RESEARCH PROTOCOL

iHIVARNA phase IIa

A phase IIa randomized, placebo controlled, double blinded study to evaluate the safety and immunogenicity of iHIVARNA-01 in chronically HIV-infected patients under stable combined antiretroviral therapy.

(14-APR-2017)

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PROTOCOL TITLE 'iHIVARNA phase IIa'

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	ABR form, General Assessment and Registration form (application form required for submission to accredited Ethics Committee; In Dutch, ABR = Algemene Beoordeling en Registratie)
AE	Adverse Event
APC	Antigen Presenting Cells
AR	Adverse Reaction
ATI	Antiretroviral Treatment Interruption
CA	Competent Authority
CA-HIV	HIV Capacity Assay
CA RNA	Cell-associated RNA
cART	Combined anti-retroviral therapy
CCMO	Central Committee on Research Involving Human Subjects (in Dutch: Centrale Commissie Mensgebonden Onderzoek)
CD40L	CD (cluster of differentiation) 40 ligand
CD70	Cluster of differentiation 70
(e)CRF	(electronic) Case Report Form
CRO	Clinical Research Organization
CV	Curriculum Vitae
DC	Dendritic Cells
DSMB	Data Safety Monitoring Board
EDTA	Ethylenediaminetetraacetic acid
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
Fluc	Firefly luciferase
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HBsAg	Hepatitis B surface antigen
HCP	Hospital Clinic Provincial
HLA	Human Leukocyte Antigen
IB	Investigator’s Brochure
IC(F)	Informed Consent (Form)
ICS	Intracellular cytokine-staining
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
ITT	Intention to treat

IUD	Intrauterine Device (contraceptive device containing copper or levonorgestrel)
IUS	Intrauterine System (see: IUD)
METC	Medical research ethics committee (MREC) (in Dutch: Medisch Ethische Toetsing Commissie (METC)
mRNA	Messenger Ribonucleic Acid
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NSAID	Nonsteroidal anti-inflammatory drug
PI	Protease Inhibitor
PrEP	Pre-Exposure Prophylaxis
PVL	Plasma Viral Load
(S)AE	(Serious) Adverse Event
SOP	Standard Operating Procedure
Sponsor	Party that commissions the organisation or performance of the research, e.g. an academic hospital. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
caTLR4	Constitutively active Toll-Like Receptor 4
UAR	Unexpected Adverse Reaction
WBP	Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)
WFI	Water for injection
WMO	Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen)

SUMMARY

Rationale: iHIVARNA-01 is a novel therapeutic vaccine for the treatment of HIV-1-infected patients based on in vivo modification of DCs. It consists of HIVACAT-TriMix: mRNA encoding a mixture of APC activation molecules (CD40L, a constitutively active variant of TLR4 and CD70) and the HIV target antigens contained in HIVACAT to be administered through the intranodal route. iHIVARNA-01 aims to achieve the ‘functional cure’ of HIV infection, i.e. controlling viral replication in the absence of anti-retroviral therapy.

Objective: To evaluate the safety and immunogenicity of iHIVARNA-01 as a new therapeutic vaccine in HIV infected patients.

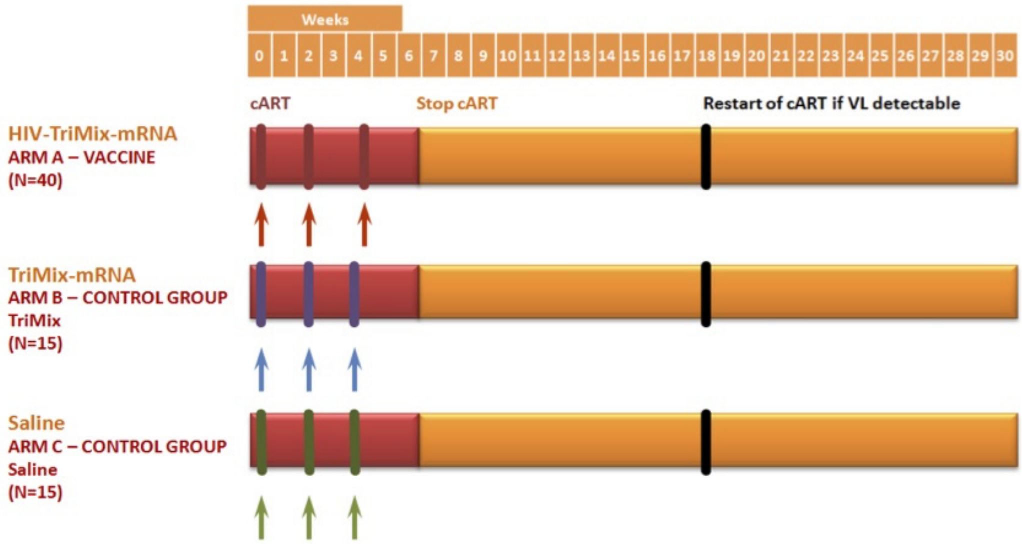
Study design and duration: Phase IIa, multicentre double-blind placebo controlled intervention study. Each patient will be followed for 30 weeks. The study duration will be 38 weeks from inclusion of the first patient.

Sites: Erasmus MC, Rotterdam The Netherlands (sponsor), Hospital Clínic de Barcelona and Institut de Recerca de la Sida – Caixa, Barcelona, Spain, Instituut voor Tropische Geneeskunde Antwerp, Belgium and Vrije Universiteit Brussel/UZ Brussel, Belgium

Study population: Chronically HIV-1- infected patients under stable cART with plasma viral load (pVL) ≤ 50 copies/ml and stable CD4+ T-cell counts ≥ 450/µl, aged 18 years or above.

Sample size: after recruitment and screening, 70 patients will be included and randomized to one of the study-arms.

Intervention: One group (n=40) receives the HIVACAT-TriMix (300 microgram TriMix + 900 microgram HIVACAT) vaccine intranodally on three occasions with a two-week interval. One control group (n=15) receives TriMix only (300 microgram TriMix) and one group (n=15) receives saline intranodally on three occasions with a two-week interval. Two weeks after the last vaccination cART treatment will be interrupted. If plasma virus is detectable, cART will be re-initiated twelve weeks after treatment interruption. cART can always be re-initiated for medical reasons, as judged by the clinical investigator.



Figure; Study design and intervention scheme (arrows indicate intranodal vaccination)

Main study parameters/endpoints:

The primary endpoints of the study are:

- safety and tolerability, as measured by the total number of adverse events.
- immunogenicity of an immunization schedule with HIVACAT-TriMix as measured by changes in frequency of the HIV-specific T-cell responses between baseline and 2 and 14 weeks after the last injection (i.e. weeks 6 and 18 of the study) as compared to both control groups (immunized with TriMix or placebo only)

The secondary endpoints are:

- magnitude and the kinetics of the HIV-specific CD4+ and CD8+ T cell responses after immunization (ELISPOT) as defined by the increase in the number of spot forming units from baseline and if positive the number of poly-functional T cells as determined by intracellular cytokine staining (ICS) at weeks 6 and 30.
- time until viral rebound after discontinuation at week 6.
- plasma viral load in vivo after analytical treatment interruption (ATI)
- functional cure: proportion of patients with viral load below detectable level after ATI
- primary immune response against vaccine: Change in frequency of at least $0.7\log^{10}$ HIV-specific T-cell responses between baseline and week 6
- the in vivo vaccine-induced capacity of ex-vivo CD8 T cells to suppress HIV growth in autologous CD4 T cells i.e. “CD8 T cell mediated viral suppression”
- effect on reservoir as measured by changes in the proviral DNA copy numbers per million cells and the intracellular viral RNA copy numbers per million cells during and after immunization.

- viral immune escape: change in % mutated epitopes from pre-cART to post-ATI (week 18)
- host protein mRNA expression profiles in whole blood

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Participation to the trial will consist of 15 visits to the hospital for a estimated total of 7 hours for vaccinations and exams, during a 30-week period. During these visits blood will be drawn for various assays. A total of 926ml blood will be taken over a 30-week period (see appendix B).

Before inclusion a physical screening will be performed. The patient will be asked to fill in a diary card to record local and systemic adverse events during one week after each vaccination.

Discomfort can be experienced as a result of intranodal vaccination including: local injection site reactions: pain, itching, redness/discoloration or fluid/blood filled blisters at the injection site. Hard swelling in skin surface at or close to site. General/ systemic reactions may include: temperature rise, chills/rigors, malaise, tiredness muscle aches, headache, nausea or vomiting.

As a result of treatment interruption a viral rebound syndrome may be experienced, with symptoms similar to those of acute HIV infection. Symptoms may include fever, fatigue, pharyngitis, lymphadenopathy, rash and weight loss.

The administration of the investigational medicinal product (IMP) has been tested in the phase I clinical trial. Based on interim analysis of July 26, 2016: twenty-one volunteers were recruited in Hospital Clinic Provincial (HCP) in Barcelona from June 23, 2015 until March 29, 2016. No patients have discontinued the trial.

ARM I	n=3	100µg TriMix mRNA
ARM II	n=3	300µg TriMix mRNA
ARM III	n=3	600µg (300µg HIV mRNA +300µg TriMix mRNA)
ARM IV	n=6	900µg (600µg HIV mRNA + 300µg TriMix mRNA)
ARM V	n=6	1200 (900µg HIV mRNA + 300µg TriMix mRNA)

The mean and median age was 46 years (range 28-59). Five (24%) were female and 16 were male (76%). Overall, the vaccine was well tolerated. A total of 17 adverse events (AE) were reported during follow-up until July 12th, 2016 (4, 4, 5, 4 and 0 in each ARM respectively). Ten AE were grade 1, 6 grade 2 and 1 grade 3. Of these AE, 2 were definitely related (2 grade 1) and 5 were possibly related (2 grade 1, 3 grade 2) to the IMP.

