A Phase II Study to Evaluate the Immunogenicity, Safety and Tolerability of a H1N1 Influenza Vaccine in Immunocompromised Adults Who Have Undergone Solid Organ Transplantation or Bone Marrow Transplantation and in Age-Matched Healthy Volunteers

Short term/title:	Focetria Tx
EudraCT number:	2009-017052-27
Investigational product:	Focetria®
Indication:	Vaccination for H1N1sw of immunocompromised adults who have undergone solid organ or bone marrow transplantation and of age-matched healthy volunteers
Study design:	Monocenter, single arm study with age-matched healthy controls
Sponsor:	Hannover Medical School Carl-Neuberg-Str. 1 D-30625 Hannover, Germany
Protocol code:	200910H1N1MHH
Development phase:	Phase II
Study initiation date:	09-March-2010
Date of early study termination:	30-April-2011
Study completion date:	21-April-2011
Principal investigator:	Prof. Dr. Michael Manns Hannover Medical School Department of Gastroenterology, Hepatology and Endocrinology Carl-Neuberg-Str. 1 D-30625 Hannover, Germany
Report version:	2.0
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Replaces previous version dated:	1.0 (03-September-2014)
Including the following amendment(s):	

The study was performed in compliance with Good Clinical Practice (GCP)

2 SYNOPSIS

Name of Sponsor/Company: Hannover Medical School Carl-Neuberg-Str. 1 D-30625 Hannover, Germany	**Individual Study Table Referring to Part of the Dossier** n/a	***(For National Authority Use only)***
Name of Finished Product: Focetria®	**Volume: -**	
Name of Active Ingredient: H1N1 influenza vaccine (adjuvanted with MF59C.1)	**Page: -**	
Title of Study: A Phase II Study to Evaluate the Immunogenicity, Safety and Tolerability of a H1N1 Influenza Vaccine in Immunocompromised Adults Who Have Undergone Solid Organ Transplantation or Bone Marrow Transplantation and in Age-Matched Healthy Volunteers		
Investigators: Prof. Dr. Michael Manns (PI) Department of Gastroenterology, Hepatology and Endocrinology Hannover Medical School Carl-Neuberg-Str. 1 D-30625 Hannover, Germany		
Study centre(s): Hannover Medical School Carl-Neuberg-Str. 1 D-30625 Hannover, Germany		
Publication (reference): None at the time of the report		
Studied period (years): **Date of first enrolment:** 09.03.2010 **Date of last completed:** 21.04.2011	**Phase of development:** Phase II study	

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Objectives:

Primary objective:

The adjuvanted H1N1 influenza vaccine, when administered twice in transplanted patients, fulfills all serological efficacy criteria as required for the elderly population (aged 60 and older) according to the respective European guidance documents. These criteria are 30% for seroconversion rate, 60% for seroprotection rate and 2 for geometric mean ratio (GMR).

Main secondary objective:

The adjuvanted H1N1 influenza vaccine in transplanted patients, when administered twice, is at least as effective as the adjuvanted H1N1 influenza vaccine in the healthy volunteers after only one administration. For orientation in the assessment a non-inferiority-margin of 0.5 for the ratio of the geometric mean titers (GMTs) of transplanted patients and age-matched healthy volunteers at day 42 will be used.

Further secondary objectives:

1. The serological efficacy criteria as outlined for the elderly in the European guidance documents (EMEA/CPMP/BWP/214/96) are fulfilled for transplanted patients at day 21 and 280.
2. Comparison of the serological efficacy criteria seroprotection and seroconversion rates between transplanted patients and age-matched healthy volunteers.
3. The serological efficacy criteria as outlined in the European guidance documents are fulfilled for age-matched healthy volunteers at day 21, 42 and 280. These criteria are 40% for seroconversion rate, 70% for seroprotection rate and 2.5 for GMR.
4. Comparison of immune response in relation to immunosuppressive medication in transplant subjects.
5. All serological assessments and group comparisons measured by microneutralization (MN) for transplanted patients and age-matched healthy volunteers will be performed in line with HI analyses at all time points.

Comparison of the safety of the adjuvanted overall, as well as in transplant patients and age-matched healthy volunteers separately.

Methodology:

Prospective, mono-center, single arm with age-matched healthy controls

Number of patients (planned and analyzed):

Planned: 120 subjects (60 Tx patients and 60 age-matched healthy volunteers).

Analysed: 13 subjects (8 Tx patients and 5 age-matched healthy volunteers) were recruited and analyzed.

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Diagnosis and main criteria for inclusion:

Diagnosis:

Adults who have undergone solid organ transplantation (renal, cardiac, liver, lung) or bone marrow transplantation and age-matched healthy adults.

Inclusion criteria:

Transplant recipients:

- Adult subjects 18-60 years of age who have undergone prior renal, cardiac, liver, lung, or bone marrow transplantation for any reason, more than 3 months prior to enrolment
- Patients able to visit the outpatient clinic with a life expectancy of at least one year
- Patients who receive any immunosuppressive treatment currently taken to prevent organ rejection

Healthy adults:

- Adult subjects 18-60 years of age
- Healthy individuals as determined by medical history, physical assessment and clinical judgment of the investigator
- Within the same age category (+/- 5 years) than the incidental transplanted patient

Transplant recipients and healthy adults:

- Individuals who are able to comply with all study procedures and are available for all clinic visits scheduled in the study
- Women of child-bearing potential (WOCBP) must have used an acceptable contraceptive method for at least 2 months prior to study entry until 3 weeks after last vaccination:
  - Female of childbearing potential is defined as an onset of menarche or pre-menopausal female capable of becoming pregnant. This does not include females who meet any of the following conditions: (1) menopause at least 2 years earlier, (2) tubal ligation at least 1 year earlier, or (3) total hysterectomy
  - Acceptable birth control methods are defined as one or more of the following:
    - Hormonal contraceptive (such as oral, injection, transdermal patch, implant, cervical ring)
    - Barrier (condom with spermicide or diaphragm with spermicide) each and every time during intercourse
    - Intrauterine device (IUD)
    - Monogamous relationship with vasectomized partner. Partner must have been vasectomized for at least six months prior to the subject's study entry

Test product, dose and mode of administration, batch number:

Focetria®, 0.5 mL injection (antigen content: 7.5 µg, MF59 content: 9.75 mg), intramuscular

Lot number: 091001D, exp. Date 08/2010

Duration of treatment:

Transplant recipients: Focetria® was administered as intramuscular injection at study day 0 and study day 21±4 days.

Healthy adults: Focetria® was administered as single intramuscular injection at study day 0.

Reference therapy, dose and mode of administration, batch number:

n/a

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Criteria for evaluation:

Efficacy:

Primary endpoint:

The observed percentage of seroconversion and seroprotection rates, as well as the observed GMR (measured by HI) in transplanted patients at day 42 will be compared with the thresholds from the guideline for adults, aged over 60 (as outlined above). This study is successful, if all three point estimates pass the corresponding efficacy criteria at day 42. For descriptive purpose two-sided 95%-confidence intervals for the rates and the GMR at day 42 will be presented.

