CONFIDENTIAL

CLINICAL STUDY PROTOCOL

A Phase I/II study of RV001V, a RhoC anticancer vaccine, against metastasis from solid tumours

Study code:	RhoVac-001	Study development phase:	Phase I/II
EudraCT number:	2016-004189-24	Investigational medicinal product:	RV001V
		Indication:	Cancer, metastatic solid tumours
Version:	Final, version 3	Date:	07 November 2017

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1 SYNOPSIS

Name of the Sponsor/Company: RhoVac Aps	**Study Code:** RhoVac-001
Name of Investigational Medicinal Product: RV001 Vaccine 0.1 mg/mL (RV001V)	**EudraCT No.:** 2016-004189-24
Development Phase of the Study: Phase I/II	**Trial under an IND:** No

TITLE OF THE STUDY:

A Phase I/II study of RV001V, a RhoC anticancer vaccine, against metastasis from solid tumours

OBJECTIVES:

Primary Objective:

The primary objective is to evaluate the safety and tolerability of RV001 Vaccine 0.1 mg/mL (RV001V) following subcutaneous (s.c.) administrations to patients who have been prostatectomised due to prostate cancer.

Secondary objectives:

The secondary objective is to investigate RV001V-specific T-cells in treated patients, and thereby the immunological response before, during and after vaccination.

Exploratory endpoints

The exploratory objectives are to investigate the Prostate-Specific Antigen (PSA) levels both in the subgroup of patients with measurable levels at baseline and in patients with no measurable levels at baseline, to explore the potential associations between PSA levels and immunological response and to preliminarily evaluate the progression-free survival (PFS) and overall survival (OS).

OVERALL STUDY DESIGN:

The trial is an open phase I/II first-in-human clinical study for a peptide cancer vaccine.

Between 10 and 25 patients prostatectomised due to prostate cancer will be enrolled.

The treatment will consist of a total of 11 s.c. vaccinations with RV001V. The first 6 vaccinations will be given every 2 weeks, whereas the remaining 5 vaccinations will be administered with 4 weeks between each vaccination. Long term follow-up of immunological response and PSA levels will be performed at 3, 6, 9, and 12 months after the last vaccination.

The first three patients will be enrolled sequentially. **Patient no 1** will have 2 vaccinations before any additional patient can be treated. Provided that no safety concern is observed after safety evaluation of the two first vaccinations, **Patient no 2** may start vaccination and a new safety readout will be performed when Patient no 2 has received 2 vaccinations. Provided no safety concern is observed from the two first patients, **Patient no 3** may start vaccination. New safety readouts will be performed when all 3 patients have received at least 2 vaccinations. Provided that no safety concern is observed, three additional patients may start vaccinations with their first vaccination taking place on separate days. Following a new safety readout, and provided no safety concern is observed, all remaining patients may start vaccinations with their first vaccination taking place on separate days. All vaccinations will be performed at a Phase I Unit.

The trial will be terminated if treatment related grade 3, 4 or 5 toxicity in accordance with Common Terminology Criteria for Adverse Events (CTCAE) is observed in more than one of the first 6 patients, with the exception of fever.

In case the patient develops disease progression, requiring surgical and/or chemotherapeutic treatment, the patient will be discontinued from the study and referred back to his urology/oncology team, from which he was referred initially, where treatment will be offered independent of participation in the study.

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Development Phase of the Study: Phase I/II	**Trial under an IND:** No

INVESTIGATIONAL MEDICINAL PRODUCT:

RV001 Vaccine 0.1 mg/mL (RV001V). RV001V consists of the peptide RV001 and the adjuvant Montanide ISA 51.

NUMBER OF PATIENTS:

At least 10 patients will be enrolled in the trial to fulfil the primary objective. Additional patients, up to a total of 25 patients, may be enrolled dependent on analysis of immune related data.

NUMBER OF STUDY CENTRES:

There will be one study centre involving two clinics; a phase I unit (Zelo Phase I Unit), approved for first-in-man trials, will be responsible for the conduct of the treatment located at the premise of Bispebjerg hospital. The principal investigator will be based at this clinic. The second clinic is located at a nearby hospital (Rigshospitalet). The investigator(s) based at Rigshospitalet will be responsible for recruitment and consenting patients to the trial. This clinic at Rigshospitalet will follow the patients throughout the study and, according to their normal practise, evaluate PSA values sampled at the Phase I Unit and provide assessment of the patients' cancer status.

MAIN INCLUSION AND EXCLUSION CRITERIA:

Inclusion criteria

The patients have to meet all of the following criteria to be eligible to enter the study:

1) Patients prostatectomised (PT) due to histologically verified adenocarcinoma of the prostate gland who currently are not being treated, or expected within the next 8 months to be treated, with any anti-cancer treatment. Patients may or may not have measurable PSA.
2) Able to understand the study procedures and willing to provide informed consent.
3) Able and willing to comply with study requirements and complete all visits.
4) Using adequate contraceptive measures.

   All non-vasectomized patients must use condoms during the study and for one month after the last vaccination with RV001V, or have a female partner who either has been post-menopausal for more than one year or is using a highly effective method of contraception (i.e., a method with less than 1% failure rate).
5) Aged 18 years and above.
6) ECOG performance status 0 or 1.
7) Recovered/stabilized at grade ≤2 from all toxicities related to prior treatment(s) in accordance with CTCAE.
8) Laboratory values obtained ≤30 days prior to first vaccination, and more than 3 weeks after potential chemotherapy.
   a) Haemoglobin ≥5.6 mmol/L.
   b) Absolute granulocyte count ≥1.5 x 10^9 /L.
   c) Platelets ≥100 x 10^9 /L.
   d) Total bilirubin ≤1.5 x upper limit of normal (ULN).

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e) Creatinine ≤1.5 x ULN.

f) Alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and alkaline phosphatase (ALP) ≤2.5 x ULN.

Exclusion criteria

Patients meeting any of the following criteria will not be permitted to enter the study:

1) Patient is a candidate for relevant therapies that are the current standard of care for their cancer disease.
2) Patient has been treated with Androgen Deprivation Therapy (ADT), or expected to receive such treatment within the next 8 months from enrolment.
3) Concurrent chemotherapy or radiotherapy within 12 weeks of 1st vaccination, or expected to receive such treatment within the next 8 months from enrolment.
4) Patients have undergone major surgery or have had major bleeding within the last month prior to the first vaccination.
5) Patients with brain or leptomeningeal metastases.
6) Prior treatment with any therapeutic cancer vaccine(s).
7) History of second malignancy (except for adequately managed basal cell carcinoma and squamous cell carcinoma of the skin).
8) Patients in need of or treated the last 30 days before the first vaccination with systemic steroids or other immune suppressive therapy. Use of inhaled steroids, nasal sprays, and topical creams for small body areas is allowed.
9) History of alcohol or substance abuse within the last 5 years
10) History of acquired immune deficiency syndrome or positive serological test for human immunodeficiency virus infection.
11) History of viral hepatitis B as determined by positive antibody immunoglobulin M (IgM) to core antigen for hepatitis B or positive for hepatitis B surface antigen, or viral hepatitis C as determined by positive antibody for hepatitis C.
12) Participation in any investigational trial or use of any investigational drug(s) within 30 days prior to inclusion in this trial.
13) Any known serious infections, e.g. tuberculosis.
14) History of significant autoimmune disease such as: Inflammatory bowel disease, Systemic lupus erythematosus, Ankylosing spondylitis, Scleroderma, Multiple sclerosis.
15) Severe medical conditions, such as but not limited to severe asthma/chronic obstructive pulmonary disease (COPD), New York Heart Association (NYHA) grading 3 or above, poorly regulated insulin dependent diabetes, any significant organ damage as judged by the Investigator.
16) Other medications or conditions that in the Investigator's opinion would contraindicate study participation for safety reasons or interfere with the interpretation of study results
17) History of drug allergies or known allergy/hypersensitivity to Montanide ISA 51, or intolerance to subcutaneous injection.

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Development Phase of the Study: Phase I/II	**Trial under an IND:** No

EFFICACY AND SAFETY VARIABLES:

Primary endpoint

The primary endpoint of the study is the proportion of patients developing treatment related grade 3, 4 or 5 toxicity in accordance with CTCAE, with the exception of fever. The study will be terminated if this toxicity is observed in more than 1 of the first 6 patients, i.e. in ≥33.3% of the patients. The study will be considered meeting the primary endpoint if the proportion of patients with this toxicity at the end of the study is not higher than 33.3%.

In addition, the tolerability will be evaluated by number of patients who discontinue the study treatment prematurely. The safety will be evaluated with frequency and severity of adverse events (AEs). The numbers and proportions of patients with any treatment-emergent adverse event, and any serious treatment-emergent adverse event will be summarised. Furthermore, changes in vital signs, laboratory variables and electrocardiogram (ECG) will be evaluated.

Secondary endpoints

For the evaluation of immunological response of the vaccine, the immunogenicity of RV001V in patients is determined by measuring anti-vaccine T cells before, during and after vaccination, and by evaluating the changes from baseline (last assessment before the first vaccination) to each vaccination and follow-up visit.

Exploratory endpoints

The PSA levels will be evaluated by:

- Changes from baseline in the PSA levels among the patients with measurable levels at baseline (the last PSA assessment before the first vaccination)
- Number of patients with measurable PSA levels at baseline and at each vaccination and follow-up visit.

The potential association between the PSA levels and immunological response will be evaluated by:

- Anti-vaccine T cell levels classified by the PSA status (measurable or non-measurable) at baseline and at each vaccination and follow-up visit
- Correlation of changes from baseline in anti-vaccine T cell levels and PSA levels among the patients with measurable PSA levels at baseline.

The PFS and OS will be evaluated as time from baseline (time of the first vaccination) to the event. For PFS, disease progression or death for any reason will be defined as an event. For OS, death for any reason is defined as an event. The data from the patients without an event will be censored at the last time when the patient is known to be free of disease progression or alive.

STATISTICAL METHODS:

All patients who received at least one vaccination will be included in the statistical analyses. A subset analysis will be done among the patients who received 3, 6 vaccinations and all 11 vaccinations.

The data will primarily be analysed with descriptive statistics. For the analysis of changes from baseline, the last assessment before the first vaccination will be used as the baseline. For the continuous endpoints, the changes from baseline may be estimated using appropriate statistical methods, e.g. Mixed Model for Repeated Measures (MMRM) in addition to the descriptive statistics. The time-to-event endpoints will be summarized using the Kaplan-Meier method.

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STUDY PERIOD: The duration of the study is expected to be approximately 2 years, beginning with first patient first visit which is anticipated to occur in Q1 2017, and ending with last patient last follow up visit in Q1 2019. All patients are expected to be enrolled and have completed vaccine treatment during the first year. The second year will consist of follow-up visits. The actual overall study duration will depend on the time required to screen and enrol patients, the number of patients needed, and whether patients complete all treatment and follow up visits.	

2 TABLE OF CONTENTS

Section **Page**

LIST OF TABLES

LIST OF FIGURES

3 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

3.1 List of Abbreviations

AE	Adverse Event
ADL	Activities of Daily Living
ADT	Androgen Deprivation Therapy
ALAT	Alanine Aminotransferase
ALP	Alkaline Phosphatase
AR	Androgen Receptor
ASAT	Aspartate Aminotransferase
CA	Competent Authority
COPD	Chronic Obstructive Pulmonary Disease
CRF	Case Report Form
CRP	C-Reactive Protein
CTC	Common Toxicity Criteria
CTCAE	Common Terminology Criteria for Adverse Events
CTL	Cytotoxic T-lymphocyte
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic CRF
ELISA	Enzyme-Linked Immunosorbent Assay
ELIspot	Enzyme-Linked Immunospot Assay
FU	Follow-up
GCP	Good Clinical Practice
GTPase	Guanosine Triphosphate Hydrolase
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
ICH	International Council for Harmonisation
ICS	Intracellular Staining
IEC	Independent Ethics Committee
IFA	Incomplete Freund’s Adjuvant
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-2	Interleukin-2
IMP	Investigational Medicinal Product

LDH	Lactate Dehydrogenase
MHC	Major histocompatibility complex
MMRM	Mixed Model for Repeated Measures
NSCLC	Non-Small Cell Lung Cancer
NYHA	New York Heart Association
OS	Overall Survival
PBMC	Peripheral Blood Mononuclear Cell
PFS	Progression Free Survival
PSA	Prostate-Specific Antigen
RhoC	Ras Homolog Gene Family Member C
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
s.c.	Subcutaneous
SOC	System Organ Class
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TAA	Tumour Associated Antigen
TEAE	Treatment-Emergent Adverse Event
ULN	Upper Limit of Normal

3.2 Definition of Terms

Competent Authority	A government body or government appointed body that has legal authority to approve or disapprove clinical studies

4 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

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5 INTRODUCTION

5.1 Background

5.1.1 Cancer Vaccination Therapy

The anticancer vaccine principle is based on the ability of the immune system to distinguish normal from malignant cells. This is due to cancer cells expressing so called tumour-associated antigens (TAAs). These TAAs can be proteins or glycoproteins. They are expressed in connection with the malignant transformation and often caused by mutations in the genes of the cell leading to changes in the protein synthesis. The expression can be of qualitatively changed cell proteins, simple overexpression of normal proteins or *de novo* expression of proteins, which are normally only presented in small amount or not at all in the tissue. The antigen is broken down intracellularly and presented on the surface of the cell. This occurs through binding of peptide fragments to tissue type molecules (Human Leukocyte Antigen [HLA]) [1,2].

Tumour-specific T lymphocytes have the ability to recognize processed tumour antigens, that is peptide bound to HLA on the surface of tumour cells, through their T-cell receptor. This interaction initiates a subsequent killing of the tumour cells.