Data handling and storage: According to international regulations, all data collected in the case report form (CRF) should be verifiable against the source documents. In addition, the sponsoring investigator will keep the trial master file that will contain all case report forms, data correction forms, templates, monitoring records and visit scheduling, regulatory filings (e.g. signed protocol, amendments, correspondence with Ethics Committee, approval, approved version of patient information sheet, signed patient consents, investigator agreement, regulatory agency authorization, etc.). These files must be kept in accordance with local regulations in each country.

Data monitoring: Data will be monitored by a CRO: CR^2O (Clinical Research Rotterdam B.V., Rotterdam, the Netherlands), based on the results of the monitoring plan. Visits to the clinical sites in The Netherlands, Belgium and Spain will be organized regularly.

Data safety monitoring board: A DSMB has been installed that will overview the results from safety monitoring of all 5 study sites.

Statistical analysis: Proportions will be compared between groups using logistic regression and continuous variables using linear or quantile regression. For longitudinal data, mixed effects models will be fitted. Time to event data will be presented by Kaplan-Meier curves and analysed using Cox proportional hazards models.

1. INTRODUCTION AND RATIONALE

The human immunodeficiency virus (HIV) is a single-stranded positive-sense RNA virus belonging to the lentivirus genus. It mainly infects human dendritic cells (DCs), macrophages and CD4+T cells where it integrates into the host genome after reverse transcription to double-stranded DNA. In the absence of antiretroviral therapy, infection progresses to acquired immunodeficiency syndrome (AIDS) as defined by progressive depletion of CD4+ T cells. Patients with advanced HIV infection have a CD4+ T-cell count below 50/mm3 and their median survival is between 12 to 18 months in the absence of antiretroviral therapy (1). The HIV pandemic has caused as much as 35 million deaths worldwide. As of December 2013, 35 million people were estimated to be infected with HIV, from which 23.5 million were living in Sub-Saharan regions (2).

Current therapy for HIV infection is based on combined anti-retroviral treatment (cART). HIV infected patients, which are currently treated with cART show a stabilized condition with no clinical progression. However, cART has to be maintained for life, since it is not able to eradicate the infection by itself (3). This represents very high costs for administrations together with difficulties for treatment adherence and widespread distribution, mainly in developing countries, but also in the developed world. Therefore, there is a clear unmet need regarding cost-effective and viable treatments for HIV-infected patients. Therapeutic vaccination has emerged as an attractive strategy aiming at achieving a 'functional cure', a situation in which the immune system is able to control viral replication without the need for cART.

In this regard, several antigenic molecules, administration routes and strategies have been employed, so far with limited success. The most promising results to date were obtained by Garcia et al. (4) (partner of the consortium iHIVARNA). In this clinical trial, HIV-infected patients were administered autologous dendritic cells pulsed with HIV viral particles, a strategy known as 'ex vivo modification of DCs'. The results of this clinical trial indicate that DC-based vaccines offer a promising strategy for therapeutic vaccination. DC-based vaccines rely on the ability of DCs to uptake and present antigens to CD4+ and CD8+ T cells, eliciting an immune response that should ultimately control viral replication by lysing infected cells. In fact, in a small percentage of individuals who are known to efficiently suppress viral replication in the absence of cART (known as 'elite controllers'), control of viral replication has been associated with effective CD8+T cell responses (5).

Although results obtained by Garcia et al. (4) demonstrated a reduction of 90-95% of pVL in HIV-infected patients, 'functional cure' (a 100% reduction of pVL) was not achieved. In addition, the use of ex vivo modification of DCs is a technical challenge for widespread use and a very costly strategy.

For that reason, iHIVARNA-01, which is the current product under development, is based on the so-called ‘in vivo modification of DCs’, in which the product is directly administered to the patient without the need to modify DCs ex-vivo.
The intended indication for iHIVARNA-01 is the therapeutic vaccination of HIV-infected patients. iHIVARNA-01 is a biological product that consists of naked mRNA to be administered through the intranodal route.
The product is a combination of mRNA sequences that fulfil two main objectives: 1) providing effective HIV antigens for specific T cell activation that will lead to protective immunity (HIVACAT mRNA sequence) and 2) providing adequate stimuli required for the activation of antigen presenting cells (APCs) (DCs) and co-activation of specific T cells (TriMix mRNA sequences) (6).

Thus, iHIVARNA-01 has been developed as a combination of two active substances:

- HIVACAT immunogen: confers specificity to key HIV regions designed to provide beneficial T cell immunity associated with viral control and ensuring broad HLA class I and class II coverage (7).
- TriMix: provides the necessary activation molecules to enhance antigen presentation by DCs and T cell activation. It is a combination of mRNA sequences corresponding to human CD40L, constitutively active TLR4 (caTLR4) and human CD70 (8)

The iHIVARNA-01 investigational product, specified as ratio TriMix:HIVACAT = 1:3 (300µg TriMix + 900µg HIVACAT in 200 µl water for injection (WFI), is the result of the Phase 1 iHIVARNA trial where iHIVARNA-01 was tested as three IMPs with mixed ratios of TriMix:HIVACAT (study) versus TriMix solution (control).

Data based on interim analysis of July 26, 2016: twenty-one volunteers were recruited in Hospital Clinic Provincial (HCP) in Barcelona from June 23, 2015 until March 29, 2016. No patients have discontinued the trial.

ARM I	n=3	100µg TriMix mRNA
ARM II	n=3	300µg TriMix mRNA
ARM III	n=3	600µg (300µg HIV mRNA +300µg TriMix mRNA)
ARM IV	n=6	900µg (600µg HIV mRNA + 300µg TriMix mRNA)
ARM V	n=6	1200 (900µg HIV mRNA + 300µg TriMix mRNA)

The mean and median age was 46 years (range 28-59). Five (24%) were female and 16 were male (76%). Overall, the vaccine was well tolerated. A total of 17 adverse events (AE) were reported during follow-up until July 12th, 2016 (4, 4, 5, 4 and 0 in each ARM respectively). Ten AE were grade 1, 6 grade 2 and 1 grade 3. Of these AE, 2 were definitely related (1 grade 1 and 1 grade 2) and 5 were possibly related (2 grade 1, 3 grade 2) to the IMP.

Table: Overview of all adverse events in phase I trial.

Time	ARM	Description	Casual relationship	Seriousnes
Before Immunizacion	I	Asthenia	Posible relationship	Grade 1
	II	Flu syndrome	No related	Grade 1
		Flu-like syndrome	Unknown	Grade 1
		Acute medium otitis	Unknown	Grade 2
	III	Palpitaciones	Unknown	Grade 1
		Naúseas	Posible relationship	Grade 2
		Asthenia	Posible relationship	Grade 2
		Esguince tobillo grado II	No related	Grade 3
	IV	Headache	Unknown	Grade 1
		Flu-like syndrome	Posible relationship	Grade 1
		Flu-like syndrome	Posible relationship	Grade 2
Weeks 0-2	I	Dizziness because painful vaccination	Obvious relationship	Grade 1
		Intense pain during vaccination	Obvious relationship	Grade 2
	III	Uretritis	No related	Grade 2
Weeks >2	I	Painful hemorroid	No related	Grade 1
	II	rash	Unknown	Grade 1
	IV	Herpes labial	No related	Grade 1

2. OBJECTIVES

Primary objectives:

- To evaluate the **safety and tolerability** of intranodal iHIVARNA-01 vaccination compared with TriMix or placebo, focusing on the nature, frequency and severity of local adverse events (pain, cutaneous reactions including induration) and systemic adverse events (temperature, chills, headache, nausea, vomiting, malaise, and myalgia).
- To evaluate the **immunogenicity** of an immunization schedule with HIVACAT-TriMix (iHIVARNA-01) to increase the frequency of HIV-specific T-cell responses between baseline and 2 and 14 weeks after the last injection (i.e. weeks 6 and 18 of the study) as compared to the control groups, immunized with TriMix-mRNA only or WFI only.

Secondary Objectives:

- To evaluate the magnitude and the kinetics of the HIV-specific CD4+ and CD8+ T cell responses generated by the immunization schedule in the 3 groups by **two methods** (**ELISPOT**, intra-cellular cytokine staining - **ICS**) at baseline and at weeks 6 and 30.
- To evaluate the ability of the immunization schedules to **prolong time until viral rebound** after discontinuation at W6 as compared to control groups TriMix or placebo.
- To evaluate the **suppressive effect on plasma viral load** in vivo after ATI from W6 to W18 compared to two control groups, receiving TriMix only or placebo.
- To assess the proportion of patients with control of viral load below detectable level 12 and 24 weeks after start ATI (**functional cure**).
- To evaluate the percentage of patients who generate a **primary immune response to previously not-recognized HIV peptides (as defined in 8.1.2)**
- To analyze induction or enhancement of the **CD8+ T-cell HIV suppressive capacity**.
- To evaluate the **effect on reservoir** as measured by proviral DNA and the intracellular viral RNA (unspliced and multiple spliced viral RNA).
- To detect **viral immune escape** by sequencing of the HIV and conducting sieve effect analyses in rebounding virus after cART interruption.
- To evaluate host protein **mRNA expression profiles** in whole blood at baseline and W6 and W18.
- To store samples for future research to relate gut microbiota composition and diversity to HIV immune status

3. STUDY DESIGN

This is a phase IIa, randomized, double-blind, placebo-controlled outpatient clinic based multicentre study in chronically HIV-1 infected patients under stable cART. Enrolled and randomized patients will be treated in 1 of the 3 study-arms, comprising 2 stages. Randomization will be stratified per center, in order for all centers to have an equal number of cases and controls.