Main secondary endpoint:

$\log_{10}$(GMT) values of transplanted and age-matched healthy volunteers will be compared with a two-sample t-test and the corresponding two-sided 95% confidence interval will be computed. Non-inferiority of the adjuvanted vaccine will be concluded, if the respective lower boundary of the two-sided 95% confidence interval for the ratio of GMTs in treatment groups does not exceed 0.50.

Further secondary endpoints:

1. Percentage of subjects with seroconversion, percentage of subjects achieving seroprotection, and GMR at day 21 and day 280 will be calculated and compared with the thresholds from the guideline. Point estimates and corresponding descriptive two-sided 95% confidence intervals will be presented, in addition.
2. Frequencies of the serological efficacy criteria seroprotection and seroconversion rates between transplanted patients and age-matched healthy volunteers will be compared. Corresponding 95% explorative confidence intervals for rate differences will be computed.
3. For age-matched healthy volunteers the success criteria according to the aforementioned guidelines will be compared per vaccination group with the thresholds for healthy individuals.
4. Success criteria will be compared in relation to immunosuppressive therapy in transplanted patients using either means and standard deviations for $\log_{10}$(GMT) values or frequencies for seroprotection and seroconversion rates. Corresponding explorative 95% confidence intervals for each of the serological variable will be considered.
5. Serological assessment of MN follows in general the aforementioned analysis-strategies for HI.

Safety:

All safety analyses will be performed within all vaccinated individuals, as well as in transplant patients and age-matched healthy volunteers separately. Thorough evaluation of all patterns of solicited events, non-solicited events and SAEs will be performed.

Statistical methods:

The planned number of 120 subjects could not be recruited. Only 13 subjects (8 transplanted patients and 5 healthy volunteers) were recruited and completed the study according to the protocol. A conclusive statistical analysis is not possible.

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SUMMARY - CONCLUSIONS

EFFICACY RESULTS:
Only 13 subjects completed the study according to protocol. Thus, no conclusive efficacy results can be reached.

SAFETY RESULTS:
Exposure: A total number of 13 participants (n= 8 patients after solid organ transplantation and n= 5 healthy volunteers) received the investigational medicinal product (IMP) Focetria® as per protocol.

Adverse Events: A total of seven non-serious adverse events (AEs) occurred in this trial. Three AEs were assessed by the investigator as not related to IMP Focetria®. The remaining four AEs were assessed as likely (n=2) or possibly related (n=2) to the IMP. These related AEs were: influenza-like illness (n=1, patient after liver transplant), hematoma (n=1, healthy volunteer), flushing (n=1, healthy volunteer), and pyrexia (n=1, healthy volunteer). Patients and volunteers recovered from all AEs.

Serious Adverse Events: A total of eleven serious adverse events (SAEs) have been reported to the Sponsor. All SAEs occurred in the group of patients who received the IMP after solid organ transplantation, none in the control group of healthy volunteers. SAEs affected a total of seven patients: one patient experienced three SAEs, three patients had two SAEs each, and two patients had a single SAE each. With the exception of one event which is still ongoing and most likely will stay chronic, all other SAEs were reported as recovered. No fatalities occurred.
All SAEs were rated as not related to the IMP Focetria® by both, the reporting investigator and the sponsor´s delegate for pharmacovigilance. Instead, SAEs were denominated as being related to the underlying disease (solid organ transplant) and/or concomitant medication (immunosuppressants).

Based on AE/SAE assessment, there have been no new findings related to the safety of the IMP in this trial. Equally, no findings that change the safety profile of the IMP as described in the investigator's brochure have been risen during this trial.

CONCLUSION:

The clinical trial was initially planned with a 4 months recruitment period and a 9 months treatment period per patient / volunteer. Therefore, the overall trial duration (FSFV to LSLV) was initially 13 months. At the end of the planned recruitment period the expected number of subjects could not be included in the trial. As a consequence the recruitment period was extended with a non-substantial amendment submitted in July 2010. By this the date the end of the trial was prolonged until the end of February 2012.

At the end of August 2010 the initial batch of study medication Focetria® expired. Due to the end of the flu season 2009/2010 a new batch of study medication was not available. Therefore, no further subjects could be included in the trial by the end of August 2010. The enrolled subjects were followed up according to the protocol. The last visit of the last enrolled subject was on 21.04.2011.

Initially 120 subjects were to be enrolled in the trial. Until the early termination of the trial only 13 subjects (8 transplanted patients and 5 healthy volunteers) were recruited. All 13 subjects received the study medication at their first study visit at day 0 and the transplanted patients received a second injection at day 21.
It was not possible to evaluate the immunogenicity, safety and tolerability of Focetria® from the available data of the few subjects participating in the prematurely terminated study.

Date of the report: 18.09.2014

Date, signature Prof. Dr. Heiko von der Leyen
(Representative of Sponsor)

Date, signature Prof. Dr. Michael Manns
(Principal Investigator)

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4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse event
ALT	Alanin-aminotransferase
AST	Aspartat-aminotransferase
BMT	Bone marrow transplantation
C	Celsius
CHMP	Committee for Medicinal Products for Human Use
CTAB	Cetyltrimethylammoniumbromide
EMEA	European Medicine Agency
GCP	Good Clinical Practice
GGT	Gamma-glutamyltransferase
GMR	Geometric mean ratio
GMT	Geometric mean titer
H1N1	Hemagglutinine 1 neurominidase 1
HbIg	Hepatitis B Immune globulin
HI	Hemagglutination inhibition
HIV	Human immune deficiency virus
ICF	Informed consent form
ICH	International Committee on Harmonization
IMP	Investigational medicinal product
IUD	Intrauterine device
MF59	Adjuvant
µg	Microgram
mg	Milligram
mL	Milliliter
MN	Microneutralization
NVD	Novartis Vaccines and Diagnostics
PI	Principal investigator
SAE	Serious adverse event
SmPC	Summary of product characteristics
SOP	Standard operating procedure
SRH	Single-Radial-Hemolysis
SUSAR	Suspected unexpected serious adverse reaction
TBC	Total Blood Count
Tx	Transplantation
WOCBP	Women of childbearing potential

5 ETHICS

This study was conducted according to the European Commission Directive on Good Clinical Practice 91/507/EEC (issued July 19, 1991 and effective January 1, 1992), the Declaration of Helsinki (see the Protocol Appendix), the ICH Guidelines, and local rules and regulations of the country.

Patients gave informed consent at the screening visit. The written patient information and the consent form are provided in the Appendix.

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Sponsor	Hannover Medical School Carl-Neuberg-Str. 1 D-30625 Hannover, Germany
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7 INTRODUCTION

Due to therapeutic immunosuppression, recipients of solid organ as well as bone marrow transplants have a higher risk of infection from the influenza virus than healthy individuals. Influenza infection in transplant recipients can lead to severe complications such as pneumonia, bacterial infections, or even acute graft rejection.