Therapeutic cancer vaccines work by a specific stimulation of the patient's immune system with cancer antigens. Immunization with a specific tumour antigen can induce an immune response, which kills the tumour cells and the expression of the tumour antigen in question. The T-lymphocytes are the cells mainly responsible for this process. Several studies ranging from experimental murine systems to human clinical trials have shown that cytotoxic T-lymphocytes (CTLs) with specificity for HLA-bound tumour peptide antigens can inhibit tumour growth. This is the reason why active immune therapy has been focusing on the activation and stimulation of tumour-specific CTL responses.

Clinical use of peptide based vaccines has been limited due to the instability and rapid degradation of the peptides in their naked form. This barrier may be overcome by the addition of adjuvants to the peptide. Many peptide vaccine studies have been completed without significant toxicity, which makes peptide vaccines easy to use in a clinical context. The use of long peptides has led to the possibility of presentation on both major histocompatibility complex (MHC) class I and II and increased efficiency compared to short peptides [3].

5.1.2 RhoC as a Vaccine Target

Ras Homolog Gene Family Member C (RhoC) belongs to a family of proteins called Rho GTPases [4] that play a crucial role in cell migration, invasion and metastasis. The three Rho proteins, A, B, and C are very homogeneous, with the exception that RhoC expresses a unique C-terminal part. It is the unique C-terminal of RhoC that is the vaccination target, thus only targeting RhoC.

RhoC seem to play a key role in cancer cell mobility, i.e., a key characteristic of metastatic cancer cells. Gene knock out of the RhoC gene has been shown to render melanoma cells incapable of metastases [5]. Thus, expression of RhoC appears to be an essential feature of metastatic disease.

A RhoC biosensor has demonstrated that RhoC is active in invadopodia, which degrade extracellular matrix, in cancer cells. RhoC is not essential for mouse development, and RhoC-null mice appear phenotypically normal [6]. RhoC has been implicated in a variety of body functions during cancer invasion [7].

Importantly, RhoC has been found to be a target for CD8 T-cells in cancer patients [8]. Moreover, RhoC-specific CD8 T cells harvested from patients are capable of killing cancer cells expressing the appropriate HLA molecule [8]. Thus, natural immune responses against RhoC are elicited in cancer patients, and RhoC-specific cytotoxic T-cells are detectable in the blood without any reported autoimmune manifestation.

RhoC is upregulated in many types of human cancer and it contributes to cancer progression and metastasis formation in mouse models [5]. Thus, several lines of evidence demonstrate a high expression of RhoC in cancer cells, and that the metastatic potential of cancer cells depends on expression of RhoC [8,9,10]. RhoC has in particular been demonstrated in metastatic breast cancer [10], non-small cell lung cancer (NSCLC) [11], melanoma [12], colorectal cancer, prostate cancer [13] and carcinoid tumours [6].

The gene encoding RhoC has not been shown to mutate in cancers, indicating that upregulated expression and thus increased protein amounts in the cell, is sufficient to contribute to metastasis, making it a suitable target for anti-cancer vaccines to eliminate RhoC-expressing metastatic tumour cells. There is also evidence that it stimulates cancer proliferation [14,15] and resistance to chemotherapy in cultured cancer cells [16,17].

5.1.3 RV001

The RV001 vaccine (RV001V) targets cancer cells by eliciting an immune response against a RhoC-derived peptide fragment containing the C-terminal [8]. It has been demonstrated that RV001 can activate cytotoxic T-cells to kill RhoC-positive tumour cells *in vitro* [8].

The RV001V will therefore have the potential for treatment of a variety of RhoC-expressing metastatic tumours.

Preclinical toxicity studies required for use of peptide vaccines in humans have been performed at doses up to 225 mcg/kg, which when recalculated to human equivalent dose, is about 400 times the dose that will be administered to patients under this protocol. At none of the doses were there findings of treatment-related systemic toxicity. Local reactions at injection sites were noticed and so were changes compatible with immunization. These changes were similar in the vaccine and the adjuvant-only group of animals, indicating that these changes are caused by the adjuvant.

5.1.4 Adjuvant

Montanide ISA 51 (Seppic Inc. Paris, France) is a modified so-called incomplete Freund's adjuvant (IFA). Montanide ISA 51 is defined as a mixture of a highly purified mineral oil (Drakeol 6VR) and a surfactant (Mannide monooleate) [18]. When mixed with an aqueous phase in a 50/50 ratio, it renders a water in oil emulsion. This water in oil emulsion is used as vaccine adjuvant, in order to enhance the immune response against antigens. IFA is used with success in combination with long peptides, where clinically relevant responses can be produced with IFA. For instance, in a vaccine with a long HPV-16 peptide against vulva cancer [19,20,21].

5.2 Study Rationale

This is an open-label first-in-human study for the peptide cancer vaccine RV001V administered to prostate cancer patients who previously have been treated with total prostatectomy. The primary objective is to assess tolerability and safety of subcutaneous administration of RV001V, comprising a synthetic peptide derived from RhoC and the adjuvant Montanide ISA 51. The secondary objective is to asses if the vaccination can induce a measurable vaccination-specific T-cell response in prostate cancer patients. Prostate-specific antigen (PSA) is monitored for exploratory purposes.

Prostate cancer

Prostate cancer is the most common cancer in the Danish male population with 4316 patients diagnosed in 2012. During the last 10 years incidence has nearly doubled, mostly due to increased unsystematic screening, whereas the mortality rate has been more or less unchanged at about 1100 deaths annually [22]. 20-30% of patients diagnosed with localized disease will develop metastatic disease and another 15% of men diagnosed with prostate cancer will present with metastatic disease at the time of diagnosis [23].

Standard treatment of prostate cancer

When limited to the prostate only, prostate cancer can potentially be cured through prostatectomy or radiotherapy. If the cancer has spread, usually in the form of bone- and/or lymph metastasis, treatment options are limited to life prolonging and palliative therapies. Androgen deprivation therapy (ADT) serves as a backbone for such treatment, including medical or surgical castration and treatment with androgen receptor (AR) targeting therapies (e.g. bicalutamide) [24]. In patients with “high-volume disease” chemotherapy (early docetaxel) can be added to ADT as this combination has demonstrated a survival benefit compared to ADT alone in recent studies [25]. In addition to improving disease related symptoms, ADT leads to a decline in PSA levels in about 80% of treated patients. Despite such a high initial response rate, the cancer will however progress within 1-2 years [26]. At this point the disease is termed metastatic castration-resistant prostate cancer, a disease stage harbouring a poor prognosis with a median overall survival (OS) of around 2-3 years [26]. Even though the disease is now termed castration-resistant, androgen synthesis and the AR are still targets for further anti-cancer therapy involving abiraterone (an androgen synthesis inhibitor) and enzalutamide (a novel AR antagonist) [27,28]. If response to ADT has been short lived however, chemotherapy with docetaxel is usually preferred before treatment with these agents (abiraterone or enzalutamide) [29,30,31]. In the case of patients who have a good performance status additional chemotherapy can be added (cabazitaxel [32]). For selected patients with symptomatic bone metastases treatment with radium-223 is also an option [33].

5.3 Potential Risks and Benefits

The RhoC peptide RV001V vaccine is tested as a ”first in man” in this study and therefore the primary objective is to assess safety and tolerability. Peptide vaccines have been tested since 1995 [34] and are generally without pharmacological activity despite induction of a biological response against the vaccination target and despite expression of the target protein in various healthy tissues. Since the biological target – the RhoC protein - is expressed in normal cells in various tissues, these cells and tissues could potentially lead to adverse effects of the vaccination. However, RhoC is supposedly expressed by much lower levels in non-cancerous tissues thus not at levels sufficient for recognition by RhoC specific T-cells.

In contrast to other cytotoxic therapies, cancer vaccines have demonstrated minimal toxicity in all clinical trials that have been reported to date [35,36]. Despite expression of many target Tumour-associated antigens in normal tissues, little evidence of autoimmunity has been observed, with the exception of vitiligo that is seen in patients receiving some melanoma vaccines [37,38]. As with all vaccines, there is a theoretical risk of unspecific autoimmune reactions. There can also be a risk of allergic reactions when administering this type of drug. Blood draws and insertion of indwelling catheters may be associated with discomfort at the puncture site with bruising, bleeding, infection, and, rarely, fainting and local nerve damage. Some people tolerate badly the adhesive and / or gel used for attaching electrocardiogram (ECG) electrodes and some skin irritation can occur.

The adjuvant Montanide ISA 51 has been tested in AIDS and cancer vaccines in thousands of patients [18]. The most common local adverse reactions are formation of granulomas, localized pain, inflammation, and erythema at the injection site. In addition, systemic side effects such as fever, headache, fatigue and GI disorders have been reported. These reactions are usually classified as mild or moderate and as being transient. It has been shown that the purified mineral oil emulsion that the Montanide ISA 51 adjuvant consists of is quickly eliminated by macrophages. Systemic adverse reactions consist of headache, chills, fewer and GI disorders and are often mild to moderate. On rare occasions when severe systemic adverse reactions occur, they are often related to the dosage of Montanide ISA 51.

This study will potentially add to existing knowledge in the field of immune therapy and help to improve the development of treatments and thus prognosis in these types of cancers. This study can contribute with descriptions of alterations in cancer specific immune reactivity associated with the given treatment, of benefit for further understanding of the immune system role in cancer treatment. The scientific background for the use of RV001V in cancer is built on the hypothesis that potential metastasis can be eliminated. There might therefore be a theoretical benefit for patients with metastatic disease, although there is no evidence of such potential effect.

The benefits for the society and the potential benefits for the individual patient are expected to outweigh the risks and it is therefore deemed appropriate to perform the study.

6 STUDY OBJECTIVES

6.1 Primary Objective

The primary objective is to evaluate the safety and tolerability of RV001 Vaccine 0.1 mg/mL (RV001V) following subcutaneous (s.c.) administrations to patients who have been prostatectomised due to prostate cancer.

6.2 Secondary Objectives

The secondary objective is to investigate RV001V-specific T-cells in treated patients, and thereby the immunological response before, during and after vaccination.

6.3 Exploratory Objectives

The exploratory objectives are to investigate the PSA levels both in the subgroup of patients with measurable levels at baseline and in patients with no measurable levels at baseline, to explore the potential associations between PSA levels and immunological response and to preliminarily evaluate the progression-free survival (PFS) and OS.

7 INVESTIGATIONAL PLAN

7.1 Study Design and Plan Description

The trial is an open phase I/II first-in-human clinical study for a peptide cancer vaccine.

Between 10 and 25 patients prostatectomised due to prostate cancer will be enrolled.

The treatment will consist of a total of 11 s.c. vaccinations with RV001V. The first 6 vaccinations will be given every 2 weeks, whereas the remaining 5 vaccinations will be administered with 4 weeks between each vaccination. Long term follow-up of immunological response and PSA levels will be performed at 3, 6, 9 and 12 months after the last vaccination.

The first three patients will be enrolled sequentially. Patient no 1 will have 2 vaccinations before any additional patient can be treated. Provided that no safety concern is observed after safety evaluation of the two first vaccinations, Patient no 2 may start vaccination and a new safety readout will be performed when patient no 2 has received 2 vaccinations. Provided no safety concern is observed from the two first patients, Patient no 3 may start vaccination. A new safety readout will be performed when all 3 patients have received at least 2 vaccinations and provided no safety concern is observed, three additional patients may start vaccinations with their first vaccination taking place on separate days. Following a new safety readout, and provided no safety concern is observed, all remaining patients may start vaccinations with their first vaccination taking place on separate days. All vaccinations will be performed at Zelo Phase I Unit.

There will in total be 17 visits; Visit 1a and 1b screening, followed by Visit 2-12 vaccinations, Visit 13 follow-up visit post dosing and long term post dose follow-up visits, Visit 14-17. Visit 1a will be done at the Department of Urology, Rigshospitalet. Remaining visits, visits 1b-17 will be done at Zelo Phase I Unit.

At each vaccination visit, patients will undergo safety assessments and provide blood samples for assessment of haematology, clinical chemistry and PSA. Before the 1st, the 5th, the 7th vaccination and 4 weeks after the 11th vaccination blood will be drawn for lymphocytic evaluation.

At the long-term follow up visits, Visit 14-17, blood will be drawn for lymphocytic evaluation and measurement of PSA.

Outside the scope of this study, the patients will be followed and treated by the investigators at the hospital on a regular basis according to the hospital clinical practice. The PSA analysis performed at the Phase I unit will be reviewed by the investigator at the hospital. For each PSA value, the investigator at the hospital will, based on their standard clinical assessment, issue a statement regarding the patient's clinical cancer status (Stable or progression).

The trial will be terminated if treatment related grade 3, 4 or 5 toxicity in accordance with Common Terminology Criteria for Adverse Events (CTCAE) is observed in more than one of the first 6 patients, with the exception of fever.

While the study is ongoing, the intensive care unit (ICU) at Bispebjerg Hospital will be informed of the vaccination schedule, as intensive care support could potentially be required if a treated patient develops anaphylactic shock.

In case the patient develops disease progression, requiring surgical and/or chemotherapeutic treatment, the patient will be discontinued from the study and referred back to the Department of Urology, Rigshospitalet, from which he was referred initially, where treatment will be offered independent of participation in the study.

7.1.1 Interim Safety Evaluation

Interim safety evaluation will be conducted after each of the 3 first patients has received their 2nd vaccination.

