CLINICAL STUDY PROTOCOL

TITLE

Characterization of humoral and cellular immunity for tick-borne encephalitis (TBE) vaccination in allogeneic blood and marrow graft recipients: a pilot study

SHORT TITLE

Immunity for TBE vaccination in allogeneic blood and marrow graft recipients

PROTOCOL No. 4

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Test drug (IMP) and Pharmaceutical Company	FSME IMMUN (FSME-Virus inaktiviert,Neudörfl strain) 2,4µg; PFIZER
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2. PROTOCOL SYNOPSIS

TITLE	**Characterization of humoral and cellular immunity for tick-borne encephalitis (TBE) vaccination in allogeneic blood and marrow graft recipients: a pilot study**
OBJECTIVES	**Primary Objective** • *To assess the humoral immunogenicity to a TBE vaccination in allogeneic blood and marrow graft (HSCT) recipients compared to healthy volunteers without previous TBE vaccination, by measuring the quantitative antibody levels using neutralization test (NT) and enzyme-linked immunosorbent assay test (ELISA)* **Secondary Objectives** • *To assess cellular immunogenicity of the TBE vaccination in allogeneic HSCT recipients, by measuring the lymphocyte proliferation and cytokine levels after in vitro stimulation with peripheral blood mononuclear cells with whole aluminium hydroxide-free and human albumin-free TBE antigen* • *To assess the immune status in HSCT recipients prior to and after vaccination, as measured by quantitative immunoglobulin levels and by immunofluorescence staining of peripheral blood T and B lymphocytes and flow cytometry analyses*
DESIGN / PHASE	*Prospective, open-label phase II study.*
STUDY PLANNED DURATION	**First patient First visit** *07/ 2014* **Last patient First visit** *12/ 2016* **Last patient Last visit** *12/ 2017*
CENTER(S) / COUNTRY(IES)	*Single center study of the Medical University of Vienna in Austria*
PATIENTS / GROUPS	*26 HSCT patients in the study group* *26 healthy volunteers in the control group*
INCLUSION CRITERIA	• *Adult patients ≥18 years who* - *had undergone an allogeneic HSCT 11 to 13 months ago or* - *healthy volunteers without previous TBE vaccination*
EXCLUSION CRITERIA	• *Previous TBE vaccination following HSCT* • *HSCT patients with extremely severe acute graft versus host disease (receiving prednisone >0.5 mg/kg bodyweight as part of a combination therapy or a three agent immunosuppressive treatment)* • *Previous TBE virus infection, previous dengue virus infection or vaccination against yellow fever or Japanese encephalitis* • *Any acute febrile illness in the 2 weeks prior to or at the time of enrolment* • *A history of severe allergic reactions or anaphylaxis after vaccination* • *If female, are pregnant or lactating* • *If belonging to the healthy control group, are immunosuppressed* • *Relapse of underlying malignant disease*
STUDY PERIODS	• *The active phase of the study for each participant is 44-56 weeks* • *No additional controls are needed*

	• *The total duration of the study is estimated to be 4 years*
STUDY DRUG	*TBE vaccine – FSME IMMUN 0.5 ml (2.4 µg)*
COMPARATIVE DRUG	*No*
EFFICACY ENDPOINTS	*Number of subjects with antibody response to TBE vaccination associated with protection 28 days after the second and third vaccination*
TOLERABILITY / SAFETY ENDPOINTS	*Number of subjects with systemic reactions after vaccination*
PHARMACOKINETIC / PHARMACODYNAMIC ENDPOINTS	**No**
QUALITY OF LIFE / PHARMACOECONOMIC ENDPOINTS	*No*
STATISTICAL METHODOLOGY	**Primary Endpoint** To assess humoral immunogenicity of the TBE vaccination in allogeneic HSCT recipients compared to healthy volunteers without previous TBE vaccination, the outcome of the neutralilzation test (NT) will be assessed four weeks after the second vaccination. Therefore, the number of subjects with NT titers against TBE virus >10 (=seroconversion rate), assumed to be the threshold for antibody-mediated protection, will be evaluated. **Null and alternative hypotheses:** *H_0: There is no statistical difference in the seroconversion rate between the study population and the control group* *H_1: There is statistical difference in the seroconverion rate between the study population and the control group* Type-I and -II errors – power: Alpha 0.05. Power 0.8. **Sample size calculation** *The calculation of the sample size was performed using nQuery 6.1. The primary endpoint is the outcome of the NT against 4 weeks after the second vaccination (positive test: Yes/No). Because of deviation of the antibody concentrations from normal distribution (NT level <=10 not detectable indicating a negative test), the outcome is reduced to a binary but clinically more relevant outcome (seroconversion rate: NT levels >10 indicates a positive test). Previously, in a study with heart transplant recipients a seroconversion rate of 35% was detected compared to 100% in the healthy control group. In our study we expect a seroconversion rate of at least 90% in the healthy control group. In the HSCT patients we expect a seroconversion not exceeding 50%. The calculation of the sample size was performed using the following assumption: Significance level alpha 0.05, Power 0.8, ratio of HSCT patients/healthy volunteers: 1/1. With a total number of 46 participants (23 HSCT patients and 23 healthy volunteers) the assumed difference in the seroconversion rate between groups can be detected (using a two-sided Fisher's Exact Test) with 80% Power. We added 10% to adjust for potential loss-to-follow-up which results in 26 HSCT patients and 26 healthy volunteers.*

	Statistical methodology *The primary endpoint (outcome of NT 4 weeks after the second vaccination) will be analysed using Fisher's Exact Test. For the seroconversion rates, 95% confidence intervals will be calculated.* *The secondary endpoints (NT 4 weeks after the third vaccination, ELISA after the second and third vaccination, cellular immunity, immunoglobulin levels, immune reconstitution and safety data) will be analysed using descriptive statistics.*

3. LIST OF ABBREVIATIONS

AE = Adverse event
CFSE = carboxyfluroescein diacetate succinimidyl ester
CRF = Case Report Form
ELISA = enzyme-linked immunosorbent assay
FACS = fluorescence activated cell sorting
GCP = Good Clinical Practice
GVHD = graft-versus-host disease
HSCT = blood and marrow transplantation
ICH = International Conference on Harmonization
NIH = National Institute of Health
NT = neutralization test
PBMCs = peripheral blood mononuclear cells
PHA = phytohemagglutinin
TBE = tick-borne encephalitis