In June 2009 the World Health Organization (WHO) declared that the current influenza pandemic caused by a novel swine-origin influenza A/H1N1 virus (H1N1sw) had reached stage 6 (i.e. active transmission on a global scale). In August 2010, they announced that the alert level would be lowered for the H1N1 pandemic. It has moved to the "post-pandemic" situation in which after an infection or immunization many people world-wide have become immune to the virus A (H1N1) 2009. In addition to this virus, other influenza viruses such as A (H3N2) or influenza B are being increasingly reported. Therefore, the WHO expects that the recent influenza pandemic patterns are transitioning towards seasonal patterns. It is assumed that A (H1N1) 2009 will circulate among other influenza viruses for several years (1). Vaccination is the most effective means of preventing influenza illness, and it is the keystone of influenza preparedness programs.

Data from the previous pandemic situation suggest that children, pregnant women, and individuals with chronic underlying illness have the greatest risk of serious infection sequelae with the strain H1N1 (2-9). In addition, a small proportion of people infected during the pandemic developed a severe form of primary viral pneumonia that is not commonly seen during seasonal epidemics and is especially difficult to treat. In the U.S., severe cases of pandemic H1N1 infections have been reported in immunocompromised transplant recipients. Individual cases were also reported in a post liver transplant patient from New Zealand and a lung transplant recipient from Canada (8-13). Further data suggest that H1N1 infection causes substantial morbidity in recipients of solid-organ transplants (14). In addition to the increased consequences of infection, transplant patients and immunocompromised individuals may have an increased risk of non-responsiveness or hypo-responsiveness to conventional vaccines (15-17). Currently, in addition to trivalent seasonal vaccines, manufacturers are preparing monovalent H1N1sw vaccines. Health authorities may prioritize various at risk populations in the event of global vaccine shortage. To date, Novartis Vaccines and Diagnostics' (NVD) candidate vaccines have been tested in healthy individuals. Current data suggest that a single dose of H1N1 vaccine adjuvanted with MF59 is protective in this population. The adjuvanted vaccine provides significantly higher neutralizing and hemagglutination inhibition antibody responses than non-adjuvanted vaccine. However, there are no data to date in immunocompromised or chronically ill persons, important vaccine target groups for public health policy.

8 STUDY DESIGN AND OBJECTIVES

8.1 Study Design

This was a monocenter, single arm phase II study with age-matched healthy controls.

8.2 Study Objectives

8.2.1 Primary Objective

The adjuvanted H1N1 influenza vaccine, when administered twice in transplanted patients, fulfills all serologic efficacy criteria as required for the elderly population (aged 60 and older) according to the respective European guidance documents at day 42. These criteria are 30% for seroconversion rate, 60% for seroprotection rate, and 2 for geometric mean ratio (GMR).

8.2.2 Secondary Objectives

Main secondary objective:

The adjuvanted H1N1 influenza vaccine in transplanted patients, when administered twice, is at least as effective as the adjuvanted H1N1 influenza vaccine in the healthy volunteers after only one administration. For orientation in the assessment a non-inferiority-margin of 0.5 for the ratio of the geometric mean titers (GMT) of transplanted patients and age-matched healthy volunteers at day 42 was used.

Further secondary objectives:

1. The serological efficacy criteria as outlined for the elderly in the European guidance documents (EMEA/CPMP/BWP/214/96) are fulfilled for transplanted patients at day 21 and 280.
2. Comparison of the serological efficacy criteria seroprotection and seroconversion rates between transplanted patients and age-matched healthy volunteers.
3. The serological efficacy criteria as outlined in the European guidance documents (EMEA/CPMP/BWP/214/96) are fulfilled for age-matched healthy volunteers at day 21, 42, and 280. These criteria are 40% for seroconversion rate, 70% for seroprotection rate, and 2.5 for GMR.
4. Comparison of immune response in relation to immunosuppressive medication in transplant subjects.
5. All serological assessments and group comparisons measured by microneutralization (MN) for transplanted patients and age-matched healthy volunteers will be performed in line with HI analyses at all time points.
6. Comparison of the overall safety, as well as safety in transplant patients and age-matched healthy volunteers separately.

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan-Description

This was a monocenter, single arm phase II study comparing patients who have undergone solid organ or bone marrow transplantation to age-matched healthy controls. Transplanted patients were vaccinated twice (Visit 1 day 0 and Visit 2 day 21±4 days), controls once (Visit 1 on day 0) using adjuvanted Focetria® according to the SmPC (see the Appendix). Thirteen patients were included (Tx group: n = 8; control group: n = 5).

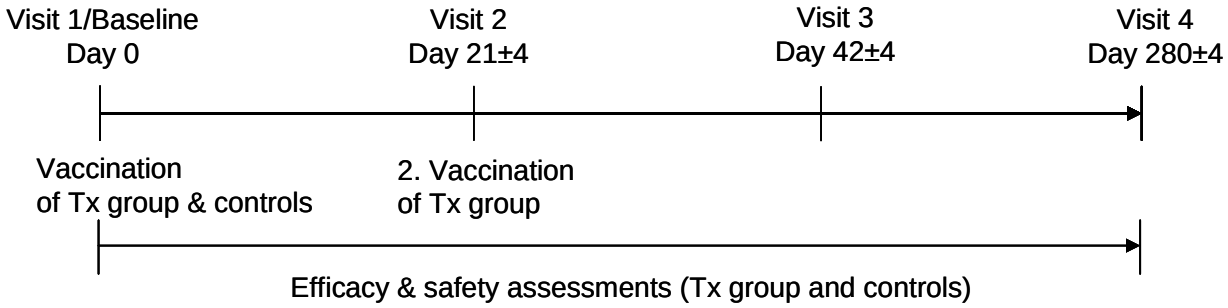

Figure 1: Study design of Tx group and controls

9.2 Discussion of Study Design, Including choice of Controls

This study was designed to evaluate immunogenicity of the novel swine-origin influenza A/H1N1 virus (H1N1sw) monovalent subunit vaccine. Furthermore, safety and tolerability were analyzed. The parameters that were to be tested reflect the efficacy and safety of the vaccination and are standard for such objectives. The study population tested was adult subjects who were immunocompromised due to prior solid organ or bone marrow transplantation. The vaccine was produced on an egg-derived platform. This study was to assess the schedule of administration in transplanted patients. A subset of age-matched healthy volunteers (including relatives and household members) were enrolled as a reference for the immune responses generated by the vaccine. They were vaccinated according to the SmPC.

Because of their increased risk for severe, life-threatening influenza viral infections and their known reduced responsiveness to plain (non-adjuvanted) influenza vaccines, transplant patients have a clear need for a more immunogenic vaccine. An adjuvanted H1N1sw vaccine was previously analyzed in healthy children and adults and has been shown to be more immunogenic in these groups than non-adjuvanted vaccine. Furthermore, the immune responses of the transplant recipients were to be compared with the immune responses of healthy adults. This study had particular urgency and relevance in the context of the current pandemic as well as for further epidemic outbreaks. Information was also required for future influenza seasons. The immune response in transplant recipients generated by the addition

of the adjuvans MF59 to the H1N1sw vaccine and by applying two vaccinations would help define the adjuvans concentration and vaccination schedule needed in possible future pandemic influenza outbreaks.