Stage I: Immunization period from W0 to W6, while on cART. The immunization will be administered by intranodal injection. Patients will be randomized to receive:

- Arm A (study treatment: HIVACAT-TriMix)
  one injection of HIVACAT-TriMix at W0, W2 and W4 while on cART (n= 40)
- Arm B (controls TriMix)
  one injection of TriMix at W0, W2 and W4 while on cART (n= 15)
- Arm C (controls buffer solution/placebo)
  one injection of buffer solution at W0, W2, and W4 while on cART (n= 15)

Stage II: Post immunization and analytical treatment interruption (ATI) period from W6 to W18, off cART, patients with undetectable level of viral load at week 18 will remain off-cART up to week 30. At week 30 the study ends, if a patient still has an undetectable viral load, he or she may continue cART interruption in consultation with the treating physician. cART can be re-initiated for medical reasons at any time, as judged by the clinical investigator.

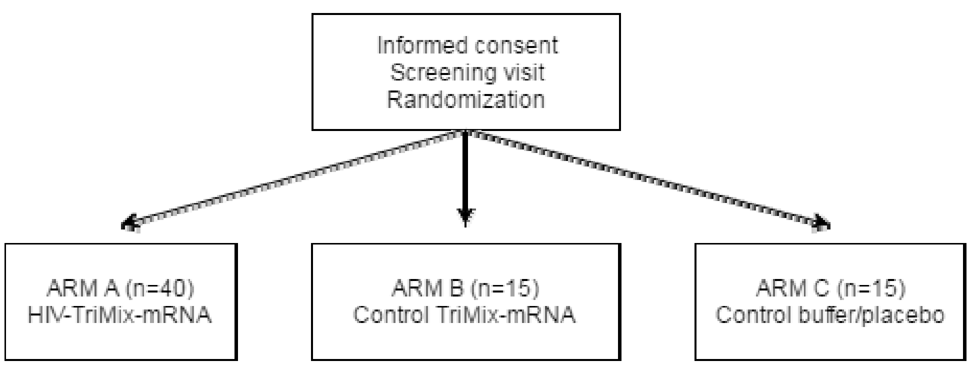
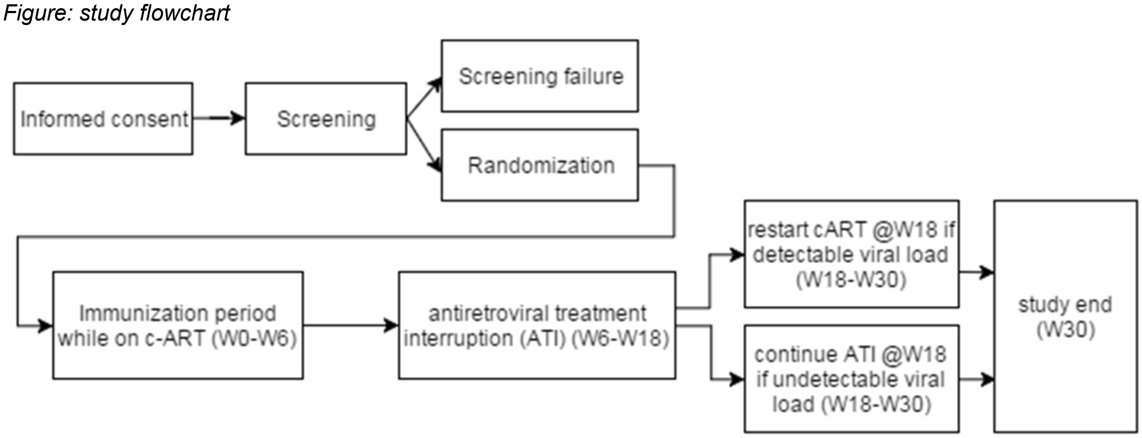


Figure: phase IIa treatment arms

Figure: study flowchart



4. STUDY POPULATION

4.1 Population (base)

The study will be conducted in chronically HIV-1 infected patients under stable cART with plasma viral load ≤ 50 copies/ml and stable CD4+ T-cell counts ≥ 450 cells. Patients will be recruited via the outpatient clinic in the five hospitals that participate in the study. Patients will be screened for eligibility after obtaining informed consent. A total of 70 patients will be randomized into 3 different groups (one study group (n=40) and two control groups (both n=15)). Every participating hospital will include 14 patients from their outpatient clinic-visiting group of 700-4000 HIV-1 infected patients.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a patient must meet all of the following criteria:

1. ≥ 18 years of age;
2. Voluntarily signed informed consent;
3. Proven HIV-1 infection (with documented antibodies against HIV-1 and a detectable plasma HIV-1 RNA before initiation of therapy);
4. On stable treatment with cART regimen (antiretroviral therapy consisting of at least three registered antiretroviral agents) for at least 3 years;
5. Nadir CD4+ ≥ 350 cells/µl (up to 2 occasional determinations ≤ 350 cells/µl are allowed);
6. Current CD4+ cell count ≥ 450 cells/µl;
7. HIV-RNA below 50 copies/mL in the last 6 months prior to randomization, during at least two measurements (occasional so called 'blips' ≤ 500 copies/mL are permitted);
8. If sexually active, willing to use a reliable method of reducing the risk of transmission to their sexual partners during treatment interruption (including PrEP).
   a. For heterosexually active female, using an effective method of contraception with partner (combined oral contraceptive pill; injectable or implanted contraceptive; IUD/IUS; consistent record with condoms; physiological or anatomical sterility (in self or partner) from 14 days prior to the first vaccination until 4 months after the last vaccination.
   b. For heterosexually active male, using an effective method of contraception with their partner from the first day of vaccination until 4 months after the last vaccination.

4.3 Exclusion criteria

A potential participant who meets any of the following criteria will be excluded from participation in this study:

1. Treatment with non-cART regimen prior to cART regimen;
2. Previous failure to antiretroviral and/or mutations conferring genotypic resistance to antiretroviral therapy;
3. Non-subtype B HIV infection;
4. Active Hepatitis B virus and/or Hepatitis C virus co-infection;
5. History of a CDC class C event (see appendix A);
6. Pregnant female (screened with a positive pregnancy test), lactating or intending to become pregnant during the study;
7. History of malignancy ≤ 30 days (extended period on the clinical assessment of the investigator) prior to screening;
8. Active infection with fever (38°C or above) ≤ 10 days of screening and/or first vaccination;
9. Therapy with immunomodulatory agents (e.g. systemic corticosteroids), including cytokines (e.g. IL-2), immunoglobulins and/or cytostatic chemotherapy ≤ 90 days prior to screening. This does not include seasonal influenza, hepatitis B and/or other travel related vaccines;
10. Congenital, acquired or induced coagulation disorders, such as thrombocytopenia (thrombocytes < 150x109/L) and/or current use of anti-coagulant medication (e.g. coumarins, inhibitors of Xa); Usage of NSAIDs (including acetylsalicylic acid) is allowed, however it is advised to interrupt therapy 10 days ahead of vaccination;
11. Usage of any investigational drug ≤ 90 days prior to study entry;
12. An employee of the investigator or study site, with direct involvement in the proposed study or other studies under the direction of that investigator or study site, or is a family member of an employee or the investigator
13. Any other condition, which, in the opinion of the investigator, may interfere with the evaluation of the study objectives

4.4 Sample size calculation

Assuming a standard deviation of 0.5 log10 in the change from baseline of cumulative **frequencies of HIV-specific PBMC**, a type 1 error of 5%, a power of 90%, as determined by a two-sided non parametric test (15% additional subjects), taking into account the weighting for case group ratio of more than 2:1, a sample of 40 in the HIVACAT-TriMix arm and 15 in each control arm will allow to detect a difference of **at least 0.7 log10 between any HIVACAT-TriMix arm and the controls arms**, allowing for 10% of non-assessable patients.

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

The Investigational Medicinal Product (IMP) under development is designated as iHIVARNA-01, and is a combination of two mRNA active substances, TriMix and HIVACAT to be administered through the intranodal route.

HIVACAT immunogen: confers specificity to key HIV regions designed to provide beneficial T cell immunity associated with control of viral replication and ensuring broad HLA class I and class II coverage.

TriMix: provides the necessary activation molecules to enhance antigen presentation by DCs and T cell activation. It is a combination of mRNA sequences corresponding to human CD40L, constitutively active TLR4 (caTLR4) and human CD70.
The optimal combination of HIVACAT and TriMix has been tested in a phase I clinical trial Combination of TriMix and HIVACAT within the manufacturing process of the IMP ensures it is done in adequate conditions to avoid mRNA degradation, avoiding extensive manipulation before use.

Table: IMP

Drug Substances	**Comments**	
TriMix	Mixture of mRNAs of human CD40L, TLR4ca and human CD70	
HIVACAT	mRNA encoding different HIV antigens	
Drug Products	**Group**	**Comments**
iHIVARNA-01 solution for injection (Arm V of phase I	Study product ARM A	300 µg TriMix + 900 µg HIVACAT in 200 µl WFI (ratio TriMix:HIVACAT = 1:3)
TriMix_300 solution for injection	Control: TriMix ARM B	Containing 300 µg of TriMix in 200 µl WFI
WFI for injection	Control: WFI ARM C	Containing 200 µl WFI

WFI: water for injection

Patients will receive 3 immunizations of the vaccine at weeks 0, 2 and 4.
The study treatment period will be 4 weeks. The total follow-up period will be 30 weeks.