4. TABLE OF CONTENTS

TABLE 1. VISIT AND ASSESSMENT SCHEDULE

PERIODS	Name	SCREENING	VACCINATION							
	Duration		*44-56 weeks*							
VISITS	Number	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
	Name	*Screening*	*Visit 1/ 1st Vaccination*	*Visit 2/ 2nd Vaccination*	*Visit 3*	*Visit 4*	*Visit 5*	*Visit 6/ 3rd Vaccination*	*Visit 7*	*Visit 8*
	Time	*Day – 28*	*Day 0*	*Day 28 (± 2 days)*	*Day 35 (7 ± 2 days after Visit 2)*	*Day 56 (28 ± 7 days after Visit 2)*	*Day 84 (± 14 days)*	*Day 270-365*	*Day 277-372 (7± 2 days after Visit 6)*	*Day 298-393 (28 ± 7 days after Visit 6)*
Informed Consent		X								
Inclusion / Exclusion Criteria		X	X							
Medical History		X	X							
Physical Examination		X	X	X				X		
Pregnancy Test		X	X	X		X	X	X		X
Blood Draw (Serology)		X				X				X
Blood Draw (Cellular immunity)		X			X				X	
Blood Draw (Immune reconstitution + serum immunoglobulin levels)		X					X	X		
Vaccination			X	X				X		
Adverse Events			X	X	X	X	X	X	X	X

5. BACKGROUND INFORMATION

5.1 Background

Allogeneic blood and marrow transplantation (HSCT) are increasingly employed in the treatment of hematological and oncological malignancies and are now standard procedures for selected diseases. During, immunosuppressive treatment is required to reduce the risk of transplant rejection and graft-versus-host disease (GVHD).

Serious infections are common in the early phase following allogeneic HSCT because immune reactivity during the first month post-transplant is very low. Effective approaches toward vaccinating patients against common pathogens are being explored but are limited by poor levels of responsiveness. Protective immunity to diseases preventable by routine vaccination is lost following allogeneic HSCT. Adoptive transfer of immunity from donors to recipients after allogeneic transplantation is not sufficient to prevent this decline. However, vaccination practices after transplantation vary and data are sparse [1, 2].

Following transplantation, cytotoxic and phagocytic functions recover by day 100, but the more specialized function of T and B lymphocytes may remain impaired for a year or even longer. Deficiencies in cellular immunity are characterized by the inversion of CD4/CD8 ratios, a decreased proliferative response to mitogens, and the development of anergy to recall antigens as measured by delayed-type hypersensitivity testing. The impact on humoral immunity consists of decreased levels of circulating immunoglobulins, impaired immunoglobulin class switching, and a loss of complexity in immunoglobulin gene rearrangement patterns. The efficacy of vaccination following HSCT is influenced by the time elapsed since transplantation, the nature of the hematopoietic graft, the use of serial immunization, the vaccine being used, and the immune status of the patient including the presence of chronic GVHD [3]. After one to three years after allogeneic HSCT patients without chronic GVHD experience complete immune regeneration whereas in patients with chronic GVHD immunodeficiency can remain for prolonged periods of time [4-6].

Chronic GVHD continues to be a major problem in long-term survivors of allogeneic HSCT, affecting 35-70% of patients [7, 8]. The pathogenesis and clinical features of chronic GVHD resemble those of several autoimmune diseases such as systemic lupus erythematosus, Sjogren's syndrome and scleroderma. Autoreactive T lymphocytes are an important effector mechanism with interferon gamma playing a central role in the increased collagen deposition that is a central histopathologic feature of chronic GVHD [9]. Since chronic GVHD only occurs in allogeneic HSCT recipients and can be prevented by T-cell depletion from the donor graft, donor T-cells responding to allogeneic antigens in the patient are of critical

importance for the development of chronic GVHD. Of note, donor B-cell responses to recipient HY antigens have been significantly associated with the development of chronic GVHD in the setting of gender mismatched HSCT.

Chronic GVHD can affect the skin (depigmentation, poikiloderma, lichen planus-like features, sclerotic features, morphea-like features, lichen sclerosus-like features lichenoid papules), the liver (hepatitis), the lung (obliterative bronchiolitis), the oral mucosa (lichen-type features, hyperkeratotic plaques, restriction of mouth opening from sclerosis, ulcerations, atrophy of the mucosa, xerostomy), eyes (keratoconjunctivitis sicca), muscles and fibers (myositis, tendinitis, and fasciitis), and the esophagus (esophageal web, strictures or stenosis), respectively. The diagnosis of chronic GVHD requires the presence of at least one diagnostic clinical sign of chronic GVHD (e.g. poikiloderma) or the presence of at least one distinctive manifestation (e.g. keratoconjunctivitis sicca) confirmed by pertinent biopsy or other relevant tests (e.g. Schirmer test) in the same or another organ. According to the consensus of the National Institute of Health (NIH) in 2005 a new clinical scoring system (0-3) was introduced to assess the extent and severity of chronic GVHD for each organ or site at any given time, with 0 representing no involvement and 3 reflecting severe impairment [10]. For mild forms, no treatment is necessary. Extended disease requires specific immunosuppressive treatment using corticosteroids +/- cyclosporine, tacrolimus, and monoclonal antibodies such as basiliximab or tumor necrosis factor-alpha inhibitor as etanercept [11]. These immunosuppressive regimens may additionally impair patients' response to vaccination. Therefore, infections have become increasingly important during GVHD therapy [12]. Immunosuppressive therapy is being improved continually and in most cases little is known about the impact of the more recently adopted regimens on durability of antibody response. Thus, studies on immune-response after vaccination in patients with different immunosuppressive regimes are still missing.

Tick-borne encephalitis (TBE) is a disease of the central nervous system caused by a tick-borne flavivirus infection. TBE can lead to severe neurological symptoms such as meningitis, meningoencephalitis, and meningoencephalomyelitis, which can result in death. There is no treatment, and prevention with the vaccine is the only intervention currently available. TBE is the most important arthropod-transmitted viral disease in Europe, and in some countries it represents a major public-health problem [13]. In Austria and the southern parts of Germany TBE is endemic and therefore it is assumed that the whole population living in those areas is at risk. In the pre-vaccination era, Austria had the highest recorded morbidity of TBE in Europe. The disease accounted for more than 50% of all viral meningoencephalitides in the eastern and southern parts of the country [14]. Flaviviruses are a large group of small, enveloped viruses responsible for a number of severe human diseases, including yellow

fever, Japanese encephalitis, dengue hemorrhagic fever and TBE. TBE virus particles are roughly spherical in shape, 40-50nm in diameter, and contain a core, 20-30nm in diameter. The genome consists of single-stranded positive-sense RNA with a relative molecular mass of about $4x10^6$. Three structural proteins and the capsid, membrane and envelope proteins are all encoded by the viral genome. The envelope glycoprotein induces neutralizing and hemagglutination-inhibition antibodies and is the most important antigen for providing protection from disease.

The first vaccine against TBE was prepared in 1941 in the brains of mice. Some 20 years later TBE vaccines derived from cell cultures (chicken embryo fibroblast cells) were developed and used for active immunization in humans in the former Soviet Union. Later, a purified, inactivated virus vaccine was developed which proved to be more immunogenic than previous TBE vaccines. The efficacy of these vaccines has been well documented. All the currently licensed TBE vaccines (Encepur children, Encepur Adults, and FSME-IMMUN "new") gave high seroconversion rates of over 87% in immunocompetent individuals. Although systemic and local adverse event were commonly reported, none were serious or life-threatening [15].