At each assessment, the treating Investigator will make a safety evaluation of all treated patients, document and make it available to a safety review committee. The safety evaluation data to be reviewed will consist of:

- AEs
- Vital signs
- Physical examination
- ECG
- Laboratory data covering 2 weeks after the 1st vaccination

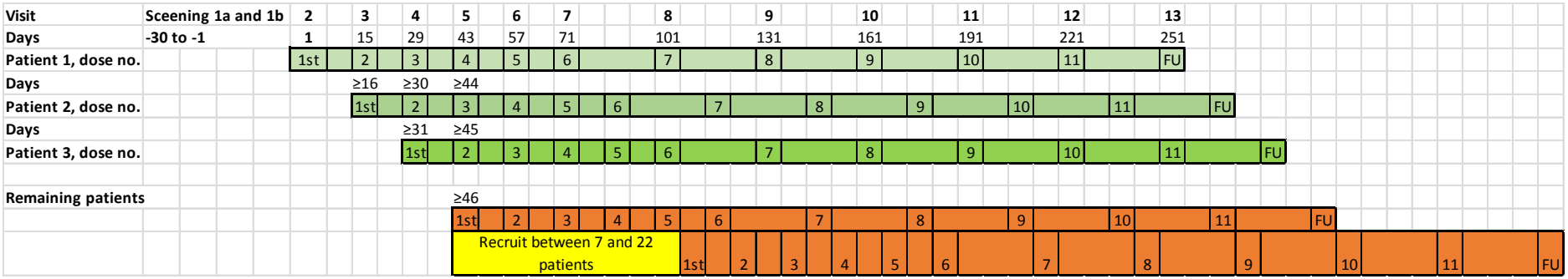
The safety review committee will, in accordance with pre-defined criteria and stopping rules, evaluate safety prior to enrolment of the next patient. Further details are given in the Safety Review Charter, This procedure will be repeated for the second and third patient treated, see Figure 1.

The safety review committee will comprise a minimum of, but is not limited to:

- Principal investigator at Zelo Phase I Unit.
- Co-Investigator from the Department of Urology, Rigshospitalet.
- Sponsor's medical responsible

Figure 1 Overall Study Design

Initial patient enrolment and treatment – Vaccination overview



7.2 Study Procedures

7.2.1 Schedule of Study Events

The study assessments described in the sections below are presented in detail in Section 10.1 (primary and secondary endpoints), Section 10.4 (demographic data and baseline characteristics) and Section 10.5 (safety assessments). Recording and reporting of AEs are described in detail in Section 11.

The timing of all study events is shown in Table 1 and Table 2 in 7.2.2.

7.2.1.1 Visit 1a and Visit 1b (Screening Visit, Day -30 to -1) at Rigshospitalet and Zelo Phase I Unit

Visit 1a performed at the Department of Urology at Rigshospitalet

Pre-screening of patient and obtaining informed consent. No study assessments may be performed before informed consent has been obtained.

- Informed consent
- Initial assessment of eligibility based on medical history, concomitant medication and available laboratory data
- Medical history
- Prior and concomitant medication
- Performance status according to the Eastern Cooperative Oncology Group (ECOG) scale
- Determination of clinical cancer status (stable disease or progression)

Visit 1b performed at Zelo Phase I Unit

No study assessments may be performed before informed consent has been obtained. The following assessments must be performed within a month prior to the start of vaccinations. Blood tests must be performed within one week prior to start of vaccinations:

- Confirmation that informed consent has been obtained
- Confirmation of eligibility
- Physical examination
- Demographic information (date of birth, sex, ethnicity)
- Vital signs: blood pressure, pulse rate and temperature
- Body measurements: body weight, height
- Medical history
- Prior and concomitant medication
- Performance status according to the ECOG scale
- Blood tests
    - Haemoglobin, red blood cell (RBC) count, haematocrit, leukocytes, differential counts and platelets

  - Sodium, creatinine, albumin, lactate dehydrogenase (LDH), alkaline phosphatase (ALP), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), total bilirubin, potassium, calcium, C-reactive protein (CRP), blood glucose, HbA1c
  - Hepatitis B core antibody, hepatitis B surface antigen, hepatitis C virus, human immunodeficiency virus (HIV)
  - PSA
- Urine dipstick: Glucose, bilirubin, ketones, specific gravity, blood, pH, protein, urobilinogen, nitrite, leucocytes
- 12-lead ECG

Visit 1a and 1b may take place on the same day.

7.2.1.2 Visit 2 (Day 1), at Zelo Phase I Unit

At Visit 2, the following activities and assessments will be performed:

- Confirmation of eligibility
- Physical examination
- Vital signs before vaccination
- Changes in concomitant medication
- Weight
- Blood tests (safety)
- 12-lead ECG before, 60 and 120 minutes after vaccination
- Blood sampling for HLA tissue type determination
- Blood sampling for Lymphocyte evaluation
- Vaccination no 1
- Heart rate and blood pressure 30, 60 and 120 min after vaccination
- Adverse events (Common Toxicity Criteria [CTC]), after first Vaccination
- Injection site reactions
- Observation in phase I unit for at least 2 hours after vaccination

7.2.1.3 Visit 3 (Day 15±2) at Zelo Phase I Unit

At Visit 3, the following activities and assessments will be performed:

- Vital signs before vaccination
- Changes in concomitant medication
- Weight
- Adverse events (CTC)
- Blood tests (safety)

- 12-lead ECG before 60 and 120 minutes after vaccination
- Vaccination no 2
- Injection site reactions
- Heart rate and blood pressure 30, 60 and 120 min after vaccination
- Observation in phase I unit for at least 2 hours after vaccination

7.2.1.4 Visit 4 (Day 29±2), at Zelo Phase I Unit

At Visit 4, the following activities and assessments will be performed:

- Vital signs before vaccination
- Changes in concomitant medication
- Weight
- Adverse events (CTC)
- Blood tests (safety)
- PSA
- 12-lead ECG before, 60 and 120 minutes after vaccination
- Vaccination no 3
- Injection site reactions
- Heart rate and blood pressure 30, 60 and 120 min after vaccination
- Request determination of clinical cancer status (stable disease or progression) from Department of Urology at Rigshospitalet
- Observation in phase I unit for at least 2 hours after vaccination

7.2.1.5 Visit 5 (Day 43±5), at Zelo Phase I Unit

At Visit 5, the following activities and assessments will be performed:

- Vital signs before vaccination
- Changes in concomitant medication
- Weight
- Adverse events (CTC)
- Vaccination no 4
- Injection site reactions
- Heart rate and blood pressure 30 and 60 min after vaccination
- Observation in clinic for at least 1 hour after vaccination

7.2.1.6 Visit 6 (Day 57±5), at Zelo Phase I Unit

At Visit 6, the following activities and assessments will be performed:

- Vital Signs before vaccination
- Changes in concomitant medication
- Weight
- Adverse events (CTC)
- Blood tests (safety)
- PSA
- Sampling for Lymphocyte evaluation
- Vaccination no 5
- Injection site reactions
- Heart rate and blood pressure 30 and 60 min after vaccination
- Request determination of clinical cancer status (stable disease or progression) from Department of Urology at Rigshospitalet
- Observation in clinic for at least 1 hour after vaccination

7.2.1.7 Visit 7 (Day 71±5), at Zelo Phase I Unit

At Visit 7, the following activities and assessments will be performed:

- Physical examination
- Vital signs before vaccination
- Changes in concomitant medication
- Weight
- Adverse events (CTC)
- PSA
- Vaccination no 6
- Injection site reactions
- Heart rate and blood pressure 30 and 60 min after vaccination
- Request determination of clinical cancer status (stable disease or progression) from Department of Urology at Rigshospitalet
- Observation in clinic for at least 1 hour after vaccination

7.2.1.8 Visit 8 (Day 101±7), at Zelo Phase I Unit

At Visit 8, the following activities and assessments will be performed:

- Vital signs before vaccination
- Changes in concomitant medication

- Weight
- Adverse events (CTC)
- Blood tests (safety)
- PSA
- Sampling for Lymphocyte evaluation
- 12-lead ECG before and 60 minutes after vaccination
- Vaccination no 7
- Injection site reactions
- Heart rate, blood pressure 30 and 60 min after vaccination
- Request determination of clinical cancer status (stable disease or progression) from Department of Urology at Rigshospitalet
- Observation in clinic for at least 1 hour after vaccination

7.2.1.9 Visit 9 (Day 131±7), at Zelo Phase I Unit

At Visit 9, the following activities and assessments will be performed:

- Vital Signs before vaccination
- Changes in concomitant medication
- Weight
- Adverse events (CTC)
- PSA
- Vaccination no 8
- Injection site reactions
- Heart rate and blood pressure 30 and 60 min after vaccination
- Request determination of clinical cancer status (stable disease or progression) from Department of Urology at Rigshospitalet
- Observation in clinic for at least 1 hour after vaccination

7.2.1.10 Visit 10 (Day161±7), at Zelo Phase I Unit

At Visit 10, the following activities and assessments will be performed:

- Vital Signs before vaccination
- Changes in concomitant medication
- Weight
- Adverse events (CTC)
- Blood tests (safety)
- PSA

- Vaccination no 9
- Injection site reactions
- Heart rate and blood pressure 30 and 60 min after vaccination
- Request determination of clinical cancer status (stable disease or progression) from Department of Urology at Rigshospitalet
- Observation in clinic for at least 1 hour after vaccination

7.2.1.11 Visit 11 (Day 191±7), at Zelo Phase I Unit

At Visit 11, the following activities and assessments will be performed:

- Vital Signs before vaccination
- Changes in concomitant medication
- Weight
- Adverse events (CTC)
- PSA
- Vaccination no 10
- Injection site reactions
- Heart rate and blood pressure 30 and 60 min after vaccination
- Request determination of clinical cancer status (stable disease or progression) from Department of Urology at Rigshospitalet
- Observation in clinic for at least 1 hour after vaccination

7.2.1.12 Visit 12 (Day 221±7), at Zelo Phase I Unit

At Visit 12 the following activities and assessments will be performed:

- Physical examination
- Vital signs
- Changes in concomitant medication
- Weight
- Adverse events (CTC)
- PSA
- Vaccination no 11
- Injection site reactions
- Heart rate, blood pressure 30 and 60 min after vaccination
- 12-lead ECG 60 min after vaccination
- Request determination of clinical cancer status (stable disease or progression) from Department of Urology at Rigshospitalet

- Observation in clinic for at least 1 hour after vaccination

7.2.1.13 Visit 13 (Day 251±14), End of Treatment Follow Up (30 days After Last Vaccination), at Zelo Phase I Unit

This end of treatment follow-up will take place in **all** patients, also those that withdraw prematurely.

At Visit 13, the following activities and assessments will be performed:

- Physical examination
- Vital signs
- Changes in concomitant medication
- Weight
- Adverse events (CTC)
- Blood tests (safety)
- Urine dipstick: glucose, bilirubin, ketones, specific gravity, blood, pH, protein, urobilinogen, nitrite, leucocytes
- PSA
- Sampling for Lymphocyte evaluation
- 12-lead ECG
- Request determination of clinical cancer status (stable disease or progression) from Department of Urology at Rigshospitalet

7.2.1.14 Visit 14 (3 months) Long Term Follow Up Post Last Dose, at Zelo Phase I Unit

At Visit 14, the following activities and assessments will be performed:

- Weight
- Adverse events (CTC). Recording of study/protocol related AEs and serious adverse events (SAEs) suspected to be related to study treatment or procedures
- PSA
- Sampling for Lymphocyte evaluation
- Request determination of clinical cancer status (stable disease or progression) from Department of Urology at Rigshospitalet
- If patients fail to return for these assessments for unknown reasons, every effort (e.g. telephone, e-mail, letter) should be made by the Investigator to contact them.

7.2.1.15 Visit 15 (6 months), Long Term Follow Up Post Last Dose, at Zelo Phase I Unit

At Visit 15, the following activities and assessments will be performed:

- Weight
- Adverse events (CTC). Recording of study/protocol related AEs and SAEs suspected to be related to study treatment or procedures
- PSA
- Sampling for Lymphocyte evaluation
- Request determination of clinical cancer status (stable disease or progression) from Department of Urology at Rigshospitalet
- If patients fail to return for these assessments for unknown reasons, every effort (e.g. telephone, e-mail, letter) should be made by the Investigator to contact them.

7.2.1.16 Visit 16 (9 months), Long Term Follow Up Post Last Dose, at Zelo Phase I Unit

At Visit 16, the following activities and assessments will be performed:

- Weight
- Adverse events (CTC). Recording of study/protocol related AEs and SAEs suspected to be related to study treatment or procedures
- PSA
- Sampling for Lymphocyte evaluation
- Request determination of clinical cancer status (stable disease or progression) from Department of Urology at Rigshospitalet
- If patients fail to return for these assessments for unknown reasons, every effort (e.g. telephone, e-mail, letter) should be made by the Investigator to contact them.

7.2.1.17 Visit 17 (12 months) Long Term Follow Up Post Last Dose, at Zelo Phase I Unit

At Visit 17, the following activities and assessments will be performed:

- Weight
- Adverse events (CTC). Recording of study/protocol related AEs and SAEs suspected to be related to study treatment or procedures
- PSA
- Sampling for Lymphocyte evaluation
- Request determination of clinical cancer status (stable disease or progression) from Department of Urology at Rigshospitalet

- If patients fail to return for these assessments for unknown reasons, every effort (e.g. telephone, e-mail, letter) should be made by the Investigator to contact them.

7.2.2 Study Flow Chart

The study flow chart for the main study period is shown in Table 1 and for the follow up period in Table 2.

Table 1 Study Flow Chart-Main Study Period

Procedures will be performed at the Zelo Phase I Unit only unless otherwise indicated.