5.2 Use of co-intervention

During the vaccination phase, i.e. until week 6, the current cART regimen should be continued. Sexually active subjects, are demanded to use a reliable method of reducing the risk of transmission to their sexual partners during treatment interruption (including PrEP). Heterosexually active female should use an effective method of contraception with their partner (combined oral contraceptive pill; injectable or implanted contraceptive; IUD/IUS; consistent record with condoms; physiological or anatomical sterility (in self or partner) from 14 days prior to the first vaccination until 4 months after the last. Heterosexually active male should use an effective method of contraception with their partner from the first day of vaccination until 4 months after the last vaccination

5.3 Escape medication

In the first 12 weeks of ATI, personal cART has to be re-started if warranted by a drop in CD4+ T cell counts below 50% of baseline or below 350 cells/µl and/or on the decision of the treating physician. If there are reasons to believe that the patient has had unprotected sex during the trial, it is advised that cART will not be interrupted or that cART will be re-started during ATI. At week 18, all patients with a pVL ≥50 cop/mL will be asked to restart cART. We have observed a rapid drop of PVL to undetectable level after cART re-initiation, in previous studies with treatment interruption, including the DCV-2 study and the DCTRN study. In a previous study Andres et al reported an increase in the viral reservoir after 12 weeks off cART (9). However, the viral reservoir drops to the same levels that the patients had before cART discontinuation, when cART is re-initiated subsequently (10,11) .

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

The main characteristics of iHIVARNA-01 are detailed in the Investigator's Brochure (IB). The drug products correspond to the active substance TriMix (control), a combination of TriMix and HIVACAT or a placebo; suspended in water for injection (WFI) and aliquoted in single dose vials. During aseptic vialing, for HIVACAT-TriMix and TriMix an extra volume of 10% is aliquoted per single dose at 220 µl per vial. Prior to injection, the vialed IMP should be reconstituted in 880 µl of Hartmann solution. 1mL of the product is administered. The IMP is produced according to the GMP guidelines. Briefly, the main steps in the aseptic production of the IMPs are:

1. mixing of the active pharmaceutical products (APIs) at pre-defined ratio's (where applicable, for iHIVARNA-01 products only),
2. purification of the mixed APIs and resuspension and
3. aseptic vialing and storage of the IMP.

More details on the manufacturing process of the active substances and drug products are given in the Investigator's Brochure.
Quality of production batches of active substances and drug products is ensured by evaluating its compliance with specifications as detailed in sections the Investigator's Brochure. eTheRNA is responsible for manufacture and supply of all clinical material according to Good Manufacturing Practice. iHIVARNA-01 will be packed and labelled by eTheRNA in accordance with European regulations.

6.2 Summary of findings from non-clinical studies

A detailed description of the pharmacodynamics for iHIVARNA-01 is provided in the IMPD section 4, Non Clinical studies, table 7 and IB.

Local biodistribution studies have been conducted in mice with eGFP and Fluc mRNAs to allow easy tracking of the product. Results from these studies indicate that intranodal injection of mRNA leads to efficient uptake and translation of injected mRNA by resident DCs (refer to IB; 4.2.1.1 Uptake and translation study of intranodally injected mRNA by resident DCs in mice), and that mRNA is stable for up to 12 hours after its administration, leading to efficient expression of functional protein up to 9 days. The only cell type observed to express functional protein derived from administered mRNA was resident DC (refer to IB; 4.2.1.2 Stability study of mRNA in vivo in mouse lymph nodes).

Immunogenicity studies were conducted in vitro and in vivo either with HIVACAT alone (refer to IB; 4.3.1 Studies with HIVACAT DNA), TriMix alone (refer to IB; 4.3.2 Studies with TriMix) or the combination of TriMix plus HIVACAT, designated as iHIVARNA-01 (refer to IB; 4.3.3 Studies with iHIVARNA-01).

<u>Immunogenicity studies with pDNA-HIVACAT</u>: some preclinical studies to test immunogenicity of HIVACAT were conducted with HIVACAT encoded in a DNA vector (named here as pDNA-HIVACAT) that was administered by the intramuscular route. These were initial studies that supported the immunogenicity of the antigens coded by the HIVACAT sequence. These studies were conducted in mice and Indian Rhesus macaques, and results indicated that HIVACAT immunogen is able to induce high frequencies of T cell responses, broadly distributed among all HIV-1 proteins included in the immunogen with central an effector memory phenotype (12) (refer to IB; sections 4.3.1.1 In vivo immune activation study of T cells by pDNA-HIVACAT in mice; 4.3.1.2 In vivo immune activation study of T cells by pDNA-HIVACAT in Indian Rhesus macaques).

<u>Immunogenicity studies with TriMix</u> have been performed in vitro and in vivo in mice. Results indicate that TriMix efficiently activates human DCs in vitro as observed by the upregulation of DCs maturation markers and IL-12 secretion, and promotes subsequent allogeneic T cell activation as measured by IFNγ secretion. (refer to IB; 4.3.2.1 In vitro maturation study of human DCs with TriMix (8).

In addition, it was shown in mice that intranodal administration of mu_TriMix does not impair in vivo uptake and subsequent translation of a co-administered mRNA (FLuc mRNA) by resident DCs (refer to IB; 4.3.2.2 In vivo maturation study of DCs with TriMix in mice (6), suggesting that co-administration of TriMix with a desired antigen is a valid strategy for adequate antigen presentation.

On the other hand, the enhancement effect of TriMix on the immunogenic potential of a given mRNA antigen has been demonstrated in a transgenic mice model bearing ovoalbumin-specific T cells. Results showed that intranodal co-administration of TriMix with OVA mRNA antigen enhances activation of specific T cell responses as compared to administration of the mRNA antigen alone (refer to IB; 4.3.2.3 In vivo effect of TriMix on specific T cell activation study in mice (6).

<u>Immunogenicity studies with iHIVARNA-01</u> were conducted in vitro with peripheral blood mononuclear cells (PBMCs) derived from HIV infected patients, in vivo in mice and ex vivo in lymph node explants. Results from in vitro studies indicate that human DCs derived from HIV-infected patients are able to efficiently uptake and translate iHIVARNA in vitro and activate CD8+T cell responses (refer to IB; 4.3.3.3 In vitro human CD8+ T-cell stimulation study with iHIVARNA-01 loaded DCs; 4.3.3.1 In vitro human PBMC stimulation

with iHIVARNA-01; 4.3.3.2 In vitro human PBMC stimulation with iHIVARNA-01 loaded DCs).

Results in mice intranodally administered with mu_iHIVARNA-01 demonstrate that HIV-1 specific T-cell responses are induced after three intranodal administrations of 50 µg of iHIVARNA-01 (12.5 µg Trimix + 37.5 µg HIVACAT, ratio 1:3) which were broadly distributed among all HIV-1 proteins included in the immunogen (refer to IB; 4.3.3.4 In vivo immunogenicity study of mu-iHIVARNA-01 in mice).

Results in lymph node explants showed that exposure to iHIVARNA mRNA contributed to DC maturation. Further, both IFNγ and IP-10 were significantly increased 24h after iHIVARNA exposition. Moreover, proinflammatory cytokines such as IL-1β were also increased whereas inflammation antagonists such as IL-305 1RA showed an opposite tendency.

Additional studies demonstrated specific lysis of splenocytes loaded with HIVACAT-derived peptides in mice treated with mu_iHIVARNA-01, indicating that T cells activated by the product are cytotoxic and capable of killing targets expressing HIV-1 derived peptides (4.3.3.5 In vivo cytotoxicity assay in mice treated with mu_iHIVARNA-01).

Efficacy studies in viral infection models have been conducted in mice models of acute influenza infection. The rational for conducting these studies was to demonstrate the efficacy of intranodal administration of mRNA-vaccines against infectious diseases, since previous published data were available only in cancer models. Because there are currently no well-established mice models of HIV infection, and because iHIVARNA-01 is species-specific, models of alternative viral infections have been used, i.e. influenza virus. Studies in mice challenged with influenza virus after vaccination with a TriMix-based mRNA influenza vaccine (TriMix-NP) showed that this was as effective as a previously described effective intramuscular DNA-based vaccine (DNA-NP) (13) in inducing T cell responses, and that TriMix-NP was able to substantially reduce mortality due to acute influenza infection in mice (from a ~20% survival to a ~95% as compared to controls) (4.4.1.1 Immunization of mice with an mRNA-based vaccine for influenza infection).

Toxicity studies were performed to assess systemic toxicity and local tolerance of iHIVARNA-01 and included a repeat-dose study in mice consisting in intranodal administration of mu_TriMix or mu_iHIVARNA-01 (3 administrations 1 week apart) (see section 4.5.2 Repeat-dose toxicity study by intranodal administration in C57BL/6 in mice). The proposed starting dose for the clinical trial bears more than an 8x safety margin with respect to the human equivalent of the dose used in this toxicity analysis (see 4.6 Calculation of the maximum recommended starting dose in humans). No treatment-related effects were noted in the toxicity study, and thus the no-observed-adverse-effect level (NOAEL) was considered to be above 10 µg/animal for Trimix and above 40 µg/animal for iHIVARNA-01.3

after repeated intranodal injection to the inguinal lymph nodes. In paragraph 4,6,3 from the IB it is concluded: The dose of iHIVARNA-01 tested in toxicity studies in mice (40 µg) can be approximated to be equivalent to a human dose of between 10 mg and 40 mg. Taking into account the lower dose for additional safety (i.e, 10 mg), and applying an 8-fold safety factor, a maximal dose of 1200 µg of iHIVARNA-01 (300 µg TriMix + 900 µg HIVACAT) would be recommended in phase IIa clinical trials. This dose is equivalent to the dose used in mRNA-based cancer treatment as is used in RBL001/RBL002 Phase I Clinical Trial (available from ClinicalTrials.gov identifier NCT01684241), no published data available yet

6.3 Summary of findings from clinical studies

Therapeutic vaccines using mRNA electroporated dendritic cells have been used in HIV infected patients with a very good safety profile (14–16). In addition, naked or protamin mRNA has been used directly in 3 clinical trials in cancer without any important side effect (17–19).