Following allogeneic HSCT, TBE vaccination should be performed as primary immunization in risk area with three vaccine-doses after the first nine to twelve months (CIII recommendation) [16].

Adequacy of the immune response to a vaccine is frequently measured by the serum level of the specific antibody. Although seroconversion does indicate an immune response, it does not necessarily signify protection. While routine determination of antibody titers prior to vaccination is not recommended in all transplant recipients, post-vaccination determination of antibody levels is useful to monitor antibody response and protection, and assess the need for additional doses of the vaccine [1]. Successful vaccination can be easily monitored by the demonstration of specific immunoglobulin G-antibodies in the serum by enzyme-linked immunosorbent assay (ELISA). However, there is a potential for misleading results, because all flaviviruses are serologically related and infections or vaccinations with one flavivirus will give rise to antibodies that also react with all other flaviviruses in ELISAs. Vaccinations against yellow fever, Japanese encephalitis or infections with dengue viruses will thus result in cross-reactive but non-neutralizing antibodies yielding a positive result in TBE ELISAs [13]. Detectable neutralizing antibodies are generally viewed as a surrogate parameter of protection against TBE infection because their formation is of decisive importance for virus elimination and entails protection against the disease [17].

Serological response to TBE vaccination has already been studied in different populations with decreased responsiveness to vaccines, the HIV-positive patients [18], in the elderly [19, 20], and in heart transplant recipients [21], respectively.
For pneumococcal polysaccharide vaccine, lack of responsiveness to vaccination in HSCT patients with chronic GVHD has been demonstrated [22]. But there is little information on the efficacy of the TBE vaccine in stem cell transplant recipients with chronic GVHD.

5.2 Study rationale

Patients undergoing HSCT experience a prolonged period of dysfunctional immunity associated with an increased risk of bacterial and viral infections. Systematic re-immunization is necessary at appropriate time intervals following transplantation to re-establish immunity [1]. TBE vaccination should be performed as primary immunization in risk area with three vaccine-doses nine to twelve months after allogeneic HSCT (CIII recommendation) [16]. However, immune response after vaccination might vary a lot among HSCT patients with and without chronic GVHD. In a study with immunosuppressed heart transplant recipients a seroconversion rate of 35% was detected compared to 100% in the healthy control group [21]. Similarly, we assume that the serum conversion rate after TBE re-immunization is significantly reduced in HSCT recipients compared to an age-matched and sex-matched control group of healthy volunteers without previous TBE vaccination. Moreover, it is not clear whether TBE vaccination in HSCT patients with chronic GVHD has a protective effect at all.
The results of the planned pilot study will provide first data to assess the efficacy of TBE vaccination in allogeneic HSCT recipients with and without chronic GVHD. These data will enable to develop new guidelines or optimize current guidelines for TBE vaccination in this special population at risk. It is expected that different guidelines for HSCT patients with and without chronic GVHD have to be established in the future.

6. STUDY OBJECTIVES

The aims of this study are to estimate the humoral and cellular immune responses after TBE vaccination in adult HSCT recipients.

6.1 Primary Objective (Hypothesis)

To assess the humoral immunogenicity to a TBE vaccination in allogeneic HSCT recipients compared to healthy volunteers without previous TBE vaccination, the quantitative antibody

levels will be measured using neutralilzation test (NT) and enzyme-linked immunosorbent assay test (ELISA).
The null hypothesis has to be rejected that there is no difference in the seroconversion rate between the HSCT patients and the healthy volunteers after TBE vaccination.

6.2 Secondary Objectives

To assess cellular immunogenicity of the TBE vaccination in allogeneic HSCT recipients, T-cell cytokine expression will be measured using flow-cytometry after in vitro stimulation with peripheral blood mononuclear cells with TBE antigen.
To evaluate the immune status in HSCT recipients prior to and after vaccination, quantitative immunoglobulin levels will be measured and immunofluorescence staining of peripheral blood T and B lymphocytes and flow cytometry analyses will be performed.

7. STUDY DESIGN

A prospective open-label phase II pilot study will be performed. A total of 52 adult male or female subjects, 26 HSCT patients (study population) and 26 healthy volunteers (control group), will be enrolled in this clinical trial. Eight study visits per patient will be planed. Eligible patients will receive at least two TBE vaccinations (study visit 1 – day 0, study visit 2 -1month after the first vaccination) with a total of two doses of the TBE vaccine FSME IMMUN®. Whenever possible, the patients will receive complete primary vaccination with a third dose of TBE vaccine FSME IMMUN® (study visit 9 - 12 months after the first vaccination). TBE vaccine will be injected intramuscularly in the Musculus deltoideus.
FSME IMMUN® contains formalin-inactivated TBE virus. One dose (0.5 ml) contains 2.4 µg of antigen inactivated by formaldehyde. Human albumin is used as stabilizer and aluminium hydroxide is used as adjuvant. The vaccine contains traces of formaldehyde, protamine sulphate, neomycin and gentamicin.
Serum samples for determination of antibody levels will be obtained immediately before the first (study visit 1), 4 weeks after the second vaccination (study visit 4), and four weeks after the third vaccination (study visit 8). Blood samples for the analysis of cellular immunity will be taken before the first (study visit 1), one week after the second vaccination (study visit 3) and one week after the third vaccination (study visit 7). Blood samples for determination of the immune reconstitution and the quantitative immunoglobulin levels will be obtained prior to the first (study visit 1) and third vaccination (study visit 6) and 12 weeks after the first vaccination (study visit 5) in HSCT recipients only.

7.1 Study population

Adult patients ≥18 years will be recruited at the bone marrow transplant center of the Medical University of Vienna. Patients will be eligible if 12 months +/- 1 month had passed since the allogeneic HSCT and if the patient is willing to sign an informed consent. Basic demographic data including age, gender, conditioning regimen, type of the hematopoietic graft (sister/brother, unrelated donor, cord blood) or bone marrow transplant, intensity of immunosuppressive therapy, medical history including time of previous TBE vaccination before HSCT and present status of chronic GVHD according to the NIH criteria [10] will be obtained. If available (only possible for sister/brother donor) the current TBE vaccination status of the donors will be requested. As control group, non-immunocompromised adult volunteers ≥18 years (age-matched with a tolerance of +/-10 years and sex-matched to the study population) without previous TBE-vaccination will be recruited at the outpatient clinic of the department of infectious diseases and tropical medicine of the Medical University of Vienna.

7.1.1 Subject population

Study entry is defined as the date of signature of the study participant (subject) on the informed consent form. All subjects enrolled will be assigned a subject code, which consists of a consecutive subject number (2 digits).