Visit	**1a+1b**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13 (EOT Follow up)**
Day	**Screen Day -30 to -1**	**1**	**15 (±2 days)**	**29 (±2 days)**	**43 (±5 days)**	**57 (±5 days)**	**71 (±5 days)**	**101 (±7 days)**	**131 (±7 days)**	**161 (±7 days)**	**191 (±7 days)**	**221 (±7 days)**	**251 (±14 days)**
Vaccination no		1	2	3	4	5	6	7	8	9	10	11	FU
Informed consent	X[a]												
Inclusion/Exclusion Criteria	X[b]	X[c]											
Demographic information	X												
Physical Examination	X	X					X					X	X
Determination of cancer status (stable or progression)	X[a]			X[a]		X[a]	X[a]	X[a]	X[a]	X[a]	X[a]	X[a]	X[a]
Vital signs (pulse rate, blood pressure, body temperature)	X	X[d]	X[d]	X[d]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X
Medical History	X[b]												
Prior and concomitant medication	X[b]	X	X	X	X	X	X	X	X	X	X	X	X
Performance status (ECOG)	X[b]												
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X												
Adverse events (CTC)		X	X	X	X	X	X	X	X	X	X	X	X
Injection site reactions		X	X	X	X	X	X	X	X	X	X	X	X
Blood tests[f]	X[g]	X	X	X		X		X		X			X
Urinalysis (dipstick)	X												X
HIV, HBV and HCV tests	X												
Lymphocyte evaluation[h]		X				X		X					X
ECG	X	X[i]	X[i]	X[i]				X[i]				X[j]	X
PSA	X			X		X	X	X	X	X	X	X	X
HLA tissue type determination		X											

EOT= End of treatment, FU= Follow-up, HBV= Hepatitis B virus, HCV= Hepatitis C virus

[a] Will be performed at Department of Urology at Rigshospitalet only.

[b] Will be performed both at Department of Urology at Rigshospitalet and at Zelo Phase I Unit.

[c] Re-check of eligibility criteria before first vaccination. Results from haematology and clinical chemistry at screening will determine eligibility.

[d] During administration of the first 3 vaccinations, the patient is observed for at least 2 hours after vaccination with measurements of pulse and blood pressure before and after the vaccine is given

[e] During administration of vaccination 4 to 11 the patient is observed for at least 1 hour after vaccination with measurements of pulse and blood pressure before and after the vaccine is given

[f] Blood tests = Haemoglobin, RBCs, haematocrit, leukocytes and differential counts, platelets, CRP, LDH, creatinine, sodium, potassium, calcium, albumin, ASAT, ALAT, total bilirubin, alkaline phosphatase. Blood glucose and HbA1c only at screening.

[g] Blood tests are to be performed within 1 week of vaccination start.

[h] See “Blood Tests for Lymphocyte Evaluation” in Section 10.2

[i] During administration of the first 3 vaccinations and vaccination number 7, ECG measurements before and after the vaccine is given

[j] At administration of vaccine 11, ECG measurement 60 minutes after vaccination

Table 2 Study Flow Chart-Post Treatment Long-Term Follow up of PSA and Immunology

Visit	14	15	16	17
Months following last vaccination	3	6	9	12
Weight	X	X	X	X
PSA	X	X	X	X
Lymphocyte evaluation[a]	X	X	X	X
Determination of cancer status (stable disease or progression)	X	X	X	X
Recording of study/protocol related AEs and SAEs suspected to be related to study treatment or procedures	X	X	X	X

[a] See “Blood Tests for Lymphocyte Evaluation” in Section 10.2

7.3 Discussion of Study Design, Including the Choice of Control Groups

It is anticipated that 10 patients will be sufficient for evaluation of the primary endpoint.

The primary and secondary objectives of the study can be answered in PT patients independent of their PSA status. However, as an exploratory aim, we will evaluate PSA kinetics during the treatment and potential association to immune response to vaccination. For this reason we aim to include patients with measurable and rising PSA levels, although, should this be difficult or result in more dropouts than anticipated, PT patients without measurable PSA will also be included to secure the primary and secondary endpoint.

Due to the exploratory nature of this open study no placebo group is included.

The 1 year long term follow up period is aimed to explore the duration of the immune response.

The adjuvant used in this study is Montanide ISA 51 which is a so called in-complete Freund's adjuvant developed specifically for use as an adjuvant in therapeutic cancer vaccines. The dose (volume) of the adjuvant Montanide ISA 51 is reported to have been used in volumes from 0.1 mL to as high as 5.0 mL. From previous experience a dose of approx. 0.5 mL of Montanide ISA 51 has shown to provide the required enhancement of antigen-specific immune responses [39]. Hence, this target volume was also chosen for this trial.

7.4 Study Period

The duration of the study is expected to be approximately 2 years, beginning with first patient first visit which is anticipated to occur in Q1 2017, and ending with last patient last follow up visit in Q1 2019. All patients are expected to be enrolled and have completed vaccine treatment during the first year. The second year will consist of follow up visits. The actual overall study duration will depend on the time required to screen and enrol patients, the number of patients needed, and whether patients complete all treatment and follow up visits.

7.5 End of Study

The end of study is defined as the date of the last patient's last visit.

The end of the treatment phase of the study will be when the last patient has completed Visit 13/end of treatment follow-up visit.

The end of the long-term follow-up phase of the study will be when the last patient has completed the last follow-up visit.

8 SELECTION OF STUDY POPULATION

8.1 Number of Patients

At least 10 patients will be enrolled in the trial to fulfil the primary objective. Additional patients, up to a total of 25 patients, may be enrolled dependent on analysis of immune related data. With an estimated dropout rate of 25% up to 20 patients may complete all vaccinations.

8.1.1 Recruitment

Patients will be recruited from the Department of Urology at Rigshospitalet. Patients will be identified from the hospitals database of patients who have undergone radical prostatectomy. Approximately, 300 patients are prostatectomised due to prostate cancer each year at Rigshospitalet and therefore it is estimated that patient inclusion can be completed within 4 months.

8.2 Inclusion Criteria

The patients have to meet all of the following criteria to be eligible to enter the study:

1) Patients prostatectomised (PT) due to histologically verified adenocarcinoma of the prostate gland who currently are not being treated, or expected within the next 8 months to be treated, with any anti-cancer treatment. Patient may or may not have measurable PSA.
2) Able to understand the study procedures and willing to provide informed consent
3) Able and willing to comply with study requirements and complete all visits
4) Using adequate contraceptive measures

   All non-vasectomized patients must use condoms during the study and for one month after the last vaccination with RV001V, or have a female partner who either has been post-menopausal for more than one year or is using a highly effective method of contraception (i.e. a method with less than 1% failure rate).
5) Aged 18 years and above.
6) ECOG performance status 0 or 1 (Appendix A).
7) Recovered/stabilized at grade ≤2 from all toxicities related to prior treatment(s) in accordance with CTCAE.
8) Laboratory values obtained ≤30 days prior to first dose, and more than 3 weeks after potential chemotherapy
   a. Haemoglobin ≥5.6 mmol/L
   b. Absolute granulocyte count ≥1.5 x 10^9 /L
   c. Platelets ≥100 x 10^9 /L
   d. Total bilirubin ≤1.5 x upper limit of normal (ULN)
   e. Creatinine ≤1.5 x ULN
   f. ALAT, ASAT and ALP ≤2.5 x ULN.

8.3 Exclusion Criteria

Patients meeting any of the following criteria will not be permitted to enter the study:

1) Patient is a candidate for relevant therapies that are the current standard of care for their cancer disease.
2) Patient has been treated with Androgen Deprivation Therapy (ADT), or expected to receive such treatment within the next 8 months from enrolment.
3) Concurrent chemotherapy or radiotherapy within 12 weeks of 1st vaccination, or expected to receive such treatment within the next 8 months from enrolment.
4) Patients have undergone major surgery or have had major bleeding within the last month prior to the first vaccination.
5) Patients with brain or leptomeningeal metastases.
6) Prior treatment with any therapeutic cancer vaccine(s).
7) History of second malignancy (except for adequately managed basal cell carcinoma and squamous cell carcinoma of the skin).
8) Patients in need of or treated the last 30 days before the first vaccination with systemic steroids or other immune suppressive therapy. Use of inhaled steroids, nasal sprays, and topical creams for small body areas is allowed.
9) History of alcohol or substance abuse within the last 5 years
10) History of acquired immune deficiency syndrome or positive serological test for HIV infection
11) History of viral hepatitis B as determined by positive antibody immunoglobulin M (IgM) to core antigen for hepatitis B or positive for hepatitis B surface antigen, or viral hepatitis C as determined by positive antibody for hepatitis C
12) Participation in any investigational trial or use of any investigational drug(s) within 30 days prior to inclusion in this trial.
13) Any known serious infections, e.g. tuberculosis.
14) History of significant autoimmune disease such as: Inflammatory bowel disease, Systemic lupus erythematosus, Ankylosing spondylitis, Scleroderma, Multiple sclerosis
15) Severe medical conditions, such as but not limited to severe asthma/ Chronic obstructive pulmonary disease (COPD), New York Heart Association (NYHA) grading 3 or above, poorly regulated insulin-dependent diabetes, any significant organ damage as judged by the Investigator.
16) Other medications or conditions that in the Investigator's opinion would contraindicate study participation for safety reasons or interfere with the interpretation of study results
17) History of multiple drug allergies or known allergy/hypersensitivity to Montanide ISA 51, or intolerance to subcutaneous injection.

8.4 Restrictions

The following restrictions apply during vaccine treatment:

Vaccination within 1 week of a major viral infection, bacterial infection requiring systemic antibiotics, or administration of other vaccinations. In such situations, it is allowed to postpone RV001V vaccinations up to 3 weeks, see Section 8.5.

8.5 Removal of Patients from Therapy or Assessment

8.5.1 Withdrawal from Study

Patients are free to discontinue their participation in the study at any time. Withdrawal from the study will not affect or prejudice the patient's further care or treatment. Patients may be withdrawn from study treatment and assessments at any time, if deemed necessary by the Investigator.

Potential reasons for withdrawal of patients from this study are:

- Screening failure
- The decision of a patient to withdraw from the study (including if the patient withdraws informed consent). The treatment can be stopped at any time the patient wishes to do so.
- Unacceptable toxicity. Treatment is terminated if AEs occur that are of a degree that makes completion of the study impossible. That is treatment related grade 3, 4 or 5 toxicity in accordance with CTCAE (except fever).
- Medical decision. The Investigators can stop treatment at any time if he/she feels it is in the patients' best interest, e.g. due to medical conditions.
- Administration of concomitant medication prohibited by this protocol.
- Need of alternative treatment. Patients will be excluded at any time a new treatment with an experimental drug or other systemic anticancer treatment is initiated after inclusion in this protocol. If Androgen Deprivation Therapy (ADT) is initiated, the patient may continue treatment and follow up when agreed between the investigator at the hospital and the sponsor's medical experts. The patient will be excluded if systemic treatment with corticosteroids is initiated unless it is agreed with the sponsor medical expert that the patient can continue in the study.
- Delayed treatment. The patient will be withdrawn from the study if a scheduled vaccination is delayed more than 3 weeks. It is the responsibility of the investigator to assess the cause of delay and define the time point for the start of the delay. A patient can be delayed several times during the cause of treatment without being excluded. I case a vaccination has to be delayed, the clock will be put on hold until the vaccination can be performed and the patients continued study related activities will be rescheduled.
- Patient is lost to follow-up

The reason and date the patient is withdrawn from the study will be documented in the case report form (CRF) (e.g. lost to follow-up, consent withdrawn, incorrect enrolment, AEs, etc.).

If a patient is withdrawn from the study, all safety data collected until the time of withdrawal will be used in the analyses, and efficacy data will be used if 3 or more vaccinations have been administered.

Patients, who are excluded from the study, will be referred back to the urology/oncology team, from which they were recruited, for standard treatment. Patients that are excluded before the 3rd vaccination will be replaced with a new patient.

8.5.2 Withdrawal from Treatment

If a patient is withdrawn from further treatment with the investigational medicinal product (IMP), the End of Treatment visit (Visit 13) should be completed.

Patients who discontinue treatment with the IMP and who received 3 or more vaccinations, should continue with the long-term follow-up visits(visits 14-17). If a patient fails to return for these assessments for unknown reasons, every effort (e.g. telephone, e-mail, letter) should be made by the Investigator to contact them.

8.6 Premature Termination of the Study

The Investigator or the Sponsor may terminate this study prematurely for any reasonable cause. The Independent Ethics Committee (IEC) and Competent Authority (CA) will be informed promptly.

Conditions that may warrant termination include, but are not limited to:

- The discovery of an unexpected, significant, or unacceptable risk to the patients enrolled in the study, or potential study patients
- A decision on the part of the Sponsor to suspend or discontinue development of the IMP

If the CA obtains information that raises doubts about the safety or scientific validity of the clinical study, the CA can suspend or prohibit the study. Before the CA reaches its decision, it shall, except where there is imminent risk, ask the Sponsor and/or the Investigator for their opinion, to be delivered within one week (Directive 2001/20/EC, Article 12, Section 1).

If the study is prematurely terminated or suspended for any reason, the Investigator should promptly inform the study patients and should assure appropriate therapy and follow-up for the patients.

9 TREATMENT OF PATIENTS

9.1 Investigational Medicinal Product

9.1.1 Treatment Regimens

The vaccine will be administered as a subcutaneous injection of 1 ml on the upper arm alternating between the right and left side. Dosage per vaccine injection will be 100 µg of RV001 peptide dissolved in in an aqueous buffer and homogenized immediately before administration with Montanide ISA 51 (adjuvant) in a 50:50 ratio.