Additionally, TriMix has been authorized in Belgium for its use in a Phase I clinical trial in combination with mRNAs encoding for hepatocellular carcinoma (HCC) antigens, in patients with metastatic HCC. The trial was initiated in 2014 (EudraCT 2012-005572-74) and is still ongoing. TriMix has also been authorized in Belgium for its use in a Phase I clinical trial in combination with mRNAs encoding for human papillomavirus (HPV) antigens and for treatment of patients with hr-HPV positive cervical intraepithelial neoplasia (CIN grade 2-3) (EudraCT 2013-005136-22). In Europe, electroporation of TriMix in DCs has also been approved in 4 other clinical trials for the treatment of myeloma or melanoma (EudrraCT 2009-015737-73, NL; 2010-023058-35, BE; 2011-001410-33, BE; 2013-000795-15, BE).

As mentioned above, therapeutic vaccinations are considered as one of the most promising strategies that could restore HIV-specific T-cell responses in HIV infected patients and help them control viral replication without cART. Expected potential risks for iHIVARNA-1 are non-serious and related to the administration of the product. Considering this, the Applicant considers that expected benefits outweigh the risks and justify the use of this novel vaccine chronic HIV-infected patients under stable cART.

iHIVARNA-01 was tested in the phase I clinical trial (iHIVARNA Study, EudraCT: 2014-004591-32). Fifteen volunteers were recruited in Hospital Clinic Provincial (HCP) from June 23, 2015 until March 29, 2016. The mean and median age was 46 years (range 28-59). Five (24%) were female and 16 were male (76%). Overall the vaccine was well tolerated. A total of 17 adverse events (AE) were reported during follow-up until July 12th, 2016 (4, 4, 5, 4 and 0 in each ARM respectively). Ten AE were grade 1, 6 grade 2 and 1 grade 3. Of these AE, 2 were definitely related (1 grade 1 and 1 grade 2) and 5 were possibly related (2 grade 1, 3 grade 2) to the IMP.

6.4 Summary of known and potential risks and benefits

iHIVARNA-01 has been tested in the iHIVARNA Phase I dose escalating trial. Overall the vaccine was well tolerated. A total of 16 AE were reported during follow-up (4, 6, 5 and 1 in the first four ARMs respectively). An increase in potential toxicity compared with standard therapy is not expected and was not observed in the phase I dose escalating trial. In addition, the assessment of safety will follow a comprehensive approach through clinical evaluation, lab analysis, biochemical, immunological and virological testing during the whole study.

The risk of virological failure during the vaccination phase in patients with inclusion criteria similar to those in the study is very small. No viral blips were observed during the phase I clinical trial. In addition, virological monitoring in this study will be more frequent than in clinical practice and the results of HIV-RNA measurements in plasma will be available two days after blood sampling. The product has been developed based on several distinctive features, which provide several advantages over previous therapeutic vaccines:

- Direct administration of nude mRNA: allows feasible widespread use and ease of production as well as a more favourable safety profile as compared to DNA vaccines or adoptive transfer of in vitro-modified autologous DCs.
- Intranodal administration: provides higher immunogenicity as compared to intradermal administration.
- Rational design of HIV antigens (HIVACAT): contains HIV regions shown to confer beneficial T cell immunity to control viral replication in the absence of cART.
- Key DC and T cell activation molecules (TriMix): allows efficient antigen presentation and T cell co-activation.

As mentioned, the product is a combination of mRNA sequences designed to specifically target DCs and activate HIV-specific T cells. Of note, direct administration of nude mRNA poses several important advantages with respect to administration of other antigenic molecules (i.e., DNA or peptides) and with respect to ‘ex vivo modification of DCs’:

- in contrast to autologous ex vivo modified DCs, mRNA can be relatively easily produced, which facilitates for feasible large-scale production and widespread use
- as compared to DNA vaccines, mRNA is safer as it does not integrate into the host genome and the expression of the delivered transgenes is transient

- mRNA is degraded very rapidly by endogenous RNases, limiting exposure to the product and associated side-effects as compared to DNA and protein antigens
- the expression of mRNA is efficient as there is no need, in contrast to DNA, to cross the nuclear envelope

Temporarily interruption of cART during ATI, may result in viral rebound, immune decompensation, and clinical progression. There is a sub study of SMART that suggests that with our inclusion criteria, to discontinue therapy for 12 weeks is safe for the patients (20). When all cART regimen components have a similar half-life; all components can be stopped and started simultaneously. However, when drugs have a different half-life (typically a non-nucleoside reverse transcriptase inhibitor [NNRTI]), it may increase the risk of selection of NNRTI-resistant mutations. It is required to stop the NNRTI 2 to 4 weeks in advance of the start of ATI. Alternatively, the NNRTI may be replaced with a ritonavir (or cobicistat)-boosted protease inhibitor (PI/r or PI/c) or integrase inhibitor for more than 4 weeks prior to ATI.

6.5 Description and justification of route of administration and dosage

iHIVARNA-01 administered through the intranodal route activates specific T cell responses after antigen presentation by DCs. This vaccination strategy is so-called 'in vivo modification of DCs' (see Investigator's Brochure for more information on previous experience with this strategy).

When administered into the lymph node, resident DCs take up the mRNA sequences contained in iHIVARNA-01 translating them to the corresponding proteins or peptides:

- Molecules encoded by TriMix sequences (CD40L, caTLR4 and CD70) are expressed on DCs cell surface. CD40L and caTLR4 function by activating immature DCs to become mature DCs, which is essential for subsequent antigen presentation. CD70 expressed on DCs' cell surface engages CD27 on T cells, a co-activation signal that is essential for efficient T cell activation and survival.
- Antigens encoded by the HIVACAT sequence are presented by DCs to T cells through MHC-I and MHC-II complexes (thus including antigen cross-presentation).

Overall, T cells specific for the antigens encoded in the HIVACAT sequence become activated, which should ultimately lead to the lysis of HIV-infected cells.

Of note, several studies suggest that a strong HIV-specific T cell-mediated immunity can indeed limit virus replication and protect against CD4 depletion and disease progression (21). In fact, the best-defined correlates of protection against disease progression as observed in patients who are able to control viral replication in the absence of cART (known as ‘elite controllers’) are low proviral load, low to absent levels of intracellular spliced and unspliced viral mRNA and potent HIV-suppressive CD8+ T-cell responses (5,22). The critical role of the CD8+T cell responses in controlling viral replication was also shown in both the infection model with macaques devoid of CD8+ T lymphocytes (23,24) and in an immune deficient murine model (25). For that reason, current therapeutic vaccines under development, such as iHIVARNA-01, are based on activation of HIV-specific T cell responses by dendritic cells (DCs) presenting target antigens.

6.6 Dosages, dosage modifications and method of administration

The study treatment period will last 5 weeks, with a total follow-up period of 30 weeks. Patients will receive 3 ultrasound guided inguinal intranodal injections of iHIVARNA-01 (HIVACAT/TriMix), or TriMix control or placebo on weeks 0, 2 and 4. The injections will be alternated between the right and the left inguinal lymph node. The dosage has been determined in the phase I dose-escalating trial of iHIVARNA and is equal to the mRNA dosage that has been used previously in the Ribological melanoma study, in which mRNA was also administered intranodally (available from ClinicalTrials.gov identifier NCT01684241), no published data available yet).
The main characteristics of iHIVARNA-01 are detailed in the Investigator’s Brochure. More details on the manufacturing process of the active substances and drug products are given in the Investigator’s Brochure. Quality of production batches of active substances and drug products is ensured by evaluating its compliance with specifications as detailed in sections the Investigator’s Brochure.

6.7 Preparation and labelling of Investigational Medicinal Product

eTheRNA is responsible for manufacturing and supplying all clinical material according to Good Manufacturing Practice. iHIVARNA-01 will be packed and labelled by eTheRNA in accordance with European regulations (as set in annex 13 of the Good Manufacturing Practice Guideline (Regulation EU no 536/2014)). Each delivered ampoule has a unique blinded label that has been generated during randomization. Batch-/lot numbers coupled to randomization numbers will be kept at eTheRNA, in order to prevent potential unblinding based on batch-/lot-numbers. The IMP is formulated in water for injection (WFI) and aliquoted in single dose vials and stored at -20ºC. During aseptic vialing, an extra volume of 10% is aliquoted per single dose, totaling at 220 µl per vial. Prior to injection the vialed IMP should be reconstituted with 880 µl of Hartmann solution (80% final). Hartman solution is provided in single-dose vials. Prior to use the vials should be thawed at room temperature. When completely thawed the vials should be gently swirled.

Care must be taken not to invert the vials (refer to IMPD and SOP provided by eTheRNA for full details). Only 1 ml of the IMPs will be administered by ultrasound-guided intranodal injection. Example labels of the IMP are given in document D3 for the METC.

6.8 Drug accountability

During the trial, product accountability will be monitored by the dispensing log, the returns, the trial register and data collected on the case report forms. The individual who administers the injection will be responsible for ensuring that the return of the used vials is recorded in the dispensing log at the end of the clinical session, and that they are placed in the participant's carton. At the end of the trial, all used and unused vials will be checked against the inventory by staff from the local clinical research organization before returning to the supplier or disposal on site according to local pharmacy guidelines and applicable regulations. Documentation of disposal will be provided to CRO and the supplier.

7. NON-INVESTIGATIONAL PRODUCT

Not applicable

7.1 Name and description of non-investigational product(s)

7.2 Summary of findings from non-clinical studies

7.3 Summary of findings from clinical studies

7.4 Summary of known and potential risks and benefits

7.5 Description and justification of route of administration and dosage

7.6 Dosages, dosage modifications and method of administration

7.7 Preparation and labelling of Non Investigational Medicinal Product

7.8 Drug accountability

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

Safety, number of adverse events and serious adverse events defined as:

- Grade 3 or above local adverse event (pain, cutaneous reactions including induration).
- Grade 3 or above systemic adverse event (temperature, chills, headache, nausea, vomiting, malaise, and myalgia).
- Grade 3 or above other clinical or laboratory adverse event confirmed at examination or on repeat testing respectively.
- Any event attributable to vaccination leading to discontinuation of the immunisation regimen.