7.1.2 Inclusion criteria

Male and female subjects will be eligible for participation in this study if they:

- Are ≥18 years on the day of screening
- Had undergone an allogeneic HSCT 11 to 13 months ago (study population)
- Are clinical healthy without previous TBE vaccination (control group)
- Have an understanding of the study, agree to its provisions, and give written informed consent prior to study entry
- If female and capable of bearing children – have a negative urine pregnancy test result at study entry and agree to employ adequate birth control measures for the duration of the study

7.1.3 Exclusion criteria

Subjects will be excluded from participation in this study if they:

- Have received a TBE vaccination following HSCT

- Suffer from extremely severe acute GVHD and therefore receive prednisone >0.5 mg/kg bodyweight as part of a combination therapy or a three agent immunosuppressive treatment (because in these HSCT patients any type of vaccination has to be postponed until immunosuppression is reduced to a double combination or prednisone <0.5 mg/kg bodyweight)
- Suffer from or have a history of previous TBE virus infection or vaccination, previous dengue virus infection or vaccination against yellow fever or Japanese encephalitis
- Have any acute febrile illness in the 2 weeks prior to or at the time of enrolment
- Have a history of severe allergic reactions or anaphylaxis after vaccination
- If female, are pregnant or lactating.
- If belonging to the healthy control group, are immunosuppressed (suffer from or have a history of immune mediated diseases, long-term use of corticosteroids, hemodialysis, chronic renal insufficiency, liver cirrhosis Child-Pugh class C, hematooncological malignant disease, solid organ transplant, HSCT)
- Suffer a relapse of their underlying malignant disease

7.1.4 Study duration

The total study duration is estimated to be four years. For the individual study participant the study duration will be 44-56 weeks.

7.1.5 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 complete the last visit (study visit 8) 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

In all cases, the reason why subjects are withdrawn must 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 patients withdrawn from the study

In case of premature discontinuation after start of vaccination, no further investigations concerning the study will be performed. Furthermore, participants 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

If the week 8 (primary endpoint) is reached, drop-outs will be included in the sample size. If the participiant drops out before the week 8 is reached, patients will be replaced and the next free subject number will be allocated.

7.1.6 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 serious that the risk-benefit 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

Active agent and characteristics: Inactived TBE-virus adsorbat vaccine. One dose (0.5 ml) contains 2.4 µg of formaldehyde inactivated TBE-virus (strain Neudörfl) and 1 mg aluminium hydroxide as adjuvant.
Trade name of the agent: FSME IMMUN 0.5 ml
Manufacturer: PFIZER
Drug supply: PFIZER

Storage Instructions: The vaccine has to be stored in a refrigerator at 2-8°C in the orginal package.
Route of administration: intramuscular injection.

8.1.1 Dosage and administration

Initial dose: All subjects will receive one dose 0.5 ml TBE vaccine FSME IMMUN on day 0 (study visit 1).
Repeated dose: All subjects will receive one additional dose 0.5 ml TBE vaccine FSME IMMUN on week 4 (study visit 2) and between week 40-52 (study visit 6).
Route of administration: TBE vaccine will be injected intramuscularly in the Musculus deltoideus.
Duration: All subjects will receive 0.5 ml TBE vaccine three times within one year.

8.1.2 Study-drug up- and down titration

Not needed for the study.

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 patient.
The interruption or premature discontinuation of study drug might be triggered by an AE, a diagnostic or therapeutic procedure, an abnormal assessment, or for administrative reasons, in particular withdrawal of the patient's consent.
The reason for study drug interruption or premature permanent discontinuation must be documented in the CRF.

8.1.4 Study-drug delivery & drug storage conditions

The drug will be ordered from the local pharmacy of the General Hospital of Vienna. After receipt the vaccine will be stored in the refrigerator at 2-8°C in the original package at the outpatient clinic of the Department of Internal Medicine I, Division of Infectious Diseases and Tropcial Medicine. The vaccine should not be frozen or stored at a temperature >8°C.

8.1.5 Study drug packaging and labeling

Packaging and labeling of the TBE vaccine is done by the producer according to local legal requirements and GMP and shipped to the local pharmacy of the General Hospital of Vienna.

8.1.6 IMP administration & handling

Before application the vaccine has to reach room temperature. Furthermore, the suspension has to be thrilled and has to be injected intramuscularly in the musculus deltoideus immediately after the protecting cap is removed from the needle. Longer, unprotected resting can lead to unsterility and/or occlusion of the needle. All vaccines have to be documented with self-adhesive label in the vaccination card.

8.1.7 Drug Accountability

The application of the drug will be documented in the clinical records of the patient by using a self-adhesive label of the vaccine and in the CRF.

8.1.8 Procedures to assess subjects compliance

Due to intramuscular injection by the doctor, the compliance of the patients has not to be additionally checked.

8.1.9 Concomitant medication

Concomitant medication has to be documented in the CRF.

8.2 Randomization

Not needed for the study.

8.3 Blinding

Not needed for the study.

8.4 Benefit and risk assessment

After vaccination with the inactivated TBE virus K23, in particular following the administration of the complete primary immunization, it is highly probable that the majority of subjects participating in the phase II clinical study will be protected against TBE, one of the most severe infections of the central nervous system. Allogeneic HSCT recipients, particularly those with chronic GVHD, have a high risk for microbial infections including TBE infection. Therefore, primary vaccination of HSCT recipients is recommended in risk areas one year

after HSCT at the latest independently of previous TBE vaccination before HSCT. The used TBE-vaccine is registered in Austria for more than one decade. Its safety and efficacy have been already demonstrated in many clinical trials. Therefore, unexpected adverse events due to the vaccination are not assumed. However, the following side effects have to be considered:

- very common (>1/10): local reactions at the site of injection, transient redness, and swelling.
- common (>1/100;<1/10): systemic reactions including fever, fatigue, headache, muscular pain, arthralgia. These side-effects usually occur within 72 hours after the vaccination, abate within 1-2 days, but do not acquire any medical treatment. All adverse events will be listed in the study documents and have to evaluated by the investigator for causality to the vaccination. After venous puncture local reactions, a hematoma or phlebitis can occur. However, the planed venous punctures withdraw only minor volumes of blood and do not impair the patient's health.

Therefore, it can be speculated that this study does not exhibit an exceptional risk for the patients.

8.5 Study procedures

8.5.1 General rules for trial procedures

- All study measures like blood sampling and measurements have to 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 should be according to the protocol. The time margins mentioned in the study flow chart are admissible. If for any reason, a study procedure is not performed within scheduled margins a protocol deviation should be noted, and the procedure should be performed as soon as possible or as adequate.
- If it is necessary for organizational reasons, it is admissible to perform procedures which are scheduled for one visit at two different time points. Allowed time margins should thereby not be exceeded.

8.5.2 Study visits

8.5.2.1 Visit 0/ Screening investigation (Day -28)

The investigator will inform the subject about the procedures, risks and benefits oft he study. Fully informed, written consent must be obtained from each subject prior to any assessment being performed. It is important that the subject is allowed sufficient time to consider his/her participation in the study.