9.3 Selection of Study Population

The study population consisted of a transplant and a control group of male and female adults. The patients in the transplant group had undergone solid organ transplantation or allogeneic or autologous BMT. These patients were medically stable, but still immunocompromised due to immunsuppressive medication used to prevent transplant rejection. The date of transplantation was at least 3 months before enrollment. The control group included male and female healthy adults, preferably relatives or household members of the transplanted subjects. Healthy adults were defined as non-transplanted individuals who were eligible in the opinion of the investigators (see also exclusion criteria). All attempts were made to achieve equal age distribution among healthy and transplanted patients.

9.3.1 Inclusion Criteria

Transplant Recipients

- Adult subjects 18-60 years of age who have undergone prior renal, cardiac, liver, lung, or bone marrow transplantation for any reason, more than 3 months prior to enrolment
- Patients able to visit the outpatient clinic with a life expectancy of at least one year
- Patients who receive any immunosuppressive treatment currently taken to prevent organ rejection

Healthy Adults:

- Adult subjects 18-60 years of age
- Healthy individuals as determined by medical history, physical assessment and clinical judgment of the investigator
- Within the same age category (+/- 5 years) than the incidental transplanted patient

Transplant Recipients and Healthy Adults:

- Individuals who are able to comply with all study procedures and are available for all clinic visits scheduled in the study
- Women of child-bearing potential (WOCBP) must have used an acceptable contraceptive method for at least 2 months prior to study entry until 3 weeks after last vaccination:
    - Female of childbearing potential is defined as a post onset of menarche or pre-menopausal female capable of becoming pregnant. This does not include females who meet any of the following conditions: (1) menopause at least 2 years earlier, (2) tubal ligation at least 1 year earlier, or (3) total hysterectomy

- Acceptable birth control methods are defined as one or more of the following:
  - Hormonal contraceptive (such as oral, injection, transdermal patch, implant, cervical ring)
  - Barrier (condom with spermicide or diaphragm with spermicide) each and every time during intercourse
  - Intrauterine device (IUD)
  - Monogamous relationship with vasectomized partner. Partner must have been vasectomized for at least six months prior to the subject's study entry

9.3.2 Exclusion Criteria (applied to all subjects)

- Individuals who received any vaccine within 30 days prior to study entry
- Individuals who received a H1N1 vaccination less than 6 months prior to the study
- Influenza diagnosed by a physician within 4 months prior to the study start
- Pregnant or lactating females
- History of an anaphylactic (i.e. life-threatening) reaction to any of the components of the vaccines, including egg and chicken proteins, ovalbumin, kanamycin and neomycin sulphate, formaldehyde and cetyltrimethylammonium bromide (CTAB)
- Subjects who are not able to comprehend and to follow all required study procedures for the whole period of the study
- History of or any current illness that, in the opinion of the investigator, might interfere with the results of the study or pose additional risk to the subjects due to participation in the study
- Temperature is ≥ 38 °C or oral temperature ≥ 38.5 °C within 3 days of intended study vaccination
- Administration of parenteral immunoglobulin compound – including HBIg, blood products, and/or plasma derivatives within 6 months prior to Visit 1 or planned during the full length of the study
- HIV infection, as previously determined or reported
- History of progressive or severe neurological disorders (including Guillain-Barré syndrome and convulsions, but excluding febrile convulsions)
- Subjects participating in another clinical trial and / or receiving investigational drug

9.3.3 Removal of Patients from Therapy or Assessment

Patients had the right to withdraw from the study at any time and for any reason. The investigator also had the right to withdraw subjects from the study in the event of intercurrent illness, (S)AEs, protocol violations, cure, administrative reasons, or other reasons. A complete final evaluation at the time of the patient's withdrawal was made with

an explanation of why the patient withdrew from the study. The investigator attempted to complete all procedures usually required at the end of the study at the time a subject was discontinued from the clinical study. As far as possible, a complete final examination was performed on all subjects who did not complete the study according to the protocol.

9.4 Treatments

9.4.1 Treatments administered

The transplanted subjects received two doses of the study vaccine administered by intramuscular injection on day 0 and day 21 ± 4 days according to the current SmPC of Focetria®. The age-matched healthy volunteers received only one vaccination on day 0 of the study.

9.4.2 Identity of Investigational Product

The vaccine studied is a highly purified subunit influenza virus vaccine manufactured by Novartis Vaccines and Diagnostics S.r.l., Siena, Italy. The vaccine is called Focetria® (7.5_MF59) and was recently approved for use in the European Union by the CHMP (http://www.emea.europa.eu/humandocs/PDFs/EPAR/focetria/Focetria-PU-05-en.pdf).

Focetria® contains MF59, which increases the immune response compared to non-adjuvanted vaccines. MF59 has been tested in more than 60 clinical trials involving more than 33,000 people. More than 45 million doses of adjuvanted vaccines were distributed and MF59 has an established safety profile. Currently, it is used as an adjuvant in the seasonal flu vaccine Fluad® for patients 65 years and older. It is intended to improve immunogenicity and was evaluated for use based on the “mock-up” approach using a different influenza virus as antigen, and a schedule of two doses 21 days apart.

The vaccine is manufactured using antigen propagated in embryonated hens’ eggs. The vaccine was developed using the same platform technology that is used to produce the seasonal trivalent inactivated subunit vaccine Agrippal® (licensed in EU and other countries for subjects ≥ 6 months of age) and the pandemic, monovalent, MF59 adjuvanted vaccine Focetria® (licensed in EU for adults ≥ 18 years of age).

Short name	Manufacturing platform	Vaccine formulation		Volume for injection (mL)
		Antigen content (in µg)	MF59 content (in mg)	
7.5_MF59 (Focetria®)	Egg-derived	7.5	9.75	0.5

9.4.3 Method of Assigning Patients to Treatment Groups

This is not applicable, because there was only one treatment group (transplanted patients and healthy controls).

9.4.4 Selection of Doses in the Study

The dose was selected according to the SmPC of Focetria®. A second dose was given to the transplant patient group because they have been reported to be poorer responders to vaccinations (16).

9.4.5 Selection and Timing of Dose for each Patient

Doses of Focetria® were not especially selected or timed for each patient. Patients in each group received the same dose at the same visit.

9.4.6 Blinding

There was no blinding in this study.

9.4.7 Prior and Concomitant Treatment

The administration of parenteral immunoglobulin compounds, including HBIg, blood products, and/or plasma derivatives within 6 months prior to Visit 1 or planned during the full length of the study was not allowed.

9.4.8 Treatment Compliance

Since the vaccination was performed by the investigator no additional measures of compliance were necessary. Patients Diary Card recordings of body temperature, any vaccine-related local reactions and general symptoms (listed on the Diary Card), any changes in health (including any serious medical problems such as hospitalizations or life-threatening medical problems), and any prescription and non-prescription (over-the-counter) medication taken were checked at the visit following the distribution of the cards (Visit 2 and Visit 3 (transplanted group only)).