The vaccine is injected into the subcutaneous tissue of the upper arm after disinfection of the skin. During administration of the first 3 vaccinations (Visits 2, 3 and 4), the patient is observed for at least 2 hours with measurements of pulse and blood pressure before vaccination, 30, 60 and 120 min after the vaccine is given. Patients are observed for acute toxicities in the form of allergic reactions, including anaphylactic shock. Allergic reactions and/or anaphylactic shock will be treated according to hospital guidelines. For the remaining vaccinations, the patient will be observed for at least 1 hour following each injection and with measurement of blood pressure and pulse rate before and 30 and 60 min after vaccination. The intensive care unit at Bispebjerg Hospital will be informed of the vaccination schedule, as intensive care support could potentially be required if a treated patient develops anaphylactic shock.

9.1.2 Identity of Investigational Medicinal Product

9.1.2.1 RV001 Vaccine 0.1 mg/mL (RV001V)

The active constituent in RV001 Vaccine 0.1 mg/mL is the synthetic peptide RV001, which has the same amino acid sequence as the C-terminal of the RhoC protein:

Ala-Thr-Arg-Ala-Gly-Leu-Gln-Val-Arg-Lys-Asn-Lys-Arg-Arg-Arg-Gly-Cys-Pro-Ile-Leu

The RV001 Vaccine 0.1 mg/mL (RV001V) is an emulsion where the aqueous phase is a solution of the RV001 peptide and the oil phase is the adjuvant (Montanide ISA 51).

The aqueous phase of the emulsion is named RV001 Injection 0.2 mg/mL, and is a sterile clear, colourless solution. It is formulated using the peptide RV001 as the active ingredient, sodium acetate and acetic acid as buffer and sodium chloride as a tonicity agent. The pH of the solution is approximately 3.5. The solution is filled at a nominal volume of 1 mL into single-use clear glass vials which is stoppered and capped.

The composition of RV001 Injection 0.2 mg/mL (the aqueous phase) is tabulated below:

Component	Content	Function
RV001 (100%, anhydrous)	0.200 mg	Active Ingredient
Sodium acetate trihydrate	0.180 mg	Buffer
Acetic acid glacial	0.142 mg	Buffer
Sodium chloride	0.292 mg	Tonicity
Water for Injection	qs to make 1 mL	Solvent

Immediately before administration to the patient (within 15 min), 1 mL of RV001 Injection 0.2 mg/mL is mixed and emulsified with 1 mL of Montanide ISA 51 using two syringes and an I-connector as described in “Instruction for Use”. The resulting emulsion (RV001V) is a water-in-oil emulsion and 1 mL of this emulsion is administered to the patient. The amount administered will contain 0.1 mg RV001.

The adjuvant Montanide ISA 51 is a so called in-complete Freund's adjuvant developed specifically for use as an adjuvant in therapeutic cancer vaccines. Montanide ISA 51 is supplied sterile, ready for use in single use vials of 3 mL. Montanide ISA 51 is defined as a mixture of a highly purified mineral oil (Drakeol 6VR) and a surfactant (Mannide monooleate). When mixed with an aqueous phase in a 50/50 ratio, it renders a water in oil emulsion.

9.1.3 Packaging and Labelling of Investigational Medicinal Product

The IMP RV001V will be supplied in kits containing the needed items for each administration:

- One vial of RV001 Injection 0.2 mg/mL
- One vial of Montanide ISA 51
- Two 2 mL disposable syringes (Luer Lock)
- Two vial adapters (13 mm, Luer Lock))
- One I-connector (Luer Lock)
- One injection needle for subcutaneous injection (Luer Lock)
- One syringe label
- One plastic bag for collection of used materials

RV001V will be supplied to the site in outer boxes, each containing 11 kits with content as described above. As the storage requirements for RV001 Injection 0.2 mg/mL and Montanide ISA 51 are different (see Section 9.1.4), RV001 Injection 0.2 mg/mL will be supplied in a separate carton labelled with the same kit number as the rest of the kit.

Replacement kits will be available on request in case the whole or part of the kit is lost.

9.1.4 Storage and Handling of Investigational Medicinal Product

RV001 Injection 0.2 mg/mL must be stored in a refrigerator (2 – 8^{o}C). Montanide ISA 51 must be stored at room temperature (15 – 30^{o}C). For further information on expiry date of the two components of RV001V, reference is made to the product label.

Following mixing and emulsification of the RV001 Injection 0.2 mg/mL and Montanide ISA 51, the vaccine RV001V shall be administered immediately (less than 15 minutes).

The vaccine is prepared and labelled by the Phase I clinic according the instructions that will be provided to the site.

9.2 Method of Assigning Patients to Treatment Groups

All patients who have provided informed consent and meet all of the inclusion criteria and none of the exclusion will be assigned to treatment with the vaccine containing RV001.

9.3 Selection of Doses in the Study

Traditionally, first-in-human clinical trials are generally conducted to identify the maximum tolerated dose or the biologically active dose using a dose-escalation design. This design may not be applied to cancer vaccines, given their unique mechanism of action. A cancer vaccine, in contrast to cytotoxic agents, generates its effect by modulating the immune system to target specific antigens on cancer cells. The selection of dose is based on previous experience, with peptide based vaccines, primarily from the Center for Cancer Immune Therapy at the University Hospital Herlev [40]. The general approach here has been

to use of 0.1 mg of the peptide. This mechanism of action requires that a certain exposure threshold is achieved [41, 42]

From a safety point of view, it is recognized that peptide vaccines generally seem inherently safe as long as the adjuvants are used in combinations and doses previously demonstrated to be safe

In addition, the completed repeated dose-toxicology study has shown that a dose (recalculated to Human Equivalent Dose) which is more than 400 times higher than the proposed could be administered to the animals without any indication of treatment related systemic toxicity.

Based on this rationale the selected dose of RV001 to be used in the study is 0.1 mg.

The dose (volume) of the adjuvant Montanide ISA 51 is reported to have been used in volumes from 0.1 mL to as high as 5.0 mL. From previous reported experience, a dose of approx. 0.5 mL of Montanide ISA 51 has shown to provide the required enhancement of antigen-specific immune responses [39, 40]. Hence, this target volume was also chosen for this trial.

The other parameter defining the volume of Montanide ISA 51 to be used is the water droplet size in the emulsion, which preferably should be around 1 micrometre in diameter. Formulation studies have demonstrated that this droplet size is obtained when the ratio between RV001 Injection 0.2 mg/mL and Montanide ISA 51 is 50/50.

In addition, Montanide ISA 51 was studied in a repeated dose toxicological study, both in combination with RV001 and alone. Local reactions at injection sites were noticed and these reactions are known and reported reactions linked to the use of Montanide ISA 51.

Administration of 1 mL of RV001V will provide a dose of 0.1 mg RV001 and 0.5 mL Montanide ISA 51.

9.4 Selection and Timing of Dose for Each Patient

There are no general consensus on the dose regiment for peptide vaccines. The dose regiment selected for this study is based on previous experience with similar peptide vaccines [40].

9.5 Blinding

This is an open-label study with no control arm.

9.6 Prior and Concomitant Therapy

Supportive treatment is given based on a clinical judgement and should be noted in the patient chart in accordance with existing practice. However, concomitant medication administered during the study may lead to withdrawal of the patient from the study (see Section 8.5).

The following medications and treatments are prohibited during the study:

- Chemotherapy and radiotherapy is prohibited from 12 weeks before the first vaccination and until 4 weeks after the last vaccination.
- For ADT; please see Section 8.5.1
- Immunosuppressive therapy. Use of inhaled steroids, nasal sprays, and topical creams for small body areas is allowed.

The use of all medication by the patient must be recorded in the appropriate sections of the CRF.

9.7 Treatment Compliance

RV001 vaccination will be administered at the Phase I Unit and by the staff under controlled conditions. The time of administration and the initials of the person administering the vaccine will be documented in the CRF and in the accountability log.

9.8 Drug Accountability

The drug accountability will be performed by checking the used vials containing RV001 and the drug accountability log.

10 STUDY ASSESSMENTS

10.1 Primary and Secondary Endpoints

Primary endpoint

The primary endpoint of the study is the proportion of patients developing treatment related grade 3, 4 or 5 toxicity in accordance with CTCAE, with the exception of fever. The study will be terminated if this toxicity is observed in more than 1 of the first 6 patients, i.e. in ≥33.3% of the patients. The study will be considered meeting the primary endpoint if the proportion of patients with this toxicity at the end of the study is not higher than 33.3%.

In addition, the tolerability will be evaluated by number of patients who discontinue the study treatment prematurely. The safety will be evaluated with frequency and severity of AEs. The numbers and proportions of patients with any treatment-emergent adverse event (TEAE), and any serious TEAE will be summarised. Furthermore, changes in vital signs, laboratory variables, physical examination and ECG will be evaluated.

Secondary endpoints

For the evaluation of the immunological effect of the vaccinations, patients will be followed with *in-vitro* analysis of antigen specific T-cell reactivity against RhoC and possibly other antigens as well.

To monitor RV001V-specific T-cells in treated patients, and thereby the immunological response, the immunogenicity of RV001 in patients is determined by measuring anti-vaccine T cells before, during and after vaccination and by evaluating the changes from baseline (last assessment before the first vaccination) to each vaccination and follow-up visit. The measurements are done on immune cells isolated from the collected blood and frozen in a research biobank.

All patients will be tested using IFNgamma enzyme-linked immunospot (ELIspot) assay. For ELIspot positive patients, intracellular staining (ICS) for dissecting CD4/CD8 responses and cytokine production profile will be performed. The ICS markers will be CD4/CD8/dead cell dye/ Interleukin-2(IL-2)/IFNgamma/TNF/CD154/CD107a. As a minimum for patients that are HLA-A3 positive, multimer staining for assessing RV001V-specific cells will be performed. For selected patients, advanced functional analysis of RV001V-specific T-cells may be performed.

Exploratory endpoints

The PSA levels will be evaluated by:

- Changes from baseline in the PSA levels among the patients with measurable levels at baseline (the last PSA assessment before the first vaccination)
- Number of patients with measurable PSA levels at baseline and at each vaccination, where measured and follow-up visit.

The potential association between the PSA levels and immunological response will be evaluated by:

- Anti-vaccine T cell levels classified by the PSA status (measurable or non-measurable) at baseline and at each vaccination where PSA is measured and follow-up visit

- Correlation of changes from baseline in anti-vaccine T cell levels and PSA levels among the patients with measurable PSA levels at baseline.

PFS and OS will be evaluated as time from baseline (time of the first vaccination) to the event. For PFS, disease progression or death for any reason will be defined as an event. For OS, death for any reason is defined as an event. The data from the patients without an event will be censored at the last time when the patient is known to be free of disease progression or alive.

10.2 Sampling Procedures, Handling and Storage

Blood Tests for Lymphocyte Evaluation

Blood samples of 100 mL heparinized blood and 8 mL blood in a dry glass will be drawn for lymphocyte function evaluation and 8 mL for HLA determination in accordance with the treatment schedule, as described in Section 7.2.1 and Table 1 and Table 2.

Immune cells will be isolated from the collected blood samples by use of Leucosep/Lymphoprep-technique and peripheral blood mononuclear cell samples and serum will be stored at -150°C or below and sent to the University of Tübingen in Germany for analysis at intervals as agreed with the sponsor. Until shipment, the blood collected for lymphocyte evaluation will be stored at Zelo Phase I Unit and constitute a research biobank.

The material will be kept at the laboratory in Tübingen for up to 5 years for potential further research in relation to the study. Thereafter the samples will be destroyed. After shipment to the University of Tübingen, the blood collected will be handled according to German laws.

A new approval from the Danish Data Protection Agency will be obtained should we wish to use the samples for other research areas.

All patient data will be transferred in a coded form in these cases. The biological material will be destroyed if the patient withdraws his/her consent and does not want the material to be used. In this case, no further analysis will be made on the collected material. Results of analyses already performed will, however, not be discarded.

10.3 Bioanalytical Method

Lymphocyte Evaluation

Antigen-specific immune reactivity will be tested by use of a panel of relevant immunological assays that may include ELIspot, enzyme linked immunosorbent assay (ELISA), proliferation assays, cytotoxic assays, ICS, and multimer staining of RhoC-specific CD8 T-cells (in patients carrying the HLA-A03.01 molecules).

10.4 Demographic and Other Baseline Characteristics

10.4.1 Demographic and Baseline Data

The following demographic and baseline data will be collected at the screening visit/Visit 1b:

- Date of birth
- Sex
- Ethnicity
- Weight and height
- Physical Examination

10.4.2 Medical History

Medical history will be recorded at the screening visit/Visit 1a (and confirmed at Visit 1b).

10.4.3 Prior and Concomitant Medication

Prior medications taken within 1 month prior to screening will be recorded at the screening visit.

Concomitant medication will be recorded on the concomitant medication log/page of the CRF throughout the study.

10.5 Safety Assessments

10.5.1 Safety Variables

The following safety variables will be measured:

- AEs
- Vital signs
- Physical examination
- ECG
- Laboratory safety assessments

10.5.2 Adverse Events

AEs will be recorded during the study period from the first dose of vaccine to the completion of the follow-up Visit 13. During the follow-up phase visit 14-17 only study/protocol related AEs and SAEs suspected to be related to the vaccination will be recorded. For further information of definitions and reporting of AEs and SAEs, see Section 11.

10.5.3 Physical Examination

All patients will undergo a standard physical examination.

The timing of the assessments is described in Section 7.2.1 and Table 1.

10.5.4 Vital Signs

The following vital signs will be monitored as safety variables:

- Resting systolic and diastolic blood pressure (mmHg), after at least 5 minutes rest in supine position
- Resting pulse (beats per minute), after at least 5 minutes rest in supine position
- Body (e.g., tympanic or temporal artery) temperature (°C)

The observed values will be recorded and assessed as “normal” or “abnormal”. Abnormal findings will be assessed as “clinically significant” or “not clinically significant”.