The following assessments will be performed:

- Inclusion and exclusion criteria.
- Demographic data including sex, age, weight and height.
- Medical history and information on planned hospitalization (including elective surgery) during the study for medical conditions existing prior to study entry.
- Physical examination including body temperature measured axillary.
- Pregnancy test in females capable of bearing children.
- Blood draw of 10 ml blood for TBE antibody level detection.
- Blood draw of 40 ml blood for cellular immunity.
- Blood draw of 10 ml blood for immune status and quantitative immunoglobulin levels in HSCT patients only.

Subjects who suffer from an acute illness with or without elevated body temperature (≥ 37.5°C, measured axillary with two weeks prior to screening will not be vaccinated. In this case Visit 1 will take place separately at a later date, as long as the study is ongoing.

8.5.2.2. Visit 1 /First Vaccination (Day 0)

The following activities will be performed before vaccination only when Visit 0 and Visit 1 are not the same day

- Review of inclusion and exclusion criteria
- Medical history update
- Physical examination including body temperature measured axillary
- Pregnancy test in females capable of bearing children.

Then the subject will receive the first vaccination. For vaccination the following procedures have to be considered:

- The vaccine has to reach room temperature before administration of the vaccine
- The vaccine has to be shaken vigorously for five to ten seconds
- The injection site has to prepared according to standard clinical procedures
- After removement of the protecting cap from the needle the 0.5 ml dose of the vaccine has to be administered immediately by intramuscular injection into the musculus deltoideus in the upper arm.

Following vaccination, the subject will be oserved for at least 30 minutes. Any infection site reactions and systemic adverse experiences will be recorded.

8.5.2.3. Visit 2 /Second Vaccination (Day 28 ± 2 days)

The following activities will be performed:

- The investigator will ask all subjects about any adverse experiences occurring since Visit 1. All adverse experiences will be documented in the CRF
- Physical examination including body temperature measured axillarily
- Pregnancy test in females capable of bearing children.
- Second vaccination: for details see above
- Post vaccination observation period (30 minutes): for details see above

8.5.2.4. Visit 3 /1. Evaluation of cellular immunity (Day 35, 7 ± 2 days after the second vaccination)

The following activities will be performed:

- The investigator will ask all subjects about any adverse experiences occurring since Visit 2. All adverse experiences will be documented in the CRF.
- Blood draw of 40 ml for determination of cellular immunity

8.5.2.5. Visit 4 /1. Evaluation of humoral immunity (Day 56, 28 ± 7 days after the second vaccination)

The following activities will be performed:

- The investigator will ask all subjects about any adverse experiences occurring since Visit 3. All adverse experiences will be documented in the CRF.
- Blood draw of 10 ml for serum antibody determination (NT, ELISA)
- Pregnancy test in females capable of bearing children.

8.5.2.6. Visit 5 /1. Evaluation of immune status (Day 84 ± 14 days)

The following activities will be performed in HSCT patients only:

- The investigator will ask all subjects about any adverse experiences occurring since Visit 4. All adverse experiences will be documented in the CRF.
- Blood draw of 10 ml for immune status and quantitative immunoglobulin levels
- Pregnancy test in females capable of bearing children.

8.5.2.7. Visit 6 /3. Vaccination and evaluation of immune status (Day 270-365)

The following activities will be performed:

- The investigator will ask all subjects about any adverse experiences occurring since Visit 5. All adverse experiences will be documented in the CRF.
- Physical examination including body temperature measured axillary.
- Pregnancy test in females capable of bearing children.
- Third vaccination: for details see Visit 1.
- Post vaccination observation period (30 minutes): for details see Visit 1.
- Blood draw of 10 ml for immune status and quantitative immunoglobulin levels

8.5.2.8. Visit 7 /2. Evaluation of cellular immunity (Day 277-372, 7 ± 2 days after the third vaccination)

The following activities will be performed in HSCT patients only:

- The investigator will ask all subjects about any adverse experiences occurring since Visit 6. All adverse experiences will be documented in the CRF.
- Blood draw of 40 ml for cellular immunity

8.5.2.9. Visit 8 /2. Evaluation of humoral immunity (Day 298-393, 28 ± 7 days after the third vaccination)

The following activities will be performed in HSCT patients only:

- The investigator will ask all subjects about any adverse experiences occurring since Visit 7. All adverse experiences will be documented in the CRF.
- Blood draw of 10 ml for serum antibody determination (NT, ELISA)
- Pregnancy test in females capable of bearing children.

After this visit, the patients will have completed the study.

8.5.3 Determination of the humoral and cellular immunity

8.5.3.1 Determination of serum antibodies against vaccination antigens

For the evaluation of antibody levels, both NT and enzyme linked immunosorbent assay (ELISA) will be performed [23]. Virus neutralization titers will be defined as the reciprocal of the serum dilution that gives a 90% reduction of virus growth compared to the control without antibody.

Neutralizing antibodies have been shown to be protective [24] and are generally viewed as a surrogate parameter of protection against TBE [17]. Neutralization tests will be performed by PFIZER. Blood samples will be also screened for TBE antibodies by ELISA [23] and will be considered seropositive when the values are above the cut-off of the assay.

8.5.3.2 Determination of cellular immune response

To investigate cellular immunity following TBE vaccination, lymphocyte proliferation and cytokine detection assays will be used. Peripheral blood mononuclear cells (PBMCs) will be isolated from heparinized venous blood by ficoll-diatrizoate centrifugation at 3 points in time as specified above (study visit 1, 3 and 7) and will be cryopreserved and stored in liquid nitrogen for later use. Lymphocyte proliferation will be analysed by [3H]thymidine incorporation and carboxyfluorescein diacetate succinimidyl ester (CFSE) labelling. PBMCs from defined time points for each subject will be thawed [25] and assayed together. As antigen to stimulate PBMCs we will use the whole aluminium hydroxide-free and human albuminfree TBE antigen at 4.8 µg/ml provided by PFIZER. Phytohemagglutinin (PHA) will be used for nonspecifically mitogenic stimulation of PBMCs at either 1 or 5 µg/ml. PBMCs will be cultured in triplicate (10^5 PBMC/well) with or without various stimuli at 37°C in a CO2 incubator for 6 days before harvesting. For the [3H]thymidine incorporation assay, PBMCs will be then pulsed with radioactive 3H (tritiated) thymidine for sixteen hours. The amount of radioactivity incorporated into DNA in each well will be measured in a scintillation counter and is proportional to the number of proliferating cells, which in turn is a function of the number of lymphocytes that were stimulated by a given antigen to enter the proliferative response. Results will be expressed as Δcpm (calculated as mean counts per minute of stimulated cell cultures minus mean counts per minute of medium control cell cultures) and as the stimulation index (SI), calculated as the ratio of the mean counts per minute of stimulated cell cultures to the mean counts per minute of medium control cell cultures [26].
For the CFSE proliferation assay, PBMCs will be CFSE-loaded (and fluorescein isothiocyanate-labeled) and cultured with medium alone (negative control), or PHA, or the whole aluminium hydroxide-free and human albumin-free TBE antigen at 4.8 µg/ml. After 6 days of incubation at 37°C and 5%CO2, collected cells will be stained with anti-CD3 monoclonal antibody (allophycocyanin), anti-CD8 (peridinin chlorophyll protein), and anti-CD4 (phycoerythrin). A total of 100,000 events in a lymphocyte gate will be analyzed by flow cytometry. Percentages of CD3+, CD8+, and CD4+ CFSE proliferating cells will be evaluated simultaneously by gating on CD3+ T cells and measuring sequentially proliferating CD8+ and CD4+ subsets within the CD3+ subsets. Net percentages of CFSE will be calculated by subtracting the negative control values as described previously [27]. Furthermore, cytokines will be measured in the supernatant (IL-2, IFN-gamma, IL-6, IL-10, TNFalpha) after in vitro stimulation with and without TBE virus antigen or PHA for 48 hours using ELISA.