Immunogenicity defined as:
Immunogenicity as measured by ELISPOT at baseline and weeks 6 and 18, i.e. two and twelve weeks after the last immunization compared to both control groups

8.1.2 Secondary study parameters/endpoints

- magnitude and the kinetics of the HIV-specific CD4+ and CD8+ T cell responses after immunization (ELISPOT) as defined by the increase in the number of spot forming units and if positive the number of poly-functional T cells as determined by intracellular cytokine staining, (ICS) at baseline and weeks 6 and 30.
- time until viral rebound (defined as two consecutive measurements of plasma viral load > 1000 copies/mL separated by at least 15 days) after discontinuation at week 6.
- plasma viral load in vivo after ATI, between week 6 and 18
- functional cure: proportion of patients with viral load below detectable level of 50 copies/mL in plasma after ATI, week 18 and 30
- primary immune response against vaccine: Change in frequency of at least $0.7log^{10}$ HIV-specific T-cell responses between baseline and week 6

- the in vivo vaccine-induced capacity of ex-vivo CD8 T cells to suppress HIV growth in autologous CD4 T cells i.e. “CD8 T cell mediated viral suppression”
- effect on reservoir as measured by changes in the proviral DNA copy numbers per million cells and the intracellular viral RNA copy numbers per million cells during and after immunization (week 4 and 6).
- viral immune escape: change in % mutated epitopes from pre-cART to post-ATI (week 18)
- host protein mRNA expression profiles in whole blood (week 6 and 18).

8.1.3 Other study parameters (if applicable)

Not applicable

8.2 Randomisation, blinding and treatment allocation

Please mind: in this paragraph the terms “randomisation code” and “random code” are used in close relationship. They serve a different purpose.

After signing the Informed Consent Form (ICF), subjects will undergo a screening to verify that they fulfil all inclusion and none of the exclusion criteria. If a patient is eligible, he or she will be randomly assigned to one of the study arms and included in the intention to treat (ITT) analysis.

It is chosen to implement central randomisation and blinded labelling of IMP. I.e. a sponsor-designated statistician will generate code lists in advance.

Six versions of the **randomisation code list** will be generated:

1. Unblinded: a list to both eTheRNA and the pharmacy of the sponsor (the latter only for emergency unblinding purposes). This list includes names of study sites, randomisation codes and treatment arms.
2. Blinded: five lists to the clinical investigators of the participating study sites (including site of the sponsor). These lists are site-specific and only contain the name of the study site and randomisation codes.

Furthermore, one **accident random code list** is generated:

1. Unblinded: a list to both eTheRNA and the pharmacy of the sponsor. This list includes names of study sites, random codes and treatment arms.

The **randomisation code list** is computer generated (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria) and will be balanced using randomly permuted blocks and is stratified by site location in order for each site to have an equal amount of subjects per treatment arm (40:15:15 ratio of HIVACAT-TriMix : TriMix alone : placebo). Every randomisation code is a unique, random 3-letter code (i.e. ABC). One randomisation code couples to one study subject. Extra random codes (**accident random code list**) are generated for accidents (see below). Refer to section 5.7 for labelling of the vials. Labels on vials, boxes etc. will only contain blinded information (refer to section 5.7 and document D3), in order to keep the blind for investigators after dispatching the IMP from eTheRNA until study-end.

Upon successful screening, the investigator assigns his/her study subject to the next free randomisation code of his/her study site. The randomisation code will be both documented in the patient files as well as in the eCRF. After assignment, no notification of the sponsor, eTheRNA or pharmacy of the sponsor is needed. In case a subject terminates study participation before the first vaccination, but after assignment of a randomisation code, the investigator may not reuse the randomisation code for another study subject. It is agreed to recruit 14 patients per site. Therefore, each site will have eight subjects assigned to the iHIVARNA vaccine arm, three subjects to the TriMix control arm and three subjects to the placebo arm. If any site fails to include 14 patients, the sponsor will decide to re-allocate part of the randomisation code list for that site to another site and demand shipping of accompanying vials to another site.

eTheRNA will supply each site with 8 boxes (3 vials each) of iHIVARNA vaccine, 3 boxes (3 vials each) of TriMix alone and 3 boxes (3 vials each) of placebo. Furthermore, backup vials are supplied (see below).

An **accident random code list** will aid in assigning the correct vials to the correct study subjects / study arms in case of accidental loss, breakage, expiration of vials etc. The list will only be available to eTheRNA and the pharmacy of the sponsor. It contains study sites, treatment arms and a unique, random 3-letter code preceded by an ‘b’ (backup) (i.e. bDEF). Each study site will be supplied with 9 extra: 3 vials for HIVACAT-TriMix, 3 vials for TriMix control and 3 vials for control. In contrast to the boxes of 3 vials **with the same randomisation code per study subject** (as mentioned above); these backup vials will have **one random code (i.e. bDEF) per vial** as labelled by eTheRNA (see above). In case of accidental loss, breakage or expiration of a vial, the investigator has to contact the pharmacy of the sponsor to request a new random code per expired vial. The investigator needs to inform the pharmacy about the name of the study site and the original randomisation code. The (unblinded) designated person at the pharmacy will check this code with the study arm and pick a code from the accident random code list corresponding to the same study site and study arm. The pharmacy of the sponsor and the local investigator register the usage of this accident random code in their logs. Since there is a one by one replacement of vials, the investigator should not alter or re-assign the original randomisation code. When the pharmacy of the sponsor notices that all random codes (bDEF) of one study arm for a site are used; they will notify eTheRNA directly, in order to deliver extra backup vials.

For reconstitution of all vials, 51 vials of Hartmann solution of the same lot/batch will be provided per site.

After immunization, the empty vial will be stored at room temperature, until final monitoring and drug accountability has been performed by the CRO. Thereafter the IMP may be destroyed as per local SOP. Non-used vials should be stored at the conditions as indicated on the vials/packages until monitoring.

Under normal circumstances the blind should not be broken until all subjects have completed and the database is locked after data completion and verification. Otherwise, the blind should be broken only if specific emergency treatment/course of action would be dictated by knowing the treatment status of the subject. In such cases, the investigator or sponsor may determine the identity of the treatment by contacting the pharmacy of the sponsor. It is recommended that the local investigator first contacts the sponsor, if possible, to discuss the particular situation, before breaking the blind. Telephone contact with the pharmacy of the sponsor will be available 24 hours per day, 7 days per week. In the event the blind is broken, the sponsor must be informed as soon as possible. The date, time, and reason for the unblinding must be documented by the clinician, in the appropriate section of the electronic case report form (eCRF), and in the source documents. The documentation received from the pharmacy indicating the code break must be retained with the subject's source documents in a secure manner.

8.3 Study procedures

Recruitment

Their treating physician will first inform patients about the phase II clinical trial and confirm their eligibility. The study team then pick patients who have indicated their willingness to participate. Patients will receive a hardcopy/digital version of the PIF and are invited for the W-4 screening visit (informed consent, eligibility and laboratory).

Informed consent and Screening

Screening visit

Screening should be performed at most 8 weeks prior to enrolment (randomization/baseline visit at week 0), an interval of 4 to 2 weeks is however advised.

At screening, the study will be discussed in detail and any questions about the study will be answered. For heterosexually active female, using an effective method of contraception with partner from 14 days prior to the first vaccination until 4 months after the last vaccination will be demanded. For heterosexually active male, using an effective method of contraception with their partner from the first day of vaccination until 4 months after the last vaccination will be demanded.
Specific attention will be paid to risks of viral rebound syndrome. Adequate measures for risk reduction of virus transmission during ATI (in order to prevent potential HIV transmission will be discussed. A potential subject for the study will be offered an optional two-week consideration period before signing informed consent. If the patient wants to make use of this consideration period, a new appointment will be scheduled for obtaining written informed consent and performing study assessments specific to the study protocol.

The principal investigator or designated collaborator will then obtain written informed consent. Genetic analysis is specifically mentioned in the patient information and consent forms. Hereafter, assessments and procedures will be undertaken according to the schedule in Appendix B. Demographic data, medical history, complete physical examination and laboratory tests will be obtained and assessed to confirm the eligibility of the patient. Patients will be monitored for active HBV/HCV infection. The patient will be given a container for collecting stool (to be brought upon inclusion at week 0). After obtaining informed consent and screening, the participant will also be registered in the eCRF database and any AE needs to be recorded into the eCRF.

Study enrolment

Review data screening visit

Review of the screening data will we performed between -4 and week 0. If, after completing the Informed consent and screening procedures, a patient is found to be eligible, the investigator will notice and reconfirm their study participation with the patient. The patient may be enrolled to the study.

Screening failure

A screening failure is considered if a potential subject did not meet to one or more of the criteria required for participation in the trial in between the period of signing informed consent and prior to study enrolment/randomization. Several reasons for a screening failure may be present, such as inclusion/exclusion criteria not met, non-compliance and consent withdrawn. Again: all study procedures after informed consent need to be entered in the eCRF. A screening failure including reason will be documented in the eCRF. A screening failure might be reconsidered for further enrolment/randomization to be eligible in agreement with the sponsor, principle investigator and CRO; in case the failure was due to temporarily factors (i.e. a laboratory error).