The timing of the assessments is described in Section 7.2.1 and Table 1.

10.5.5 Electrocardiogram

A standard 12-lead ECG will be recorded after the patient has rested in supine position for at least 5 minutes.

The observed values will be recorded and assessed as “normal” or “abnormal”. Abnormal findings will be assessed as “clinically significant” or “not clinically significant”.

The timing of the assessments is described in Section 7.2.1 and Table 1.

10.5.6 Laboratory Safety Assessments

The laboratory safety analyses (haematology, clinical chemistry and urinalysis) will be performed by Department of Clinical Biochemistry at Bispebjerg Hospital. The analysis will be done immediately (typically on the day of collection) and any remaining material will be discarded immediately after analysis.

The following laboratory safety parameters will be measured (Table 3):

Table 3 Laboratory Safety Parameters

Category	Laboratory Parameter
Haematology	Haemoglobin, RBCs, haematocrit, leukocytes and differential counts, platelets (3 ml)
Clinical Chemistry	Sodium, potassium, calcium, creatinine, albumin, LDH, alkaline phosphatase, ASAT, ALAT, total bilirubin, CRP (4 ml) Blood glucose (3 ml, only at screening) HbA1c (3 ml, only at screening)
Serology	Hepatitis B core antibody, hepatitis B surface antigen, hepatitis C virus (4 ml, only at screening) HIV (4 ml, only at screening)
Urinalysis	Glucose, bilirubin, ketones, specific gravity, blood, pH, protein, urobilinogen, nitrite, leucocytes

The observed values will be recorded and assessed as “normal” or “abnormal”. Abnormal findings will be assessed as “clinically significant” or “not clinically significant”.

The timing of the assessments is described in Section 7.2.1 and Table 1.

10.5.7 Other Safety Measurements

Injection site reactions will be recorded.

10.6 PSA Measurements

PSA analysis will be performed by Department of Clinical Biochemistry at Bispebjerg Hospital. The analysis will be done immediately (typically on the day of collection) and any remaining material will be discarded immediately after analysis.

The timing of the assessments is described in Section 7.2.1 and Table 1. A volume of 4 mL will be collected at each time point.

10.7 Total Blood Volumes

The total volume of blood taken during the study will be approximately 1000 mL. This includes all safety laboratory assessments, cancer specific assessment (PSA) and immunological assessments.

For details of blood volumes, see Appendix B

The total amount of blood collected in this study corresponds to approximately to two blood donations during a period of approximately 20 months. This is considered acceptable. Furthermore, the haemoglobin, RBC count, and haematocrit are measured at frequent intervals during the study, allowing the investigator to take appropriate measures (e.g., to decide not to collect blood for lymphocyte evaluation at some visits) in case the patient shows early signs of anaemia.

10.8 Appropriateness of Measurements

Standardised methods for measurements of safety variables and analysis of PSA (such as Electrochemiluminescence Immunoassay will be used. For analysis of immune response, state of the art assays and methodology will be used as described in Section 10.3.

11 ADVERSE EVENTS

11.1 Definitions

11.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical trial patient administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

Comment: An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an IMP, whether or not considered related to the IMP.

11.1.2 Adverse Reaction

All untoward and unintended responses to an IMP related to any dose administered.

Comment: All AEs judged by either the Investigator or the Sponsor as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

11.1.3 Unexpected Adverse Reaction

An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator's Brochure for an unauthorised IMP or summary of product characteristics for an authorised product).

Comment: When the outcome of the adverse reaction is not consistent with the applicable product information this adverse reaction should be considered as unexpected.

11.1.4 Serious Adverse Event

Any untoward medicinal occurrence or effect that at any dose:

- Results in death
- Is life-threatening
- Requires hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect

Comments: Life-threatening in the definition of an SAE or serious adverse reaction (SAR) refers to an event in which the patient was at risk of death at the time of event; it does not refer to an event which hypothetically might have caused death if it was more severe.

Medical judgement should be exercised in deciding whether an adverse event/reaction is serious in other situations. Important adverse events/reactions that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

11.1.5 Suspected Unexpected Serious Adverse Reaction

A suspected unexpected serious adverse reaction (SUSAR) is an AE that is assessed as serious, related and unexpected.

11.2 Reporting of Adverse Events

All study patients will be carefully monitored for the occurrence of AEs during the study period from the first vaccination to the completion of the follow-up visit (Visit 13). During the follow-up phase visit 14-17 only study/protocol related AEs and SAEs suspected to be related to the vaccination will be recorded The Investigator will collect AEs with a non-leading question such as “have you experienced any new health problems or worsening of existing conditions” as well as reporting events directly observed or spontaneously volunteered by patients.

Clearly related signs, symptoms and abnormal diagnostic procedure results should be grouped together and reported as a single diagnosis or syndrome whenever possible.

All AEs including but not limited to events reported by the patient, or reported in answer to an open question by the Investigator or member of this team, which fall into any of the above definitions must be recorded as an AE in the CRF and should include the following information:

- Brief description of the event (diagnosis)
- Start date (and time, if relevant)
- Stop date (and time, if relevant) (or resolution)
- Severity
- Action taken regarding study drug
- Opinion on causality
- Seriousness
- Outcome

Severity

Grading of Adverse Events

The severity of an AE refers to the intensity of the reaction.

Events are graded using CTCAE version 4.03 [43].

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL)[1].
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL[2].
- Grade 4: Life-threatening consequences; urgent intervention indicated.

[1] Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
[2] Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

- Grade 5: Death related to AE

Patients experiencing AEs will be monitored with the relevant clinical evaluations and laboratory investigations assessed by the attending physician. All AEs must be monitored until satisfactory restitution or stabilization. Results of the monitoring must be recorded in the patient chart and electronic CRF (eCRF).

If an AE changes in severity, the worst severity should be reported.

Causality

Causality will be assessed as:

Probable

A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the IMP, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (de-challenge). Re-challenge information is not required to fulfil this definition.

Possible

A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the IMP, but which could also be explained by concurrent disease or other drugs or chemicals. Information on IMP withdrawal may be lacking or unclear.

Unlikely

A clinical event, including laboratory test abnormality, with a temporal relationship to IMP administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations.

Follow-up of Patients after Adverse Events

Any AE that is ongoing when the patient is withdrawn from the study should be followed up until the AE is resolved or the Investigator decides that the AE is stable and needs no further follow-up. The date when the Investigator considers one of these outcomes to have occurred for the last ongoing AE for a patient will be considered the last visit for this patient, and the outcome should be recorded in the CRF.

Abnormal Laboratory Values/Vital Signs

The reporting of abnormalities as both laboratory/vital signs findings and AEs should be avoided.

An asymptomatic abnormal laboratory/vital sign finding should only be reported as an AE if it is clinically significant, if it fulfils the criteria for an SAE or if it causes the patient to discontinue the study.

If an abnormal laboratory/vital sign value is associated with clinical signs and symptoms, the sign/symptom should be reported as an AE and the associated laboratory/vital sign result should be considered additional information.

11.3 Reporting of Serious Adverse Events

The Investigator is responsible for ensuring that all SAEs are reported to the Sponsor immediately, but in any event no later than 24 hours of any site staff becoming aware of the event. Initial reports should be followed as soon as possible by detailed written reports. The

initial and follow-up reports should identify patients by unique code numbers assigned in the study. The patients' names, personal identification numbers, and/or addresses must not be included. The following information is **mandatory** for the initial report:

- Patient study ID
- Study treatment (blinded, if applicable)
- Start date (time, if relevant) of the study treatment
- Brief description of the event (diagnosis)
- Start date (time, if relevant) of the event
- Seriousness criteria
- Causality assessment

For reported deaths, the Investigator should supply the Sponsor and the IEC (if applicable) with any additional requested information (e.g. autopsy reports and terminal medical reports).

SAE REPORTING CONTACT DETAILS

TFS

Drug Safety

Fax: +46 (0) 46 280 19 19

Phone: +46 (0) 46 280 18 00 (switchboard)

E-mail: safety.tfs@tfscro.com

Note: If there is local legislation requiring Investigators to report AEs to the CA or the IEC, the Investigator should also comply with this legislation. If any such reporting is planned, this must be stated in the SAE report, and once the reporting has been performed, a copy of the reporting documentation must be enclosed with the follow-up SAE report to the Sponsor.

11.4 Reporting of Suspected Unexpected Serious Adverse Reactions

The Sponsor is responsible for informing the CA(s), the European Medicines Agency and IEC(s) of any individual case reports of SAEs that are determined to be reportable by the Sponsor (i.e. SUSARs). The Investigator will ensure that all relevant information is provided to the Sponsor to allow the Sponsor to meet their obligations to report the SUSAR to the CA and IEC. For a SUSAR that is fatal or life-threatening, this should be reported as soon as possible and not later than 7 days after the Sponsor was first advised, for any other SUSAR this should be within 15 days.

11.5 Adverse Events of Special Interest

Treatment is terminated if AEs occur that are of a degree that makes completion of the study impossible, e.g., treatment related grade 3 or 4 toxicity in accordance with CTCAE (except for transient (< 48 hours) fever, fatigue, headache, flue like symptoms).

11.6 Precautions/Overdose

There is no known antidote to RV001V. In the event of overdose symptomatic management is indicated.

11.7 Pregnancy

Male patients will be instructed to notify the Investigator immediately if their partner becomes pregnant.

A pregnancy as such is not an AE, unless there is a possibility that the IMP has interfered with the efficiency of any contraceptive measures. However, the Investigator should report pregnancies according to the procedures and timelines described for reporting of SAEs (Section 11.3). The pregnancy report form should be used instead of the SAE form.

The pregnant partner will be followed until the end of the pregnancy. Any complication during the pregnancy should preferably be reported as an AE. The outcome of the pregnancy must be reported on the pregnancy report form. Any spontaneous abortion, stillbirth, birth defect/congenital anomaly, death, or other serious infant condition must be reported and followed up as an SAE.

12 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

12.1 Statistical and Analytical Plans

The first version of the Statistical Analysis Plan (SAP) for this open-label study will be finalized before the first vaccination. The first SAP version can either be a stand-alone document or this section of the study protocol. Subsequent versions of the SAP may be developed during the course of the study. The final SAP will be finalised before locking the study database.

12.1.1 Data Sets to be Analysed

All patients who received at least one vaccination will be included in the statistical analyses. A subset analysis will be done on selected variables among the patients who received 3, 6 and all 11 vaccinations. Additional subgroup analyses may be performed as defined in the SAP.

12.1.2 Statistical Issues

Level of Significance, Multiple Comparisons and Multiplicity

No formal statistical testing is planned. The confidence intervals will be calculated with 2-sided 95% coverage.

Adjustment for Covariates

No adjustments for covariates are planned.

Handling of Dropouts and Missing Data

The study data will be primarily evaluated as observed cases. For continuous endpoints, likelihood-based modelling approach, e.g. Mixed Model for Repeated Measures (MMRM) may be used to handle incomplete data.

Examination of Subgroups

Subgroup analyses may be defined in the SAP.

Data Monitoring

This is an open label study.

12.1.3 Summary Statistics

In general, data will be summarised by means of summary statistics. Continuous data will be presented with the number of observations, mean value, standard deviation, standard error of the mean, minimum, Q1, median, Q3 and maximum value. Categorical data will be presented as counts and percentages. The data will be presented by visit.

Individual patient data will be listed.

12.1.4 Analysis of the Primary Endpoint

The primary endpoint of the study is the proportion of patients developing treatment related grade 3, 4 or 5 toxicity in accordance with CTCAE, with the exception of fever and local reactions that can be attributed to the vaccine. The study will be terminated if this toxicity is

observed in more than one of the first 6 patients, i.e. in <33.3% of the patients. The study will be considered meeting the primary endpoint if the proportion of patients with this toxicity at the end of the study is not higher than 33.3%. The primary endpoint will be evaluated by calculating the percentages of patients with the toxicity as defined above out of all patients who got at least one vaccination. In addition to the percentage, an exact 95% confidence interval will be calculated using the Clopper-Pearson method.

The tolerability will be evaluated by number of patients who discontinue the study treatment prematurely. The following will be reported:

- Number and proportion of patients who discontinue the study prematurely
- Reasons for premature discontinuation
- Number of vaccination received prior to the premature discontinuation
- The time from the first vaccination to premature discontinuation illustrated with a Kaplan-Meier plot. In this analysis, the patients who do not discontinue the study prematurely will be censored at the time of the last visit.

AEs will be captured and graded by CTCAE. All AEs captured during the study will be coded with the Medical Dictionary for Regulatory Activities (MedDRA). TEAEs are defined as signs or symptoms that emerge after the first vaccination, including those signs and symptoms that are absent pre-vaccination or that have worsened relative to the pre-vaccination status. TEAEs will be summarized by MedDRA system organ class (SOC) and preferred term (PT). Both event and patient counts, where applicable, will be summarized. The counts will be complemented by percentages calculated for the patient counts unless otherwise specified. The following summaries will be produced:

- An overall summary of the number and percentage of patients reporting TEAEs and the number of TEAE events, drug-related TEAEs, severe TEAEs, serious TEAEs, TEAEs leading to premature study treatment discontinuation and TEAEs leading to death
- TEAEs by SOC and PT, both as event and patient counts
- TEAEs by PT, both as event and patient counts, sorted in descending frequency of the events
- TEAEs by SOC, PT, and relationship to study treatment, as event counts only (percentages calculated as % of total number of reported events)
- TEAEs by SOC, PT, and severity, as event counts only (percentages calculated as % of total number of reported events)
- Serious TEAEs by SOC and PT, both as event and patient counts
- TEAEs leading to study treatment discontinuation, both as event and patient counts
- TEAEs leading to death, both as event and patient counts

The time course of the most frequent TEAEs will be described as a function of the number of vaccinations / as a function of time:

- The time to first onset of the most frequent TEAEs (on PT level) will be classified based on the number of vaccinations received prior to the onset (after the first and before the second vaccination, after the second and prior to the third vaccination, etc.). In addition, the time curves will be illustrated with Kaplan-Meier plots, in which the patients without the TEAE in question will be censored at the time of the last visit.