8.5.3.3 Determination of immune reconstitution in HSCT recipients

The status of immune reconstitution in HSCT recipients prior to and after vaccination will be assessed by immunofluorescence staining of peripheral blood (PB) T and B lymphocytes and flow cytometric analyses. Therefore, PB cells will be stained with monoclonal antibodies and analyzed by FACS for the following cell populations: T lymphocytes (CD3+), B lymphocytes (CD19+), T helper cells (CD3+CD4+), T suppressor cells (CD3+CD8+), natural killer cells (CD3-CD16&56+) and cytotoxic cells (CD3+CD16&56+).
In addition, serum immunoglobulin levels will be analysed. Immune reconstitution and serum immunoglobulin levels will be determined in all HSCT patients prior to the first and third vaccination and 12 weeks after the first vaccination (study visits 1, 5 and 6).

8.5.4 *Study endpoints*

8.5.4.1 Primary study endpoint

To assess the humoral immunogenicity of the TBE vaccination, the outcome of NT will be analysed four weeks after the second vaccination. Therefore, the number of subjects with NT titers against TBE virus >10, assumed to be the threshold for antibody-mediated protection, will be evaluated.

8.5.4.2 Secondary study end points

The secondary end points of this study are:

- Antibody concentrations of TBE ELISA (validated in-house test, Department of Virology, Medical University of Vienna) before and four weeks after the second and third vaccination.
    - Increase of antibody response.
    - Number of subjects with seroconversion defined when patients reach antibody levels above the cut-off of TBE ELISA.
- Antibody concentrations of NT titers four weeks after the third vaccination compared to four weeks after the second vaccination.
    - Increase of antibody response.
    - Number of subjects with NT titers against TBE virus >10.
- Evaluation of cellular immunity prior to and one week after the second and third vaccination.
    - Lymphoproliferative response after vaccination induced by TBE antigen.
    - Increase in cytokine levels induced by TBE antigen

- Evaluation of immune reconstitution and quantitative immunoglobulin levels before first and third vaccination and 12 weeks after first vaccination in HSCT recipients only.

9. SAFETY DEFINITIONS AND REPORTING REQUIREMENTS

9.1 Adverse events (AEs)

9.1.1 Summary of known and potential risks of the study drug

The following side effects have to be considered:
- very common (>1/10): local reactions at the site of injection, transient redness, and swelling.
- common (>1/100;<1/10): systemic reactions including fever, fatigue, headache, muscular pain, arthralgia. These side-effects usually occur within 72 hours after the vaccination, abate within 1-2 days, but do not acquire any medical treatment. All adverse events will be listed in the study documents and have to evaluated by the investigator for causality to the vaccination. After venous puncture local reactions, a hematoma or phlebitis can occur. However, the planed venous punctures withdraw only minor volumes of blood and do not impair the patient's health.

9.1.2 Definition of adverse events

An AE is any untoward adverse change from the subject's baseline condition, i.e., any unfavorable and unintended sign including an abnormal laboratory finding, symptom or disease which is considered to be clinically relevant by the physician that occurs during the course of the study, whether or not considered related to the study drug.
Adverse events include:

- Exacerbation of a pre-existing disease.
- Increase in frequency or intensity of a pre-existing episodic disease or medical condition.
- Disease or medical condition detected or diagnosed after study drug administration even though it may have been present prior to the start of the study.
- Continuous persistent disease or symptoms present at baseline that worsen following the start of the study.
- Lack of efficacy in the acute treatment of a life-threatening disease.
- Events considered by the investigator to be related to study-mandated procedures.

- Abnormal assessments, e.g., ECG and physical examination findings, must be reported as AEs if they represent a clinically significant finding that was not present at baseline or worsened during the course of the study.
- Laboratory test abnormalities must be reported as AEs if they represent a clinically significant finding, symptomatic or not, which was not present at baseline or worsened during the course of the study or led to dose reduction, interruption or permanent discontinuation of study drug.

Adverse events do not include:

- Pre-planned interventions or occurrence of endpoints specified in the study protocol are not considered AE´s, if not defined otherwise (eg.as a result of overdose)
- Medical or surgical procedure, e.g., surgery, endoscopy, tooth extraction, transfusion. However, the event leading to the procedure is an AE. If this event is serious, the procedure must be described in the SAE narrative.
- Pre-existing disease or medical condition that does not worsen.
- Situations in which an adverse change did not occur, e.g., hospitalizations for cosmetic elective surgery or for social and/or convenience reasons.
- Overdose of either study drug or concomitant medication without any signs or symptoms. However, overdose must be mentioned in the Study Drug Log.

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 is not considered an SAE and should be reported as an AE only:

- Treatment on an emergency or outsubject basis for an event not fulfilling the definition of seriousness given above and not resulting in hospitalization.

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

- Hospitalizations for cosmetic elective surgery, social and/or convenience reasons.
- Standard monitoring of a pre-existing disease or medical condition that did not worsen, e.g., hospitalization for coronary angiography in a subject with stable angina pectoris.
- Elective treatment of a pre-existing disease or medical condition that did not worsen, e.g., hospitalization for chemotherapy for cancer, elective hip replacement for arthritis.

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, heart catheterization), 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 SmPC - Summary of Product Characteristics or 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.

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 drug and the AE using his/her clinical expertise and judgment according to the following algorithm that best fits the circumstances of the AE:

Unrelated

- May or may not follow a reasonable temporal sequence from administration of the study product
- Is biologically implausible and does not follow known response pattern to the suspect study drug (if response pattern is previously known).
- Can be explained by the known characteristics of the subject's clinical state or other modes of therapy administered to the subject.
- Unlikely
- May or may not follow a reasonable temporal sequence from administration of the study product
- Is biologically not very plausible
- May be explained by the known characteristics of the subject's clinical state or other modes of therapy administered to the subject.

Possible related

- Follows a reasonable temporal sequence form administration of the study drug.

- May follow a known response pattern to the study drug (if response pattern is previously known).
- Could not be reasonably explained by the known characteristics of the subject's clinical state or other modes of therapy administered to the subject, if applicable.
- Probable
- Follows a reasonable temporal sequence form administration of the study drug.
- Follows a known response pattern to the study drug (if response pattern is previously known).
- other causes for the event are unlikely

Definitely related

- Follows a reasonable temporal sequence form administration of the study drug.
- Follows a known response pattern to the study drug (if response pattern is previously known).
- No other reasonable cause is present.