Study visits for enrolled and randomized subjects

Baseline visit (trial enrolment)

During the baseline visit, the investigator will ensure that all patient selection procedures were performed and the results confirm that the patient is eligible for participation in the study. During the visit, a complete physical exam will be performed and blood will be drawn (see: Appendix B). Female patients will undergo a urine pregnancy test. A negative test allows for further study continuation. If all criteria for study participation are met, the subject will be randomized. The investigator picks the next free randomisation code on the site specific randomisation code list and assigns the study subject. The randomisation code should also be noted in the patient record and eCRF. Explicit notification of an assignment to the sponsor, pharmacy of the sponsor is not needed. Hereafter, the patient will be included to the intention to treat (ITT) analysis.

The participant will receive the first immunization. If immunization within the same visit is not possible due to logistic reasons, it is preferred to let immunization occur within 24 hours of the first visit, but always after all blood samples of the baseline visit were taken. The treating physician(s) and/or general practitioner of the patient will be notified about the study participation.

Immunization

Patients will receive 3 inguinal intranodal injections of iHIVARNA-01 on weeks 0, 2 and 4 in the Radiology Service of participating hospitals. Prior to each immunization, women of childbearing age will undergo a pregnancy rest (urine test) and will only be allowed to receive intranodal vaccination if they obtained a negative pregnancy result within the last 48 hours prior to vaccination. Injections will be performed with ultrasound guidance using a multifrequency gauge for soft tissue. This procedure should be done by a radiologist trained both in ultrasound imaging and tissue biopsy. The injections will be given alternated between the right and the left inguinal lymph region, as described in SOP030-iHIVARNA. Date, time and location of the injection will be recorded in the CRF and on the patient diary card. The visits of week 0, 2, 4, including drawing of blood, should be performed prior to vaccination. If a female participant becomes pregnant along study, she will discontinue the study, but will be asked for consent for the pregnancy and delivery follow up.

Follow-up after immunization

Subjects will be closely monitored during the first hour after immunization, at which point vital signs (pulse, blood pressure and respiratory rate) will be recorded on the case record form, as well as any local reactions and systemic events. A diary card will be given to all the subjects, with instructions and a full verbal explanation to record local and systemic adverse events following one week after immunization.

24 hours and 1 week after the second immunization (week 2+1day and week 3) and 24 hours and 1 week after the third immunization (week 4+1day and week 5) a short visit for drawing blood for the cell-associated HIV RNA (CA-HIV) assay will be scheduled.

Telephone contact

The clinical team will hold a telephone contact with the study subject 7 days (+/- 2 days) after the first immunization. This contact will inquire about adverse events, up to resolution of solicited local and systemic events. Additional visits may be recommended at the discretion of the clinical and principal investigators, if clinically indicated or in order to clarify observations.

Follow-up visits

Patients will return to the clinical centre in weeks 6, 8, 10, 14, 18, 24 and 30 of the study. At each follow-up visit, information on concomitant medication, a complete physical examination, and specific laboratory tests (see: appendix B) will be obtained. Subjects that have not resumed cART at week 30, because their pvl remains undetectable and it is their preference not to re-initiate cART, will be closely monitored for PVL and CD4 T cells (every 8 weeks) in the follow-up period until cART is resumed.

Treatment interruption at week 6

Two weeks after the third vaccination (week 6 visit), cART will be interrupted. Female subjects will be allowed interruption after a negative pregnancy test.

Assessment of safety

Safety assessments will take place at fixed time points during the follow-up visits and telephone consultation. The patients will be informed of potential safety issues. Patients are able to contact the investigator or a member of the investigational team 24 hours a day, 7 days a week.

During all visits, the following will be assessed as a minimum: vital signs (blood pressure, heart rate, temperature) inspection of lymph nodes and in particular the site of injection. Full physical examination (general appearance, eyes, ears/nose/throat, cardiovascular, respiratory, gastrointestinal, neurological, musculoskeletal, skin) will be performed at screening and baseline (W0) and will only be repeated at follow-up visits on clinical indication (thus when a patient has systemic or local complaints).

Special attention to immunization related signs and symptoms:

After each immunization, close attention must be paid to the following immunization related signs and symptoms:

Local injection site reactions:

- Pain at injection site
- Itching at injection site
- Redness/discoloration
- Fluid filled blisters
- Blood filled blisters
- Hard swelling in skin surface at or close to site

General/systemic reactions:

- Temperature;
- Chills/rigors;
- Malaise/ tiredness;
- General muscle aches;
- Headache;
- Nausea;

- Vomiting.

Viral rebound during Analytical treatment interruption (ATI):
cART interruption (ATI) is needed in the search for HIV cure (26). During ATI subjects will be monitored for adverse events of plasma viral rebound such as fever, fatigue, lymphadenopathy, pharyngitis, rash and/or weight loss. If the CD4+ T cell counts drop below 50% of baseline level or below 350 cells/ml, an extra CD4+ T cell count will be performed within 7 days. If the results are below 50% of baseline or below 350 cells/ml, cART will be re-initiated, immediately. cART will also be re-initiated if the physician or investigator judges that this is in the best interest of the patient.
Safety laboratory (all visits):
Haematology (except W0): red blood cell count, leukocytes, haemoglobin, haematocrit, thrombocytes. Required amount of blood: 4 ml EDTA blood;

Chemistry (except W0): creatinine, ASAT, ALAT, alkaline phosphatase, GGT, total bilirubin, amylase or lipase, CPK, glucose.
Required amount of blood: 9 ml serum.

Immunology (except W0): Lymphocyte subsets (CD4+ and CD8+ T cells) (all visits).
Required amount of blood: 4 mL EDTA blood.

HLA typing (only at inclusion).
Required amount of blood: 2-10 ml of EDTA blood.

Elispot (week -4, weeks 0, 6, 10, 18, 30) and intracellular cytokine staining (if Elispot is positive).
Required amount of blood: 27 ml EDTA blood.

CD8+T-cell suppressive capacity assay (week -4, week 0, week 4)
45 ml EDTA blood

HIV-1 plasma viral load (except week 0)
9ml EDTA blood

Systems biology (week -4, week 6 and week 18)
3 ml blood in Tempus tube

Virus sequencing (week week 10, week 18)
one ml EDTA plasma

Other laboratory
Faeces microbiota (week week 0, week 6, week 18) one sample

To give the clinical sites some flexibility in visit planning the following visit windows are allowed: W2 until W6 +/-1day, W8 until W10 +/- 2days, W14 until W30 +/- 1 week.
See appendix B for scheme of sampling

8.4 Treatment discontinuation and study withdrawal of individual subjects

Treatment discontinuation: A patient will prematurely discontinue the study in case of withdrawal of Informed Consent or if the investigator considers it in the best interest of the patient to withdraw. All terminations of study patients and the reasons for them must be reported immediately to monitor and sponsor and be duly documented in both the medical records and the case report form.

Study withdrawal: The patient may discontinue study participation if he/she is unwilling or unable to meet the protocol requirements in terms of the visit schedule or if the patient or the investigator considers it best to end his/her participation in the study. All patients have the right to withdraw their consent at any time during the study without prejudice to them.
If a female participant becomes pregnant along study, she will discontinue the study treatment (i.e. intranodal vaccination), but will be asked for consent for the pregnancy and delivery follow up.
In case a patient withdraws the study, all efforts will be made to complete the week 30 procedures as termination visit.
This will be reported immediately to the study monitor and sponsor.

8.4.1 Specific criteria for withdrawal (if applicable)

A patient will not receive the first, second and/or third immunization if:
- there is a need to start concomitant treatment with medication that is not compatible with the use of the study immunization (e.g. corticosteroids, as it is an exclusion criterium).

- a severe adverse event occurs which is considered as a (possible) result of the immunization and/or SAE not resolved at time of vaccination.
- a female participant becomes pregnant along study, she will discontinue the study, but will be asked for consent for the pregnancy and delivery follow up.
- it is in her or his best interest in the opinion of the investigator.

8.5 Replacement of individual subjects after withdrawal

Subjects who do not receive the second or third immunization (for any reason) will be asked to stay in follow up for safety and immunological assessments according to the protocol whenever possible. In case of an adverse event, subjects will be followed until the adverse event has been resolved or stabilized. Safety data of these patients will be collected according to the protocol where possible.
The date that the participant is discontinued from further immunizations and the reason will be recorded in the eCRF.
Subjects, who have not received any of the study vaccinations, after randomization, will not be replaced. Unblinding of replaced participants will occur after the database lock, or earlier if necessary (see also: 8.2).
Patients that have received 1 or more immunizations will not be replaced. All patients that were randomized will be analyzed in the ITT approach.

8.6 Follow-up of subjects withdrawn from treatment

All randomized subjects, including those who do not receive the second and/or third immunization (for any reason), will be asked to stay in follow up for safety and immunological assessments according to the protocol where possible. In case of an adverse event subjects will be followed until the adverse event has been resolved or stabilized. Safety data of these patients will be collected according to the protocol whenever possible. Subjects that have not received all three immunizations will not interrupt cART. The date that the participant is discontinued from further immunizations and the reason will be recorded in the eCRF.

8.7 Premature termination of the study

Premature termination of the study will occur when two or more grade 3 or above SAE's, related to the vaccine or SUSARs occur or when subjects are exposed to unacceptably high risk in the investigators opinion or when advised by the DSMB and accepted by the sponsor. Premature termination will be reported to the local EC and competent authorities within 15 days.

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise patient health or safety. The sponsor will notify the accredited METC with undue delay of a temporary halt including the reason for such an action. The study will be suspended pending further review by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the IP. The study period is defined as the time from inclusion, after signing the informed Consent Form (ICF) until the end of the study at week 30 after the first vaccination. Any medical condition that was present before the study treatment and that remains unchanged or improves should not be considered or recorded as an AE. A worsening of that medical condition will be considered as an AE.
All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded. Notes of the attributability and causal relationship to the IP or other study related procedures should be made.
An adverse reaction (AR) is any noxious and unintended reaction to an investigational drug, regardless of the dose administered.
An unexpected adverse reaction (UAR) is an adverse reaction, the nature or severity of which is not consistent with the information about the medicinal product set out in the Investigator Brochure (IB).