- Increases or decreases of the TEAE incidence over time will be illustrated with plots of mean cumulative frequencies of the TEAEs as function of time, calculated using Proportional Means Regression model for recurrent data, allowing the count of multiple events per patient. This analysis will be performed for the most frequent TEAEs (on PT level, for event level data).

The pre-vaccination vital signs at baseline, each vaccination and follow-up visit and changes from baseline to each vaccination and follow-up visit will be summarized with descriptive statistics. In addition, the changes from pre-vaccination to post-vaccination will be summarized for visits at which post-vaccination vital signs are captured.

The physical examination findings will be summarized by body system using shift tables. The shift table includes columns for baseline data (normal or abnormal) and rows for each post-baseline visit (normal or abnormal).

All collected ECG data will be summarized. In addition, the corrected QT (QTc) times will be derived using both the formula by Bazett ($QT * [60/HR]^{1/2}$) and by Fridericia ($QT * [60/HR]^{1/3}$). The pre-vaccination ECG parameters at baseline, each vaccination and follow-up visit and changes from baseline to each vaccination and follow-up visit will be summarized with descriptive statistics. In addition, the changes from pre-vaccination to post-vaccination will be summarized for visits at which post-vaccination ECG data are captured.

In addition, the following categorical analyses will be performed at each visit/time point:

- Clinical interpretation: proportion of ECGs which are normal, abnormal and not clinically significant or abnormal and clinically significant (as shift table by visit)
- Proportion of clinically significantly abnormal QTc values, defined as values ≤450 ms, >450 – 480 ms, >480 – 500 ms, and >500 ms (as shift tables by visit, separately for QTcB and QTcF)
- Proportion of clinically significantly abnormal changes in QTc values from baseline, defined as changes >30 msec or >60 msec (as frequency tables by visit, separately for QTcB and QTcF).

The safety laboratory at baseline, each vaccination and follow-up visit and changes from baseline to each vaccination and follow-up visit will be summarized with descriptive statistics. The number and proportion of normal/abnormal values will be summarized with shift tables.

12.1.5 Analysis of Secondary Endpoints

For the evaluation of the immunological effect of the vaccinations, patients will be followed with in-vitro analysis of antigen specific T-cell reactivity against RhoC and possibly other antigens as well.

To monitor specific RV001V T-cells in treated patients, and thereby the immunological response, the immunogenicity of RV001 in patients is determined by measuring anti-vaccine T cells before, during and after vaccination and by evaluating the changes from baseline (last assessment before the first vaccination) to each vaccination and follow-up visit. These values will be summarized with descriptive statistics by visit. In addition, MMRM may be used to estimate the changes from baseline with 95% confidence intervals.

All patients will be tested using IFNgamma ELIspot assay. For ELIspot positive patients, ICS staining for dissecting CD4/CD8 responses and cytokine production profile will be performed. The ICS markers will be CD4/CD8/dead cell dye/IL-2/IFNg/TNF/CD154/CD107a. For minimum all HLA-A3 positive patients, multimer staining for assessing specific RV001V cells

will be performed. For selected patients, advanced functional analysis of RV001V specific T-cells may be performed.

If feasible, the ICS markers at baseline, each vaccination and follow-up visit will be summarized with descriptive statistics. In addition, the changes from baseline will be summarized.

In addition, thresholds for immunological response may be defined and the proportions of patients above or below the thresholds will be summarized by visit.

12.1.6 Analysis of Exploratory Endpoints

The PSA levels will be evaluated by:

- Among the patients with measurable PSA levels at baseline: the PSA values at baseline, at each vaccination and follow-up visit will be summarized with descriptive statistics. In addition, the changes from baseline will be summarized. If feasible, the changes from baseline may be estimated with MMRM, including 95% confidence intervals for the changes.
- Number of patients with measurable PSA levels at baseline and at each vaccination and follow-up visit will be summarized with a shift table.

The potential association between the PSA levels and immunological response will be evaluated by:

- Anti-vaccine T cell levels classified by the PSA status (measurable or non-measurable) at baseline and at each vaccination and follow-up visit will be summarized with descriptive statistics
- Correlation of changes from baseline in anti-vaccine T cell levels and PSA levels among the patients with measurable PSA levels at baseline will be illustrated with scatter plots and by calculating correlation coefficients (e.g. Spearman's correlation coefficient).

The PFS and OS will be evaluated as time from baseline (time of the first vaccination) to the event. For PFS, disease progression or death for any reason will be defined as an event. For OS, death for any reason is defined as an event. The data from the patients without an event will be censored at the last time when the patient is known to be free of disease progression or alive. The data will be summarized as Kaplan-Meier plots.

12.1.7 Demographic and Other Baseline Characteristics

Patient disposition, demographic and other baseline data will be presented using summary statistics.

12.1.8 Exposure to Treatment

Exposure to the study treatment will be summarized as number of vaccinations received. The data will be summarized as cumulative number of patients receiving at least 1, 2, and so forth up to 11 vaccinations.

In addition, the cumulative dose vaccinated will be summarized.

12.1.9 Concomitant Treatment

Concomitant medication and concomitant therapy will be summarised as number of patients being treated with each type of medication/therapy classified according to ATC level 1 and World Health Organization (WHO) Drug Dictionary preferred term.

12.2 Determination of Sample Size

The study is designed as a phase I/IIa trial and the primary objective is to determine safety and tolerability related to the treatment. A 3+3 design is a standard design to assess the safety and tolerability in dose-response oncology studies. As the present study is a vaccination study not assessing dose-response and only one dose level will be tested, additional patients are included to improve the precision of the evaluation of the safety and tolerability. It is judged that 10 patients is sufficient for such evaluation.

The expected variability of the measurements associated with the primary objective is unknown. The number of patients needed is therefore uncertain. It is judged that 10 patients (fully evaluable) is a relevant number to meet the primary objective of the study.

For evaluation of immune response of the treatment, the variability is unknown. An adequate sample size to provide sufficient information can therefore not be estimated, however from previous experiences of early clinical trials, it is found appropriate to evaluate up to 20 patients. Given a dropout rate of some 20% the total number of patients that may be included is set to 25.

All patients, who are included and treated with at least one dose of RV001V and in accordance with the protocol, will be part of the safety analyses.

12.3 Procedures for Reporting any Deviation(s) from the Original Statistical Analysis Plan

Any deviation(s) from the original statistical analysis plan (as described in the study protocol or in the SAP) will be described and justified in a protocol amendment and/or in a revised SAP and/or in the final report, as appropriate.

12.4 Interim Analysis

A formal interim analysis is not planned for this study. Evaluation of safety will be conducted as described in Section 7.1.1. Results from immune analysis will be evaluated during the study.

13 INVESTIGATOR/SPONSOR RESPONSIBILITIES

13.1 Ethics

13.1.1 Independent Ethics Committee (IEC)

This protocol and any amendments will be submitted to a properly constituted IEC, in accordance with the International Conference on Harmonisation (ICH) guidelines, the applicable European Directives and local legal requirements, for approval/favourable opinion of the study. Approval/favourable opinion must be obtained in writing before the first patient can be recruited.

13.1.2 Ethical Conduct of the Study

The study will be conducted in compliance with the protocol, regulatory requirements, good clinical practice (GCP) and the ethical principles of the latest revision of the Declaration of Helsinki as adopted by the World Medical Association.

13.1.3 Patient Information and Consent (Appendix C)

All patients will receive written and verbal information regarding the study at a prior interview. This information will emphasise that participation in the study is voluntary and that the patient may withdraw from the study at any time and for any reason. All patients will be given the opportunity to ask questions about the study and will be given sufficient time to decide whether to participate in the study.

Before any study-related procedures the informed consent form will be signed and personally dated by the patient (or their legally acceptable representative and/or witness, as applicable) and by the person who conducted the informed consent discussion.

A copy of the patient information including the signed consent form will be provided to the patient.

13.2 Patient Records and Source Data

No data will be recorded directly in the CRF. The origin of source data in the study will be specified for the Phase I unit and the hospital in separate documents (“Origin of Source Data”).

It is the responsibility of the Investigator to record essential information in the medical records in accordance with national regulations and requirements.

The following information should be included as a minimum in the medical records at the hospital:

- A statement that the patient is in a clinical study
- The identity of the study e.g. Study code
- That informed consent was obtained and the date

In addition the investigator at the hospital will fill in a study specific Form for determination of cancer status (stable disease or progression). A copy of the Form will be kept at the hospital and the original will be sent to the phase I unit.

The following information should be included as a minimum in the medical records at the Phase I unit:

- A statement that the patient is in a clinical study
- The identity of the study e.g. Study code
- Patient screening number and/or patient number
- That informed consent was obtained at the hospital and the date (verified by a copy of the signed consent Form)
- Dates of all visits during the study period
- Any information relating to AEs
- All treatments and medications prescribed/administered (including dosage)
- Date of study termination
- Patient health service identification number (CPR)

In addition to the medical records the phase I unit will use “work sheets” to record data. The Investigator is responsible for ensuring the accuracy, completeness, legibility and timeliness of the data recorded in the CRFs. Data reported in the CRF that are derived from source documents should be consistent with the source documents or the discrepancies should be explained. Signed sections of CRFs will be monitored and collected on a regular basis.

13.3 Access to Source Data and Documentation

The Investigator should guarantee access to source documents for the monitor and auditors as well as for inspection by appropriate regulatory agencies, and the IEC, if required. In addition, the monitor, the principal investigator at Zelo Phase I Unit, and the investigator at the Department of Urology at Rigshospitalet will have access to the patient’s entire medical records at the Department of Urology at Rigshospitalet. The aforementioned parties will also have access to all study-specific notes made about the patient in the study files at Zelo Phase I Unit.

13.4 Monitoring

The monitor will visit the study site (both clinics) on a regular basis to ensure that the study is conducted and documented in accordance with this protocol, ICH GCP guidelines, regulatory requirements and any study specific documents such as CRF completion guidelines.

Monitoring visits will be conducted to confirm that e.g.:

- The investigational team is adhering to the study protocol
- Informed consent has been obtained from all participants
- AEs have been reported as required
- Data are being accurately recorded in the CRFs
- IMP is being stored correctly and drug accountability is being performed on an on-going basis
- Facilities are, and remain, acceptable throughout the study

- The Investigator and the site are receiving sufficient information and support throughout the study

Moreover, during monitoring visits the data recorded in the CRFs, source documents and other study-related records will be compared against each other in order to ensure accurate data that reflect the actual existence of the patient in the study i.e. source data verification.

13.5 Data Management

Data management and handling of data will be conducted according to the study specific Data Management Plan with ICH guidelines and TFS standard operating procedures (SOPs).

Viedoc, an eCRF system, will be used to capture data from the study. Data entry will be performed by the study site personnel. Validation and data queries will be handled by the TFS Data Management Team. The data will be subjected to validation according to TFS SOPs in order to ensure accuracy in the collected eCRF data.

Changes to the data in the eCRF will be made at the site by the study site personnel. The eCRF will have an audit trail with appropriate functionality for data capture, tracking and documentation of any queries or changes. Electronic signatures will be used to lock the data and identify the person entering or changing the data.

Before database closure a reconciliation will be performed between the SAEs entered in the safety database and the study database. After database closure, the database will be exported as SAS® data sets.

Any deviations, i.e. discrepancies and additions from the process defined in the Data Management Plan, will be described in a study specific Data Management Report.

13.6 Quality Assurance and Audit

Audits or inspections, including source data verification, may be performed by representatives of TFS, the Sponsor, a CA and/or an IEC.

13.7 Record Retention

The Investigator/institution should maintain essential documents (as defined in ICH E6 GCP, Section 8) as required by the applicable regulatory requirement(s). The Investigator/institution should take measures to prevent accidental or premature destruction of the documents.

Essential documents should be retained according to applicable regulatory requirements of the country(ies) where the product is approved, and/or where the Sponsor intends to apply for approval.

It is the responsibility of the Sponsor to inform the Investigator/institution in writing as to when the documents no longer need to be retained.

13.8 Protocol Deviations

Deviations to the study protocol will be documented in a Protocol Deviation Log.

The classification of patients into protocol violators will be made during a meeting before database lock. The classification will be mutually agreed between the Sponsor and TFS. Listings will indicate the allocation of patients by analysis set and the number of patients per analysis set will be recorded in the clinical study report.

Any Serious Breaches that substantially affect the integrity or the safety of the patients or the scientific validity of the study will be reported to the relevant authorities in accordance with local regulatory requirements

13.9 Insurance

The Sponsor must provide insurance or must indemnify (legal and financial coverage) the Investigator/the institution against claims arising from the study, except for claims that arise from malpractice, negligence or non-compliance with the protocol.

Patients are covered by mandatory insurance as per the Danish law “Lov om klage- og erstatningsadgang inden for sundhedsvæsenet” (Complaint Access and Compensation Entitlement within the Health System), Consolidated Act No. 1113 of 07 July 2011, Chapter 4.

In addition, DanTrials has taken out an insurance at the Danish insurance company Tryg, covering injuries to research subjects caused by the study staff (“lægeansvarsforsikring”). Research subjects do not lose any legal rights or benefits they would otherwise have been entitled to by participating in the study.

13.10 Report and Publication

After completion of the study, a clinical study report will be prepared according to the ICH Guideline for Structure and Content of Clinical Study Reports (ICH E3). The report will be developed after all patients have performed the follow up visit 1 month after the last vaccination. An addendum to the report will be written when the last patient has had the last follow up visit (End of long-term follow up).