9.5 Reporting procedures

A special section is designated to adverse events in the case report form. The following details must thereby be entered:

- Type of adverse event
- Start (date and time)
- End (date and time)
- Severity (mild, moderate, severe)
- Serious (no / yes)
- Unexpected (no / yes)
- Outcome (resolved, ongoing, ongoing – improved, ongoing – worsening)
- Relation to study drug (unrelated, possibly related, definitely related)

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, the investigator has to use all supportive measures for best patient treatment. A written report is also to be prepared and made available to the clinical investigator immediately. The following details should at least be available:

- Patient initials and number
- Patient: 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.

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 study management and the following details should be at least available:

- Patient initials and number
- Patient: 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 the 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

No follow-up of the patient is required for this study.

11. STATISTICAL ANALYSIS

Statistical analysis will be planed and performed by a statistician of the Institute for Medical Statistics, Medical University of Vienna, Dr. Alexandra Graf.

11.1 Sample size considerations

For our phase II pilot study a calculation of the sample size was performed using nQuery 6.1. The primary endpoint is the outcome of the NT against 4 weeks after the second vaccination (positive test: Yes/No). Because of deviation of the antibody concentrations from normal distribution (NT level <=10 not detectable indicating a negative test), the outcome is reduced to a binary but clinically more relevant outcome (seroconversion rate: NT levels >10 indicates a positive test). Previously, in a study with heart transplant recipients a seroconversion rate of 35% was detected compared to 100% in the healthy control group [21]. In our study we expect a seroconversion rate of at least 90% in the healthy control group. In the HSCT patients we expect a seroconversion not exceeding 50%. The calculation of the sample size was performed using the following assumption: Significance level alpha 0.05, Power 0.8, ratio of HSCT patients/healthy volunteers: 1/1. With a total number of 46 participants (23 HSCT patients and 23 healthy volunteers) the assumed difference in the seroconversion rate between groups can be detected (using a two-sided Fisher's Exact Test) with 80% Power. We added 10% to adjust for potential loss-to-follow-up which results in 26 HSCT patients and 26 healthy volunteers.

11.2 Relevant protocol deviations

All protocol deviations will be listed in the study report. Major deviations regarding to subjects safety will lead to withdrawal.

11.3 Endpoints analysis

The primary endpoint (outcome of NT 4 weeks after the second vaccination) will be analysed using Fisher's Exact Test. For the seroconversion rates, 95% confidence intervals will be calculated. All subjects with available antibody responses fulfilling the eligibility criteria will be included in the analysis

The secondary endpoints (NT 4 weeks after the third vaccination, ELISA prior to the first vaccination and 4 weeks after the second and third vaccination, cellular immunity, immunoglobulin levels and immune reconstitution) will be analysed using descriptive statistics.

11.4 Missing, unused and spurious data

Only subjects for whom data are available will be included in the statistical analysis. Missing values will neither be replaced nor estimated.

11.5 Interim analysis

No interim analysis will be performed.

11.6 Software program(s)

Sample size calculation was performed using nQuery 6.1.

Statistical analysis will be prepared using SPSS Statistics (Version 17.0 or higher).

12. DOCUMENTATION AND DATA MANAGEMENT

12.1 Documentation of study results

A subject screening and enrollment Log will be completed for all eligible or non-eligible subjects with the reasons for exclusion.

12.1.1 Case report form (CRF)

For each subject enrolled, regardless of study drug initiation, a Paper-CRF must 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 must be noted on the CRF. Case report forms are to be completed on an ongoing basis.

CRF entries and corrections will only be performed by study site staff, authorized by the investigator.

In the “Paper-CRF” all forms should be completed and must be legible. Errors should be crossed out but not obliterated, the correction inserted, and the change initialed and dated by the investigator, co-investigator or study nurse. The original CRFs are passed first by Dr. Forstner and then the entries will be checked by the monitor Dr. Ramharter and any errors or inconsistencies will be checked immediately.

The monitor will collect original completed and signed CRFs at the end of the study.

12.1.2 Data Collection

Data collected at all visits are entered into an interactive form. The CRFs will be source documents verified following guidelines established before study onset as detailed in the Monitoring Plan. Maintenance of the study database will be performed by Dr. Forstner.

12.2 Safekeeping

The investigator will maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified (according to ICH-GCP “essential documents”). These documents will be classified into two different categories: investigator's file, and subject clinical source documents.

The investigator's file will contain the protocol/amendments, EudraCT forms, CRFs (eCRF printout), standard operation procedures (SOPs), data clarification and query forms, EC/IRB and Health Authority approval with correspondence, informed consent, drug records, staff curriculum vitae and authorization forms, screening and enrollment logs, and other appropriate documents/correspondence as per ICH/Good Clinical Practice (GCP) and local regulations.

Subject clinical source documents include, but are not limited to subject hospital/clinic records, physician’s and nurse’s notes, appointment book, original laboratory reports, ECG,

X-ray, pathology and special assessment reports, consultant letters, etc.

These two categories of documents must be kept on file by the investigator for as long as needed to comply with national and international regulations (in Austria 15 years after discontinuing clinical development or after the last marketing approval). If source documents are not durable as long as needed they must be preserved as a copy. No study document should be destroyed without prior written approval from the Department of Internal Medicine I.
When source documents are required for the continued care of the subject, appropriate copies should be made for storing outside of the site.

12.3 Quality Control and Quality Assurance

The following quality control and quality assurance measures will be taken to ensure the adherence to GCP and applicable regulatory requirements as well as the accuracy and integrity of data obtained from the study:

- Periodic visits will be performed by the monitor to assure integrity and accuracy of study data. Discrepancies will be addressed and appropriate corrective action will be implemented.
- Training for study personnel as well as for monitors will be provided on the correct handling and use of the CRF. Investigators will also be trained on GCP and Good Documentation Practice (GDP) relevant issues.

12.3.1 Periodic Monitoring

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 monitoring plan 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. Depending on the quality of the data, additional monitoring visits

may be necessary according to the sponsor's discretion. The investigator will resolve discrepancies of data.

Monitoring will be performed by Dr. Ramharter. Three visits are planned, an initiation visit, one routine visit after the first year and a close out visit after the last patient has finished the study or data base lock. 100% of source data will be checked by the monitor (which means 100% Source Data Verification).

12.3.2 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.

12.4 Reporting and Publication

12.4.1 Publication of study results

The findings of this study will be published by the investigator) in a scientific journal and will be 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 patient 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 patient information sheet in comprehensible language, explaining the nature and purpose of the study and its progress.
The patients must agree to the possibility of study-related data being passed on to relevant authorities.
The patients 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 investigator (or a designated CRO) will submit this protocol and any related document provided to the subject (such as subject information used to obtain informed consent) to an Ethics Committee (EC) or Institutional Review Board (IRB). 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 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.