9.5 Efficacy and Safety Variables

9.5.1 Efficacy and Safety Measurements Assessed

Efficacy and safety assessments were planned according to the following schedule:

Table 1: Schedule of efficacy and safety assessments

	Visit 1 Baseline	Visit 2	Visit 3	Visit 4
Study day / weeks	0 / 0	21 / 3	42 / 6	280 / 40
Study visit window		± 4 days	± 4 days	± 15 days
ICF	x			
Exclusion/Inclusion	x			
Medical history	x			
Concomitant medications	x	x	x	x
Physical assessment	x	x		x
Vital signs	x	x		x
Urine pregnancy test[a]	x	x[e]		
Serology blood draw	x	x	x	x
Hematology[b]	x	x		x
Biochemistry[c]	x	x		x
Vaccination	x	x[e]		
Diary card dispensed	x	x[e]		
Diary card collected and reviewed		x	x[e]	
Assess local reactions[d]	x	x	x[e]	
Asses systemic reactions	x	x	x[e]	
Assess AEs and SAEs	x	x	x	x
Study termination				x

a. Urine pregnancy tests were performed for females of childbearing potential at Visit 1 (all females) and 2 (only transplanted females)
b. Differential blood count
c. AST, ALT, GGT, bilirubin, creatinine, creatinine kinase
d. Local reactions were assessed by the patients for 7 days including the day of vaccination
e. Only applicable to transplanted patients

Physical Assessment

During the baseline visit (Visit 1, screening), a physical assessment was performed by the investigators to assure that the subject was eligible for study participation. The medical history was evaluated. A complete physical assessment was to be conducted at Visit 1, Visit 2, and Visit 4. Concomitant medication was documented in the patient's record at every visit. Vital signs such as heart rate, blood pressure, and body temperature, as well as weight were measured and documented in the medical records.

Urine Pregnancy Test

WOCBP had to use an acceptable contraceptive method for at least 2 months prior to study entry until 3 weeks after the second vaccination. At Visit 1 (all females) and Visit 2 (only transplanted females) urine was collected for pregnancy testing (defined in Exclusion criteria 9.2). The tests were provided by Novartis Vaccines.

Blood Draw

At all visits 20mL of peripheral blood for serology was drawn. At visit 1, visit 2, and visit 4 additional blood was to be drawn: approximately 3mL for differential blood count (TBC) and 5.5mL for clinical chemistry.

Vaccination

At Visit 1, the vaccine was administered intramuscularly (i.m.) in the upper part of the arm that is used less or in the anterolateral thigh. Only transplanted patients received a second vaccination at Visit 2. After the vaccination, patients stayed in the clinic for at least 30 minutes so that the study staff could monitor any reactions. 30 minutes after the vaccination, the study staff checked the injection site and asked about any reactions since the vaccination. The temperature was also measured and recorded. In addition, the tenderness or pain of the vaccine injection site and its intensity was assessed.

Diary Card Dispense, Collection, and Review

Staff at the site provided two Diary Cards (only one for healthy individuals) and explained how to handle them. Completion of the Diary Cards throughout the study (starting on the evening of the first vaccination and each day for 6 days after each vaccination) was required to record body temperature, any vaccine-related local reactions and general symptoms listed in the Diary Card, any changes in health (including any serious medical problems such as hospitalizations or life-threatening medical problems), and any prescription and non-prescription (over-the-counter) medication. Study staff also provided a digital thermometer to measure the body temperature and a ruler to measure the size of the local reactions. The study staff answered any questions about the Diary Card.

The study staff scheduled the visits as outlined in Table 1 and reminded the subjects to complete the Diary Card for review and collection during the clinic visits.

Subjects were asked to call the study staff as soon as possible if any serious medical problem (e.g. hospitalization, any life-threatening medical problem, or any events that could lead to discontinuation of the study) occurred.

Serology

Blood samples were collected and stored at -20 °C until they were shipped to Novartis for testing. It was planned to do strain-specific hemagglutination inhibition (HI) and microneutralization (MN) assays.

Efficacy Assessments

The following efficacy assessments were planned:

Immunogenicity Endpoints

The measures for immunogenicity as determined by HI were planned as follows:

1. Geometric mean HI titer on Day 0, Day 21, Day 42, and Day 280 for the primary course;

2. GMR of HI: Day 21/Day 0, Day 42/Day 0, Day 42/Day 21, Day 280/Day 0, Day 280/Day 21, and Day 280/Day 42;
3. Percentage of subjects achieving seroconversion, defined as a significant increase of HI (HI ≥1:40 for subjects negative at baseline [<1:10]; or a minimum 4-fold increase in HI titers for subjects positive at baseline [HI≥1:10]) on Day 21, Day 42, and Day 280;
4. Percentage of subjects achieving seroprotection, defined as a HI titers ≥1:40 on Day 0, Day 21, Day 42, and day 280.

The measures of immunogenicity, as determined by MN, were planned as follows:

1. Geometric mean MN titer on Day 0, Day 21, Day 42, and Day 280 for the primary course;
2. GMR of MN: Day 21/Day 0, Day 42/Day 0, Day 42/Day 21, Day 280/Day 0, Day 280/Day 21, and Day 280/Day 42;
3. Percentage of subjects with a MN titers ≥1:40, 1:80, and 1:160 on Day 0, Day 21, Day 42, and Day 280;
4. Percentage of subjects achieving at least a 4-fold increase in MN titers on Day 21, Day 42 and Day 280.

Criteria for success as determined by HI

The immunogenicity criteria for success, as determined by HI, related to the following guidelines:

- EMEA/CPMP/BWP/214/96 (influenza vaccines guideline) and
- EMEA/CPMP/VEG/4717/2003-Rev.1 (pandemic guideline) and
- EMEA/CHMP/VWP/263499/2006 (pre-pandemic guideline) are:

For adult subjects aged 18-60 years:

- The percentage of subjects with seroconversion in HI antibody is > 40%
- The percentage of subjects achieving seroprotection > 70%
- The GMR is >2.5

For adult subjects aged over 60 years:

- The percentage of subjects with seroconversion in HI antibody is > 30%
- The percentage of subjects achieving seroprotection > 60%
- The GMR is >2.0

No predefined criteria for success are available for MN.

Safety Assessments

All safety analyses were planned to be performed in all vaccinated individuals, as well as separately in transplant patients and age-matched healthy volunteers.

The safety of the study vaccine was to be analyzed based on the number of subjects exposed to the vaccine with respect to (1) solicited events within the first 7 days after each injection and (2) non-solicited adverse events at least 21 days after each injection. Unsolicited events included SAEs, AEs, all other non-solicited AEs, which are routinely collected including onset of chronic diseases.

Underlying diseases/conditions which did not fulfill exclusion criteria at the study entry and any newly diagnosed diseases/conditions which did not lead to the subject's exclusion during the study course were recorded and followed-up throughout the study.

9.5.2 Appropriateness of Measurements

The assessments done in this study were appropriate because they are standard tests of efficacy and safety in vaccine studies. Some serum parameters tested are also standard for monitoring transplant patients.