All publications and presentations must be based upon the clinical study report.

All information supplied by the Sponsor in connection with this study will remain the sole property of the Sponsor and is to be considered confidential information. No confidential information will be disclosed to others without obtaining prior written consent from the Sponsor and will not be used except in the performance of this study.

The sponsor recognises the traditional freedom of scientists to publish and present promptly the results of their studies and the sponsor is committed to presenting or publishing the results of this study, both if the results are positive, inconclusive, or negative. The presented or published data should be done using clean, checked and validated data only, in order to ensure the accuracy of the results.

If an Investigator wishes to publish results from this clinical study, written permission to publish must be obtained from the Sponsor in advance. As some of the information regarding the IMP and development activities at the Sponsor may be of a strictly confidential nature, the Sponsor must first review any publication manuscript prior to their submission to journals, meetings or conferences.

The Sponsor may choose to publish or present data from this study. If an Investigator is offered authorship, he/she will be asked to critically review the article for important intellectual content and approve the version to be published. The Sponsor has the right to use the results for registration and internal presentation and for promotion of the Sponsor’s commercial interests.

13.11 Subject Confidentiality

The Sponsor will ensure that the use and disclosure of protected health information obtained during this clinical study complies with the legislation related to the privacy and protection of personal information, among these the Danish law on protection of private information ("Lov om behandling af personoplysninger") which implements the EU directive 95/46/EC.

Individual patient medical information obtained as a result of this clinical study is considered confidential and disclosure of this information to third parties is prohibited. Such medical information may be given only after approval of the patient to the patient's physician or to other appropriate medical personnel responsible for the patient's well-being.

The Sponsor affirms the patient's right to protection against invasion of privacy. Only a patient identification number and/or initials will identify patient data retrieved by the Sponsor.

This is a private research project and therefore exempted from notification to the Danish Data Protection Agency.

14 REFERENCE LIST

1. Berke G. The binding and lysis of target cells by cytotoxic lymphocytes: molecular and cellular aspects. Annu. Rev. Immunol. 1994;12, 735–73.
2. Knuth A, Wölfel T, Meyer zum Büschenfelde, KH. T cell responses to human malignant tumours. Cancer Surv. 1992;13, 39–52.
3. Melief CJ, van Hall, T, Arens R, Ossendorp F. van der Burg SH. Therapeutic cancer vaccines. J. Clin. Invest. 2015;125, 1–12.
4. Ridley AJ. Rho GTPase signalling in cell migration. Curr. Opin. Cell Biol. 2015;36, 103–112.
5. Clark EA, Golub TR, Lander ES, Hynes RO. Genomic analysis of metastasis reveals an essential role for RhoC. Nature 2000;406, 30–33.
6. www.proteinatlas.org. The Human Protein Atlas.
7. Thumkeo D, Watanabe S, Narumiya S. European Journal of Cell Biology Physiological roles of Rho and Rho effectors in mammals. Eur. J. Cell Biol. 2013;92, 303–315.
8. Wenandy L, Sørensen RB, Svane IM, Thor Straten P, Andersen MH. RhoC a new target for therapeutic vaccination against metastatic cancer. Cancer Immunol Immunother. 2008;1871–78.
9. Islam M., Sharma S, Teknos TN. RhoC Regulates Cancer Stem Cells in Head and Neck Squamous Cell Carcinoma by Overexpressing IL-6 and Phosphorylation of STAT3. PLoS One. 2014 Feb 12;9(2):e88527. doi: 10.1371/journal.pone.0088527. eCollection 2014.
10. Rosenthal DT, Zhang J, Bao L, Zhu L, Wu Z, Toy K, et al. RhoC Impacts the Metastatic Potential and Abundance of Breast Cancer Stem Cells. PLoS One. 2012;7(7):e40979. doi: 10.1371/journal.pone.0040979. Epub 2012 Jul 23.
11. Villadangos JA. Antigen-specific impairment of adoptive T-cell therapy against cancer : players, mechanisms, solutions and a hypothesis. Immunol Rev. 2016 Jul;272(1):169-82.
12. Boone B, Van Gele M, Lambert J, Haspeslagh M, Brochez L. The role of RhoC in growth and metastatic capacity of melanoma. J Cutan Pathol. 2009 Jun;36(6):629-36. doi: 10.1111/j.1600-0560.2008.01117.x. Epub 2009 Feb 5
13. Reymond N, Im JH, Garg R, Cox S, Soyer M, Riou P, et al. RhoC and ROCKs regulate cancer cell interactions with endothelial cells. Mol Oncol. 2015 Jun;9(6):1043-55. doi: 10.1016/j.molonc.2015.01.004. Epub 2015 Jan 22.
14. Griner EM, Dancik GM, Costello JC, Owens C, Guin S, Edwards MG, et al.. RhoC Is an Unexpected Target of RhoGDI2 in Prevention of Lung Colonization of Bladder Cancer. Mol Cancer Res. 2015;13(3):483-92,
15. Wu Y, Tao Y, Chen Y, Xu W. RhoC regulates the proliferation of gastric cancer cells through interaction with IQGAP1. PLoS One. 2012;7(11):e48917. doi: 10.1371/journal.pone.0048917. Epub 2012 Nov 7.
16. Paul D, Chanukuppa V, Reddy PJ, Taunk K, Adhav R, Srivastava S, et al. Global proteomic profiling identifies etoposide chemoresistant markers in non-small cell

lung carcinoma. J Proteomics. 2016; 138:95-105.

17. Kawata H, Kamiakito T, Omoto Y, Miyazaki C, Hozumi Y, Tanaka A. RhoC upregulation is correlated with reduced E-cadherin in human breast cancer specimens after chemotherapy and in human breast cancer MCF-7 cells. Horm Cancer. 2014;5(6):414-23.

18. van Doorn E, Liu H, Huckriede A, Hak E. Safety and tolerability evaluation of the use of Montanide ISA™51 as vaccine adjuvant: A systematic review. Hum Vaccin Immunother. 2016;12(1):159-69.

19. Bijker MS, van den Eeden SJ, Franken KL, Melief CJ, Offringa R, van der Burg SH. CD8+ CTL priming by exact peptide epitopes in incomplete Freund's adjuvant induces a vanishing CTL response, whereas long peptides induce sustained CTL reactivity. J. Immunol. 2007;179(8), 5033–40.

20. Bijker MS, van den Eeden SJ, Franken KL, Melief CJ, van der Burg SH, Offringa R. Superior induction of anti-tumor CTL immunity by extended peptide vaccines involves prolonged, DC-focused antigen presentation. Eur. J. Immunol. 2008:38(4), 1033–42.

21. Kenter GG, Welters MJ, Valentijn AR, Lowik MJ, Berends-van der Meer DM, Vloon AP. et al. Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. N. Engl. J. Med. 2009;361(19), 1838–47.

22. NORDCAN, Association of the Nordic Cancer Registries

http://www-dep.iarc.fr/NORDCAN/English/frame.asp

23. FB Thomsen, MK Mikkelsen, RB Hansen, AH Krug, A Glenthøj, P Stattin et al. Clinical characteristics and primary management of patients diagnosed with prostate cancer between 2007 and 2013: status from a Danish primary referral center. Acta Oncol. 2016 Jun 22:1-5

24. Dansk Urologisk Cancer Gruppe. Prostatacancer retningslinjer 2016. (2016).

http://ducg.dk/daproca-prostatacancer/kliniske-retningslinjer/

25. Vale CL, Burdett S, Rydzewska LH, Albiges L, Clarke NW, Fisher D, et al. Addition of docetaxel or bisphosphonates to standard of care in men with localised or metastatic, hormone-sensitive prostate cancer : a systematic review and meta-analyses of aggregate data. Lancet Oncol. 2016;17(2), 243–56.

26. James ND, Spears MR, Clarke NW, Dearnaley DP, De Bono JS, Gale J, et al. Survival with Newly Diagnosed Metastatic Prostate Cancer in the 'Docetaxel Era': Data from 917 Patients in the Control Arm of the STAMPEDE Trial (MRC PR08, CRUK / 06 / 019). Eur Urol. 2015 Jun;67(6):1028-38.

27. Ryan CJ, Smith MR, de Bono JS, Molina A, Logothetis CJ, de Souza P et al. Abiraterone in metastatic prostate cancer without previous chemotherapy. N Engl J Med. 2013 Jan 10;368(2):138-48.

28. Beer TM, Armstrong AJ, Rathkopf DE, Loriot Y, Sternberg CN, Higano CS et al. Enzalutamide in metastatic prostate cancer patients before chemotherapy. N Engl J Med. 2014 Jul 31;371(5):424-33.

29. Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, et al. Docetaxel plus Prednisone or Mitoxantrone plus Prednisone for Advanced Prostate Cancer. N Engl J Med. 2004 Oct 7;351(15):1502-12.)

30. de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, Chu L, et al. Abiraterone and Increased Survival in Metastatic Prostate Cancer. N Engl J Med. 2011 May

26;364(21):1995-2005.

31. Scher HI, Fizazi K, Saad F, Taplin ME, Sternberg CN, Miller K, et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med. 2012 Sep 27;367(13):1187-97.

32. de Bono JS1, Oudard S, Ozguroglu M, Hansen S, Machiels JP, Kocak I, et al. Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment : a randomised open-label trial. Lancet. 2010 Oct 2;376(9747):1147-54.

33. Parker C, Nilsson S, Heinrich D, Helle SI, O'Sullivan JM, Fosså SD, et al. Alpha emitter radium-223 and survival in metastatic prostate cancer N Engl J Med. 2013 Jul 18;369(3):213-23.

34. Marchand M, Weynants P, Rankin E, Arienti F, Belli F, Parmiani G, et al. Tumor regression responses in melanoma patients treated with a peptide encoded by gene MAGE-3. I Int J Cancer. 1995 Dec 11;63(6):883-5.

35. Guo C1, Manjili MH, Subjeck JR, Sarkar D, Fisher PB, Wang XY. Therapeutic cancer vaccines: Past, Present and Future. Adv Cancer Res. 2013;119: 421-475.

36. Wang M, Cao JX, Liu YS, Xu BL, Li D, Zhang XY,et al. Evaluation of tumour vaccine immunotherapy for the treatment of advanced non-small cell lung cancer: a systematic meta-analysis. BMJ Open 2015 Apr 14;5(4).

37. Banchereau J, Palucka AK, Dhodapkar M, Burkeholder S, Taquet N, Rolland A, et al. Immune and clinical responses in patients with metastatic melanoma to CD34(+) progenitor-derived dendritic cell vaccine. Cancer Res. 2001 Sep 1;61(17):6451-8

38. Luiten RM, Kueter EW, Mooi W, Gallee MP, Rankin EM, Gerritsen WR, et al. Immunogenicity, including vitiligo, and feasibility of vaccination with autologous GM-CSF-transduced tumor cells in metastatic melanoma patients. J Clin Oncol. 2005 Dec 10; 23(35):8978-91.

39. Rosenberg SA, Yang JC, Schwartzentruber DJ. Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. Nat Med. 1998 March ; 4(3): 321–327

40. Iversen TZ, Engell-Noerregaard L, Ellebaek E, Andersen R, Larsen SK, Bjoern J, et al. Long-lasting Disease Stabilization in the Absence of Toxicity in Metastatic Lung Cancer Patients Vaccinated with an Epitope Derived from Indoleamine 2,3 Dioxygenase. Clin Cancer Res;2013:20(1); 221–32.

41. Rahma OE, Gammoh E, Simon RM, Khleif SN. Is the "3.3" Dose-Escalation Phase I Clinical Trial Design Suitable for Therapeutic Cancer Vaccine Development? A Recommendation for Alternative Design. 2014. Clin Cancer Res; 20(18);4758-67

42. Simon R. Clinical Trial Designs for therapeutic cancer vaccines. Cancer Treat Res 2005;123, p. 339-350.

43. U.S. Department of health and human services, National Institutes of Health, N. C. I. Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. 2009, (2010).

15 SIGNATURES

This Clinical Study Protocol is approved by:

SPONSOR'S REPRESENTATIVE:

DATE: _____

Jens Kristensen, MD, PhD
Chief Medical Officer
RhoVac AB

PRINCIPAL INVESTIGATOR at Zelo Phase I Unit:

DATE: _____

Jesper Sonne, MD, DMSc
Zelo Phase I unit

PROJECT LEADER:

DATE: _____

Kerstin Danielson
RhoVac AB

STUDY PROTOCOL AUTHORS:

DATE: _____

Jens Kristensen,
Chief Medical Officer
RhoVac AB

DATE: _____

Catherine Heddle
Medical Writer
TFS

16 CLINICAL STUDY PROTOCOL AGREEMENT FORM

I have read the clinical study protocol entitled: A Phase I/II study of RV001V, a RhoC anticancer vaccine, against metastasis from solid tumours

and verified that it contains all necessary information for conducting the study.

I hereby confirm that:

- I have carefully read and understood this clinical study protocol
- my staff and I will conduct the study according to the study protocol and will comply with its requirements, including ethical and safety considerations.

I understand that, should the Sponsor decide to prematurely terminate or suspend the study for whatever reason, such decision will be communicated to me in writing. Conversely, if I decide to withdraw from execution of the study I will immediately communicate such a decision to the Sponsor.

I agree not to publish any part of the results of the study carried out under this clinical study protocol without consulting the Sponsor.

Principal Investigator/Investigator: _____

Date: _____

Signature: _____

17 APPENDICES

Appendix A: ECOG Function/Performance Status

Appendix B: Sampling Blood Volumes

Appendix C: Informed Consent Form

Appendix D: Financial Arrangements