsor: Biomedizinische Forschungund Bio-Produkte AG
Lazarettgasse 19, A 1090 Wien

Placebo
1 mg $Al(OH)_3$ in 0,5 ml
eine Dosis (0,5 ml) enthält 1 mg $Al(OH)_3$
Suspension zur i.m. Injektion
Nur zur einmaligen Entnahme

Ch. Bez.: 51XYYYZ
Lagertemperatur: 2-8°C

Proband #: _____

OUTER LABEL

Prüfer: Prof. Dr. Bernd Jilma
Prüfplan Code: BioMed 0515
Eudra CT 2015-003714-24

Sponsor: Biomedizinische ForschungH
Lazarettgasse 19, A 1090 Wien

Placebo
1 mg $Al(OH)_3$ in 0,5 ml
eine Dosis (0,5 ml) enthält 1 mg $Al(OH)_3$

Suspension zur i.m. Injektion
Nur zur einmaligen Entnahme

Ch. Bez.: 51XYYYZ
verwendbar bis: XX.YY.ZZZZ

Lagertemperatur: 2-8°C
NICHT EINFRIEREN
VOR GEBRAUCH SCHÜTTELN

Nur zur klinischen Prüfung