9.5.3 Primary Efficacy Variables

Primary endpoint:

The observed percentage of seroconversion and seroprotection rates, as well as the observed GMR (measured by HI) in transplanted patients at day 42 were planned to be compared with the thresholds from the guideline for adults aged over 60 years (as outlined above). This study would have been successful, if all three point estimates had passed the corresponding efficacy criteria at day 42. For descriptive purposes two-sided 95%-confidence intervals for the rates and the GMR at day 42 were to be presented.

Main secondary endpoint:

Log10(GMT) values of transplanted and age-matched healthy volunteers were to be compared using a two-sample t-test and the corresponding two-sided 95% confidence interval. Non-inferiority of the adjuvanted vaccine would have been concluded, if the respective lower boundary of the two-sided 95% confidence interval for the ratio of GMTs in treatment groups had not exceeded 0.50.

Further secondary endpoints:

1. Percentage of subjects with seroconversion, percentage of subjects achieving seroprotection, and GMR at day 21 and day 280 were to be calculated and compared with the thresholds from the guideline. In addition, point estimates and corresponding descriptive two-sided 95% confidence intervals would have been presented.
2. Frequencies of the serological efficacy criteria seroprotection and seroconversion rates between transplanted patients and age-matched healthy volunteers were to be compared. Corresponding 95% explorative confidence intervals for rate differences would have been computed.

3. For age-matched healthy volunteers the success criteria according to the aforementioned guidelines was to be compared per vaccination group with the thresholds for healthy individuals.
4. Success criteria would have been compared in relation to immunosuppressive therapy in transplanted patients using either means and standard deviations for log10(GMT) values or frequencies for seroprotection and seroconversion rates. Corresponding explorative 95% confidence intervals for each of the serological variable would be considered.
5. Serological assessment of MN would follow in general the aforementioned analysis-strategies for HI.

9.5.4 Drug Concentration measurements

Pharmacokinetics were not done in this study.

9.6 Quality Assurance

The data quality was assured by source data on-site monitoring, double data entry, and SAE-reconciliation. In addition, a final quality control of analysis data sets was done.

9.7 Statistical Methods Planned in the Protocol and Determination of Sample Size

9.7.1 Statistical and Analytical Plan

Analysis of Demographic and Baseline Characteristics

Demographic characteristics of all individuals (e.g. age, gender) were to be evaluated separately for transplanted and age-matched healthy volunteers. In addition, for transplanted patients type of transplantation, immunosuppressive agent(s) and dose (planned), as well as time since transplantation were planned to be examined.

Quantitative variables were to be displayed with means and standard deviations. Assessments of qualitative data were to be presented using frequency tables.

Primary analysis

The primary analysis was to be done only in transplanted patients. The analysis was to be performed in all transplanted patients that had evaluable blood samples at day 0 and 42.

The observed percentage of seroconversion and seroprotection rates, as well as the observed GMR at day 42 was to be compared with the thresholds from the guideline for adults, aged over 60, given under Determination of Sample Size. This study would have been successful, if all three point estimates had passed the corresponding efficacy criteria at day 42.

For descriptive purposes two-sided 95%-confidence intervals for the rates and the GMR at day 42 were to be presented.

Key secondary analyses

For the main secondary endpoint, log_{10}(GMT) values of transplanted and age-matched healthy volunteers were planned to be compared with a two-sample t-test and the corresponding two-sided 95% confidence interval was to be computed. Non-inferiority of the adjuvanted vaccine would have been concluded, if the respective lower boundary of the two-sided 95% confidence interval for the ratio of GMTs in treatment groups did not exceed 0.5.

The following further secondary analyses were planned and would have been evaluated descriptively:

Percentage of transplanted patients with seroconversion, percentage of transplanted patients achieving seroprotection, and GMR at day 21 and day 280 was to be calculated and compared with the thresholds from the guideline for adults, aged over 60. In addition, point estimates and corresponding descriptive two-sided 95% confidence intervals were to be presented.

Frequencies of the serological efficacy criteria seroprotection and seroconversion rates between transplanted patients and age-matched healthy volunteers were to be compared. Corresponding 95% confidence intervals for rate differences were to be computed and assessed descriptively.

For age-matched healthy volunteers the success criteria according to the guidelines mentioned above was to be compared per vaccination group with the thresholds for healthy individuals (seroconversion rate: 40%, seroprotection rate: 70%, GMR: 2.5).

Success criteria were to be compared in relation to immunosuppressive therapy where two groups (calcineurin inhibitors, others) had been investigated in transplanted patients using either means and standard deviations for log_{10}(GMT) values or frequencies for seroprotection and seroconversion rates. Corresponding 95% confidence intervals for each of the serological variable were to be considered descriptively.

Serological assessment of MN followed in general the aforementioned analysis-strategies for HI.

Safety analyses

All safety analyses were planned to be performed in all vaccinated individuals. In addition, safety analyses were to be performed separately in transplant patients and age-matched healthy volunteers. Thorough evaluation of all patterns of solicited events, non-solicited events, and SAEs were to be done. Particularly, occurrence and frequency of events was to be presented using frequencies and corresponding 95% confidence intervals.

9.7.2 Determination of Sample Size

Sample size calculation was feasibility driven: transplantation medicine is orphan; in a reasonable time frame a maximum of 60 transplant patients were expected to be included

in this study at the Hannover Medical School. Due to close medical attendance, dropouts, and discontinuations were not expected.

Until now it is not clear, whether serological efficacy criteria are reduced in immunosuppressed patients. We assumed that immunosuppressed patients would show slightly reduced serological assessments as compared to the elderly and that acceptance criteria for the elderly would also be valid for transplant patients.

According to the influenza vaccine guideline (EMEA/CPMP/BWP/214/96) and the pandemic guideline (EMEA/CPMP/VEG/263499/2006), the following three criteria had to be met for the respective point estimates in adult subjects aged over 60 years:

- Observed percentage of subjects with seroconversion should be > 30%, and
- Observed geometric mean ratio should be > 2, and
- Observed percentage of subjects achieving seroprotection should be > 60 %.

According to the European guideline documents only the point estimates of the three efficacy variables have to meet the criteria. Thus, this study would have been successful if the three point estimates of the efficacy variables exceeded the criteria of the guideline.

To provide reassurance that each of the three CHMP-criteria would be obtained with a single power of 80%, the overall power was set at 93%.

Sample size was mainly influenced by seroprotection and seroconversion rates. We assumed that the rates of the transplanted patients would be a maximum of 3% smaller than the rates of the elderly.

The three efficacy criteria that were observed according to SmPC of Focetria®, for adults (18-60 years) and elderly subjects (>60 years), as well as our assumptions for transplanted patients (reduction compared to the elderly by 3%) are shown in Table 2.

Table 2: Criteria Assumed for Transplanted Patients Compared to Elderly and Healthy Individuals

	132 Adults (18-60 years)	**122 Elderly (> 60 years)**	**Own assumptions transplanted patients**
Seroprotection rate (Day 22)	96%	72%	69%
GMR (Day 22 to Day 1)	18	4	3.5
Seroconversion rate (Day 22)	88%	43%	40%
Sample size (n)	-	-	58

Based on a normal distribution, a total of 58 patients would have been necessary to have a 93% chance to observe a point estimate for the seroprotection rate larger than 0.6 if a seroprotection rate of 0.69 was assumed. To have a 93% chance to observe a point estimate for the GMR larger than 2 under the assumption of a comparable standard deviation of 4.06 (calculated via back-transformed $\log_{10}$-transformed confidence interval) and an assumed true GMR of 3.5, a sample size of 17 would have been sufficient. 53 patients would have been enough to have a 93% chance to observe a point estimate for the seroconversion rate larger than 0.3 if the true seroconversion rate was 0.4.