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 patients will be insured as defined by legal requirements. The investigator of the clinical trial will receive a copy of the insurance conditions of the 'patients 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 patients insurance are given in the patient information sheet.

13.4 Confidentiality

The information contained in this document, especially unpublished data, is the property of the project leader. 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 of the project leader.

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, if applicable, the Code of Federal Regulations (USA). In other countries in which GCP Guidelines exist, the investigators will strictly ensure adherence to the stated provisions.

Therefore this study follows the EU Directive embedded in the Austrian drug act.

14. APPENDICES

Appendix 1. Informed Consent Form (Version 5.0; Date 30.01.2014)

Appendix 2. Summary of medicinal products characteristics of FSME-IMMUN 0,5 ml

15. REFERENCES

1. Singhal S, Mehta J. Reimmunization after blood or marrow stem cell transplantation. Bone Marrow Transplant 1999; 23: 637-646.
2. Wilck MB, Baden LR. Vaccination after stem cell transplant: a review of recent developments and implications for current practice. Curr Opin Infect Dis 2008; 21: 399-408.
3. Avigan D, Pirofski LA, Lazarus HM. Vaccination against infectious disease following hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2001; 7: 171-183.
4. Storek J, Witherspoon RP, Webb D, Storb R. Lack of B cell precursors in marrow transplant recipients with chronic GVHD. Am J Hematol 1996; 52: 82-89.
5. Maury S, Mary JY, Rabian C, Schwarzinger M, Toubert A, Scieux C, Carmagnat M, Esperou H, Ribaud P, Devergie A, Guardiola P, Vexiau P, Charron D, Gluckman E, Socie G. Prolonged immune deficiency following allogeneic stem cell transplantation: risk factors in complications in adult patients. Br J Haematol 2001; 115: 630-641.
6. Hammarstrom V, Pauksen K, Svensson H, Lonnqvist B, Simonsson B, Ringden O, Ljungman P. Serum immunoglobulin levels in relation to levels of specific antibodies in allogeneic and autologous bone marrow transplant recipients. Transplantation 2000; 69: 1582-1586.
7. Lee SJ, Vogelsang G, Flowers ME. Chronic graft-versus-host disease. Biol Blood Marrow Transplant 2003; 9: 215-233.
8. Socie G. Graft-versus-host disease --from bench to bedside? N Engl J Med 2005; 353: 1396-1397.
9. Vogelsang GB, Lee L, Bensen-Kennedy DM. Pathogenesis and treatment of graft-versus-host-disease after bone marrow transplant. Annu Rev Med 2003; 54: 29-52.
10. Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, Martin P, Chien J, Przepiorka D, Couriel D, Cowen EW, Dinndorf P, Farrell A, Hartzman R, Henslee-Downey J, Jacobsohn D, McDonald G, Mittleman B, Rizzo JD, Robinson M, Schubert M, Schultz K, Shulman H, Turner M, Vogelsang G, Flowers MED. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant 2005; 11:945-956.

11. Wolff D, Gerbitz A, Ayuk F, Kiani A, Hildebrandt GC, Vogelsang GB, Elad S, Lawitschka A, Socie G, Pavletic SZ, Holler E, Greinix H. Consensus conference on clinical practice in chronic graft-versus-host disease (GVHD): first-line and topical treatment of chronic GVHD. Biol Blood Marrow Transplant 2010; 16: 1611-1628.
12. Young JA. Infectious complications of acute and chronic GVHD. Best Pract Res Clin Haematol 2008; 21: 343-356.
13. Holzmann H. Diagnosis of tick-borne encephalitis. Vaccine 2003; 21: 36-40.
14. Kunz C. TBE vaccination and the Austrian experience. Vaccine 2003; 21 (Suppl 1): S50-55.
15. Demicheli V, Debalini MG; Rivetti A. Vaccines for preventing tick-borne encephalitis. Cochrane Database Syst Rev 2009;(1):CD000977.
16. Hilgendorf I et al. Vaccination of allogeneic haematopoetic stem cell transplant recipients. Report from the International Consensus Conference on Clinical Practice in chronic GVHD. Vaccine 2011, in press.
17. Kaiser R, Holzmann H. Laboratory findings in tick-borne encephalitis – correlation with clinical outcome. Infection 2000; 28: 78-84.
18. B Panasiuk, D Prokopowicz, A. Panasiuk. Immunological response in HIV-positive patients vaccinated against tick-borne encephalitis. Infection 2003; 31: 45-46.
19. Jilkova E, Vejvalkova P, Stiborova I, Skorkovsky J, Kral V. Serological response to tick-borne encephalitis (TBE) vaccination in the elderly--results from an observational study. Expert Opin Biol Ther 2009; 9: 797-803.
20. Weinberger B, Keller M, Fischer KH, Stiasny K, Neuner C, Heinz FX, Grubeck-Loebenstein B. Decreased antibody titers and booster responses in tick-borne encephalitis vaccinees aged 50-90 years. Vaccine 2010; 28: 3511-3515.
21. Dengler TJ; Zimmermann R; Meyer J; Sack FU; Girgsdies O; Kubler WE. Vaccination against tick-borne encephalitis under therapeutic immunosuppression. Reduced efficacy in heart transplant recipients. Vaccine 1999; 17: 867-874.
22. Hammarstrom V, Pauksen K, Azinge J, Oberg G, Ljungman P. Pneumococcal immunity and response to immunization with pneumococcal vaccine in bone marrow transplant patients: the influence of graft versus host reaction. Support Care Cancer 1993; 1: 195-199.
23. Stiasny K, Holzmann H, Heinz FX. Characteristics of antibody responses in tick-borne encephalitis vaccination breakthroughs. Vaccine 2009;27:7021-7026.
24. Kreil TR, Eibl MM. Pre- and postexposure protection by passive immunoglobulin but no enhancement of infection with a flavivirus in a mouse model. J Virol 1997; 71:2921-2927.

25. Disis ML, dela Rosa C, Goodell V, Kuan LY, Chang JC, Kuus-Reichel K., Clay TM, Kim, Lyerly H, Bhatia S, Ghanekar SA, Maino VC, Maecker HT. Maximizing the retention of antigen specific lymphocyte function after cryopreservation. J Immunol Methods. 2006; 308: 13-6.
26. Gorse GJ, Simionescu RE, Patel GB. Cellular immune responses in asymptomatic human immunodeficiency virus type 1 (HIV-1) infection and effects of vaccination with recombinant envelope glycoprotein of HIV-1. Clin Immunol 2006; 13: 26-32.
27. Valor L, Sarmiento E, Navarro J, Gallego A, Fernandez-Yanez J, Fernandez-Cruz E, Carbone J. Evaluation of lymphoproliferative responses by carboxy fluorescein succinimidyl ester assay in heart recipients with infections. Transplantation Proceedings 2012; 44: 2649-2652.