Thus, to demonstrate that all point estimates met the respective criteria a sample size of 58 patients would have been sufficient. A total of 60 patients were to be recruited. It is noted, however, that the procedure that was based on observed estimates did not control the type-1-error.

9.8 Changes in the Conduct of the Study

The study's recruitment period was originally planned for 4 months. However, because the expected number of patients could not be recruited in the trial by this time, the recruitment period was extended in a non-substantial amendment submitted July 2010. The study was terminated early, because the flu season was over and no more vaccine was available. The last person was recruited in August 2010.

10 STUDY PATIENTS

10.1 Disposition of Patients

Overall, 13 patients were screened and included in the study between November 2009 and August 2010. Three patients withdrew during treatment or were lost to follow-up (2 Tx patients and 1 healthy volunteer, Table 3).

Table 3: Study Completion and Withdrawal (n)

	Transplanted	Not transplanted	Total
Completed according to protocol	6	4	10
Early withdrawal or lost to follow up	2	1	3
Total	8	5	13

10.2 Deviations from the Protocol

There were no deviations from the protocol.

11 EFFICACY EVALUATION

11.1 Data Sets Analyzed

The plan was to enroll a total of 120 subjects age 18-60 years, 60 Tx patients and 60 age-matched healthy adults. However, only 13 subjects were actually recruited and analyzed (8 Tx patients and 5 age-matched healthy volunteers).

11.2 Demographics and Other Baseline Characteristics

Baseline characteristics are shown in Table 4 below.

Table 4: Baseline characteristics

	Tx group (n=8)	Control group (n=5)
Age, years, median (range)	52 (19–70)	47 (20–58)
Sex, % male	38	60
Male/female, n	3/5	3/2
Type of transplantation, n:		
Liver	6	
Kidney	1	
Liver & kidney	1	
Bone marrow	0	
Immunosuppressants, n:		
Tacrolimus	1	
Tacrolimus & Corticosteroid	2	
Tacrolimus & Corticosteroid & Azathioprin	1	
Ciclosporin & Corticosteroid	1	
Ciclosporin & Corticosteroid & Mycophenolate-Mofetil	2	
Mycophenolate-Mofetil	1	
Time since transplantation, years, median (range)	4.65 (1.28-18.57)	

11.3 Measurement of Compliance

Compliance to study drug vaccination was not measured, because the investigator injected the vaccine. Patients recorded temperature, any vaccine-related local reactions, general symptoms listed, any change in health, and any prescription or non-prescription medication on their Diary Cards. Overall, the compliance of the patients enrolled in the study was good. 77% (10/13 patients) finished the study according to protocol.

11.4 Efficacy Results and Conclusions

Only 13 subjects were recruited and treated. Thus, efficacy parameters were not assessed and conclusions on the efficacy of the vaccination cannot be made.

12 SAFETY EVALUATION

Safety analyses were performed in all vaccinated individuals, as well as separately in transplant patients and age-matched healthy volunteers. Thorough evaluation of SAE patterns were performed. Occurrence and frequency of events were presented.

12.1 Exposure

A total of 13 participants (n= 8 patients after solid organ transplantation or BMT and n= 5 healthy volunteers) were exposed to the IMP Focetria® as per protocol. Each patient received a dose of 7.5 µg antigen and 9.75 mg MF59 at Visit 1 day 0. Transplanted patients were given a second dose 21 ± 4 days after the first.

12.2 Adverse Events

A total of seven non-serious adverse events (AEs) occurred in this trial. Three AEs were assessed by the investigator as not related to IMP Focetria®. The remaining four AEs were assessed as likely (n=2) or possibly related (n=2) to the IMP. As shown in Table 5, these related AEs were: influenza-like illness (n=1, patient after liver transplant), hematoma (n=1, healthy volunteer), flushing (n=1, healthy volunteer), and pyrexia (n=1, healthy volunteer). Both patients and volunteers recovered from all AEs.

Table 5: Summary of Adverse Events

System Organ Class: AE (Patient No.)	Group	Mild		Moderate		Severe		Total
		Related	Not related	Related	Not related	Related	Not related	
Injury, poisoning & procedural complications:								
Fatigue fracture (1101)	Transplanted	0	1 (8%)	0	0	0	0	1(8%)
Infections & infestations:								
Nasopharyngitis (1101)	Transplanted	0	1 (8%)	0	0	0	0	1(8%)
General disorders & administration site conditions:								
Flu-like illness (1353)	Transplanted	1 (8%)	0	0	0	0	0	1(8%)
Pyrexia (1354)	Not transplanted	1 (8%)	0	0	0	0	0	1(8%)
Vascular disorders:								
Flushing (1354)	Not transplanted	1 (8%)	0	0	0	0	0	1(8%)
Hematoma (1354)	Not transplanted	1 (8%)	0	0	0	0	0	1(8%)
Nervous system disorders:								
Migraine (1354)	Not transplanted	0	1 (8%)	0	0	0	0	1(8%)

12.3 Deaths, other SAE, and other significant AE

A total of 11 cases of serious adverse events (SAEs) were reported to the Sponsor/pharmacovigilance. All SAEs occurred in the group of patients who received the IMP after solid organ transplantation, none in the control group of healthy volunteers. One patient experienced three cases, three patients had two, and two patients had a single case of SAEs (Table 6). With the exception of one event which was still ongoing (b-cell lymphoma) and will most likely stay chronic, all other cases of SAEs were reported as recovered. No fatalities occurred.

All SAEs were rated as not related to the IMP Focetria® by both the reporting investigator and the sponsor´s delegate for pharmacovigilance. Instead, SAEs were designated as being related to the underlying disease (solid organ transplant) and/or concomitant medication (immunosuppressants).

Table 6: Summary of SAE according to SOC

System organ class (SOC)	N	Case Numbers[a]
Preferred term (PT)		
Gastrointestinal disorders	**3**	
Diarrhoea	1	DE-HCTC-000014
Flatulence	2	DE-HCTC-000100, DE-HCTC-000101
General disorders and administration site conditions	**3**	
Pain	1	DE-HCTC-000101
Pyrexia	2	DE-HCTC-000100, DE-HCTC-000144
Hepatobiliary disorders	**7**	
Bile duct stone	2	DE-HCTC-000084, DE-HCTC-000085
Cholangitis	5	DE-HCTC-000079, DE-HCTC-000080,DE-HCTC-000082 DE-HCTC-000100,DE-HCTC-000101
Infections and infestations	**3**	
Cytomegalovirus infection	1	DE-HCTC-000083
Infection	1	DE-HCTC-000144
Pneumonia	1	DE-HCTC-000014
Injury, poisoning and procedural complications	**1**	
Incisional hernia	1	DE-HCTC-000081
Investigations	**2**	
Transaminases increased	2	DE-HCTC-000079, DE-HCTC-000080
Metabolism and nutrition disorders	**1**	
Dehydration	1	DE-HCTC-000014
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	**1**	
B-cell lymphoma	1	DE-HCTC-000144
Renal and urinary disorders	**1**	
Renal failure acute	1	DE-HCTC-000014
Skin and subcutaneous tissue disorders	**1**	
Night sweats	1	DE-HCTC-000100
Total	**23**	11
[a]Case numbers refers to the number of SAE reports to pharmacovigilance		

12.4 Clinical Laboratory Evaluation

Laboratory values were not evaluated for this study.

12.5 Vital Signs

The vital signs for each patient at baseline are shown in Table 7 below.

Table 7: Patients' Vital Signs

Patient	Visit	Age (years)	Sex	Weight (kg)	Height (cm)	Heart rate (bpm)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	Body temperature (°C)
01101	Visit 1/ Baseline	38	Male	103	180	64	118	70	35.6
01351	Visit 1/ Baseline	60	Male	74	183	.	130	65	35.6
01352	Visit 1/ Baseline	58	Male	89	180	63	120	70	.
01353	Visit 1/ Baseline	57	Female	71	164	72	135	80	.
01354	Visit 1/ Baseline	22	Female	73	177	72	120	70	31.1
01356	Visit 1/ Baseline	48	Female	59	167	70	120	80	36.2
01357	Visit 1/ Baseline	47	Male	80	180	70	110	70	36.4
01358	Visit 1/ Baseline	70	Female	81	172	76	120	60	35.7
01359	Visit 1/ Baseline	44	Female	80	162	70	130	80	36.3
01360	Visit 1 / Baseline	19	Female	50	154	70	120	70	36.2
01361	Visit 1 / Baseline	20	Male	70	185	65	130	80	.
01362	Visit 1 / Baseline	52	Male	92	177	72	120	60	36.2
01363	Visit 1 / Baseline	53	Female	52	165	66	130	80	36.3

12.6 Safety Conclusions

Based on the AE/SAE assessment, there have been no new findings related to the safety of the IMP in this trial. At the same time, no findings that change the safety profile of the IMP as described in the investigator's brochure have arisen during this trial.

13 DISCUSSION AND OVERALL CONCLUSIONS

This clinical trial was initially planned with a 4 month recruitment period and a 9 month treatment period per patient. Therefore, the overall trial duration (FSFV to LSLV) was initially 13 months. At the end of the planned recruitment period the expected number of subjects had not been included in the trial. As a consequence the recruitment period was extended with a non-substantial amendment submitted in July 2010. The date of the end of the trial was prolonged until the end of February 2012.

At the end of August 2010 the initial batch of study medication Focetria® expired. Due to the end of the flu season 2009/2010 a new batch of study medication was not available. Therefore, no further subjects were included in the trial by the end of August 2010. The enrolled subjects were followed-up according to protocol. The last visit of the last enrolled subject was on April 21, 2011.

Initially, 120 subjects were to be enrolled in the trial. Until the early termination of the trial only 13 subjects (8 transplanted patients and 5 healthy volunteers) were recruited. All 13 subjects received the study medication at their first study visit at day 0 and the transplanted patients received a second injection at day 21.

Safety evaluations were done, but efficacy parameters were not tested. Therefore, from the available data obtained for the few subjects from this prematurely terminated study, the evaluation of immunogenicity, safety, and tolerability of Focetria® was not possible.

14 REFERENCE LIST

1. http://www.who.int/csr/disease/swineflu/en/index.html
2. Vaillant L, La Ruche G, Tarantola A et al. Epidemiology of fatal cases associated with pandemic H1N1 influenza 2009. Eurosurveillance 2009p; 14: 1-6.
3. Jain S, Kamimoto L, Bramley AM et al. Hospitalized patients with 2009 H1N1 influenza in the United States, April-June 2009. New Eng J Med 2009; 361: online
4. ANZIC influenza investigators. Critical care services and 2009 H1N1 influenza in Australia and New Zealand. New Eng J Med 2009; 361: online.
5. ANZECMO influenza investigators. Extracorporeal membrane oxygenation for 2009 influenza A (H1N1) acute respiratory distress syndrome. JAMA 2009 303:1888-1895.
6. Kumar A, Zarychanski R, Pinto R et al. Critically ill patients with 2009 influenza (H1N1) infection in Canada. JAMA 2009; online Oct 12, 2009.
7. Dominguez-Cherit G, Lapinsky SE, Macias AE et al. Critically ill patients with 2009 influenza (H1N1) infection in Mexico. JAMA 2009; online Oct 12, 2009.
8. CDC. Oseltamivir-resistant novel influenza A (H1N1) virus infection in two immunosuppressed patients – Seattle, Washington. MMWR 2009; 58: 893-896.
9. Siston AM; Rasmussen SA, Honein MA et al. Pandemic 2009 Influenza A(H1N1) Virus Illness Among Pregnant Women in the United States. JAMA 2010; 303: 1517-1521.
10. Shieh WJ, Blau DM, Amy M. Denison AM et al. 2009 Pandemic Influenza A (H1N1) Pathology and Pathogenesis of 100 Fatal Cases in the United States. Am J Pathol 2010; 177: 166-175.
11. Jurawan R, de Almeida M, Smith A, Weilert F. Swine H1N1 influenza in a post liver transplant patient. N Zealand Med J 2009; 122: 107-111.
12. Ajlan AM, Quiney B, Nicolaou S, Muller NL. Swine-origin influenza A (H1N1) viral infection: radiographic and CT findings. Am J Roentgen 2009; 193: 1-6.
13. Danziger LA, Husain S, Mooney ML, Hannan MM. ISHLT Infectious Diseases Council. The novel 2009 H1N1 Influenza virus pandemic: unique considerations for programs in cardiothoracic transplantation. J Heart Lung Transplat. 2009; doi:10.1016/jhealun.2009.10.001 (on line)
14. Kumar D, Michaels MG, Morris MI et al. Outcomes from pandemic influenza A H1N1 infection in recipients of solid-organ transplants : a multicentre cohort study. Lancet Infect Dis 2010; 10: 521-526.
15. Fraund S, Wagner D, Pethig K, Dreshcer J, Girgsdies OE, Haverich A. Influenza vaccination in heart transplant recipients. J Heart Lung Transplant. 1999; 18: 220-5.

16. Dengler TJ, Strnad N, Buhring I et al Differential immune response to influenza and pneumococcal vaccination in immunosuppressed patients after heart transplantation. Transplantation 1998; 66: 1340-7.
17. Beran J, Ambrozaitis A, Laiskonis A, Mickuviene N, Bacart P, Calozet Y, Demanet E, Heijmans S, Van Belle P, Weber F, Salamand C: Intradermal influenza vaccination of healthy adults using a new microinjection system: a 3-year randomised controlled safety and immunogenicity trial. BMC Med. 2009, 7:13.

15 APPENDICES