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospital admission or prolongation of existing inpatients' admission;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect;
- requires medical or surgical intervention to preclude of

An elective hospital admission will not be considered as a serious adverse event.

AEs will be recorded at each visit based on careful clinical observation of the patient, laboratory tests and spontaneous reports by the patient and also by open-ended questioning by the investigator.

All AEs (serious or not) occurring during the study must be noted in the medical history and recorded in the CRF. The investigator will also decide whether the adverse event is, based on his/her judgment, related or not to the study drug—this decision should also be noted in the medical history and CRF.

At each visit, all AEs experienced by the patient since the previous visit should be recorded in the specific adverse event form of the CRF.

The following will be recorded for each event: description, severity (grade 1, 2, 3, 4 and 5), duration (start and end dates), causal relationship with the drug (according to the previously attributed criteria) and study drug(s) for which this causal relationship is suspected, need for treatment (if applicable) or the actions taken, possible alternative explanations, predisposing factors, and outcome. For a pre-existing AE that has worsening in terms of severity or frequency, the meaning of the change should be specified.

The degree of severity of an adverse event provides a qualitative assessment of the extent or intensity of an adverse event elicited by the investigator or reported by the patient. Severity does not reflect the clinical seriousness of the event, only the grade or extent of the complaint or incidence.

The study will utilize the Division of AIDS Table for grading the severity of adverse events (CTCAE table v4, dd14-JUN-2010) (see Appendix C).

The investigator will report SAEs to the sponsor within 24 hours of the investigator becoming aware of the events. The investigator must complete the SAE form and send the form BOTH by email to r.gruters@erasmusmc.nl AND by fax to +31 10 70 33441 AND by mail to CR2O: ihivarnasafety@cr2o.nl AND by phone to CR2O: +31 6 2061 0123 (safety physician). It is advised to contact the sponsor by phone (+31 613 50 80 33) as well, to avoid any unnecessary delay in the notification.

The sponsor will report these SAEs through the accredited METC that approved the protocol through the web portal *ToetsingOnline* and this, within 7 days for SAEs that result in death or are life threatening. Thereafter, a period of maximum 8 days is allowed to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 9.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
    - Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report the following SUSARs to the METC through the web portal *ToetsingOnline*:

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.

The expedited reporting of SUSARs through the web portal Eudravigilance or ToetsingOnline is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

9.3 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States, once a year throughout the clinical trial.

This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the patients, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.
- A line listing of all SAE's which occurred in the 2 Belgian sites will be sent to all the Belgian EC's and FAGG on a yearly basis.

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported until the end of study within the Netherlands, as defined in the protocol.

9.5 Data Safety Monitoring Board (DSMB)

The DSMB is an independent group of experts that advises the sponsor of this study. The advice(s) of the DSMB will only be sent to the sponsor of the study. Should the sponsor decide not to fully implement the advice of the DSMB, the sponsor will send the advice to the reviewing METC, including a note to substantiate why (part of) the advice of the DSMB will not be followed.

DSMB composition:

Dr. P.J.J. (Perry) van Genderen (president), dept. of Internal Medicine, Havenziekenhuis Rotterdam

Dr. C (Kees) Brinkman, Onze Lieve Vrouwe Gasthuis, Amsterdam

Dr. D. (Dimitris) Rizopoulos, dept. of Biostatistics, Erasmus MC, Rotterdam

The DSMB will meet at least on four occasions, preferably face to face, or alternatively by teleconference. These meetings are scheduled

- before the start of the phase II clinical trial
- after completion of immunization of the first two patients at each clinical site,
- after the last immunization of the last study subject and
- at the end of the clinical trial.

The DSMB will review at least:

- this protocol prior to study start
- cumulative study data to evaluate safety, study conduct, and scientific validity and integrity of the trial.
- interim/cumulative data for evidence of study-related adverse events
- interim/cumulative data for evidence of efficacy according to pre-established statistical guidelines, if appropriate.
- factors that might affect the study outcome or compromise the confidentiality of the trial data (such as protocol violations, unmasking, etc.).
- factors external to the study such as scientific or therapeutic developments that may impact participant safety or the ethics of the study.

A more detailed description of the DSMB tasks is available in form K5 from the METC submission documents.

10. STATISTICAL ANALYSIS

In general, analyses will be performed using an "intent-to-treat" approach. All subjects randomized to either group will be included in the analysis under the group they are randomized to, even if they didn't receive all vaccinations. All analyses will be adjusted by center.

Every effort will be made to minimize the amount of missing data in the trial. However it is realistic to assume a priori that missingness may be informative. Sensitivity analyses, including multiple imputations, will be undertaken to assess the robustness of the conclusions to the missing data.

10.1 Primary study parameter(s)

- All non-serious and serious adverse events will be grouped according to a pre-specified side-effect coding system and tabulated. The number (and percentage) of patients experiencing any grade 3 or above local adverse event, any grade 3 or above systemic adverse event, any grade 3 or above other clinical or laboratory adverse event, any adverse event attributable to vaccination leading to discontinuation of the immunization regimen will be compared between treatment groups using logistic regression. Safety will be analyzed using the all-patients-treated approach.
- Difference in frequency of log^{10} HIV-specific T-cell responses as determined by SFU/$10^{\hat{}6}$*cells between baseline and 2 and 14 weeks after the last injection will be compared between the three study arms (HIVACAT-TriMix; TriMix; placebo) using linear or quantile regression as appropriate.

10.2 Secondary study parameter(s)

- The magnitudes and kinetics of the HIV-specific CD4+ and CD8+ T cell responses at week 6 and week 30 will be compared between the three study arms (HIVACAT-TriMix; TriMix; placebo) using linear or quantile regression as appropriate.
- Time from discontinuation at W6 until viral rebound is compared between the three intervention arms using a Cox proportional hazards model
- Linear mixed effects model to compare the evolution of log10 plasma viral load over time between the three intervention groups
- Logistic regression to compare the proportion of patients with functional cure at week 18 and week 30 between the three intervention groups

- The proportion of patients with a primary immune response against vaccine, at week 6, after 3 vaccinations, will be compared between the three intervention groups using logistic regression
- Mixed effects regression to compare the evolution of CD8+ T-cell HIV suppressive capacity from baseline to two weeks after the last vaccination between the three intervention groups
- Mixed effects regression to compare the evolution of log10 proviral DNA and log10 intracellular viral RNA from baseline to week 30 after re-start cART, between the three intervention groups
- Compare viral immune escape (change in % mutated epitopes from pre-cART to post-ATI) between the three intervention groups using linear or quantile regression as appropriate
- Principal component analysis will be applied on the mRNA expression profiles. The evolution over time (baseline, after 3 vaccinations and after ATI) of selected principal components will be compared between the three intervention groups using linear mixed effects models

10.3 Other study parameters

Not applicable

10.4 Interim analysis

Rationale:

The phase I clinical trial has proven safe (primary outcome). However, the secondary outcome, immunogenicity as measured by peptide Elispot showed limited effect of the vaccine on the number of spot forming units. This was also due to high baseline values. Based on these results the consortium wants to be cautious in the phase II trial and an interim analysis has been proposed.

Proposal

We will include 35 patients into the phase II trial (this is half of the total study population). The interim analysis is the same as the primary analysis in the protocol, i.e. comparison of change from baseline to week 6 of cumulative frequencies of HIVACAT-specific PBMC (on log10 scale) between HIVACAT-TriMix arm and placebo arm. Thus we test H0: mu1=mu2 vs H1: mu1≠mu2. We assume a standard deviation of 0.5 in both arms.)

Time point	N	Z_I	Z_f	Probability stopping under H0	Probability stopping under H1 (p1)	Power	Type I error
0.5	20,8	2,23	1.71	99%	15%	85%	1.6%

Column ‘N’ shows the number of patients included in the interim analysis in both arms. If the test statistic at the interim analysis is smaller than ‘Z_I’ the trial is stopped for futility, otherwise the rest of the patients will be enrolled and the test statistic of the final analysis will be compared to cut-off value ‘Z_f’. The power is calculated under the alternative that the difference in means is 0.7.

The analysis will be performed by an unblinded statistician, who is not a member of the study consortium.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

The clinical trial will be conducted in accordance with the principles contained in the Declaration of Helsinki.

For participating Dutch sites the following is applicable as well:
The clinical trial will be conducted in accordance with the Medical Research Involving Human Subjects Act (WMO, BWBR0009408, 26-FEB-1998).

For participating Spanish sites the following is applicable as well:
Royal Decree 1090/2015, CREC, authorization from the Spanish Agency for Medicinal Products and Medical Devices, and approval by the Director of the Institution. The investigator agrees to comply with the rules set forth in the applicable clinical trial regulations: Medicines Act 29/2006 (Official State Journal No. 178, 27-07-06) and Royal Decree 1090/2015 on Clinical Trials. For participating Belgian sites, the law on experiments on the human person of May 7th, 2004 is applicable.

11.2 Recruitment and consent

Patients will be informed verbally by their treating doctor and in writing via the patient information form. All relevant information will be reported to the subjects adapted to their level of understanding. After informed consent they will have a minimum of two weeks before they will be asked to sign the form for study participation.

11.3 Objection by minors or incapacitated subjects

Not applicable

11.4 Benefits and risks assessment, group relatedness

Vaccination in HIV-infected patients against other pathogen (e.g. influenza virus) is common practice and has not shown extra risk for these patients. cART although effective requires lifelong medication with side effects and long-term toxicities including cardiovascular and renal problems. Therapeutic vaccination may complement or even replace daily treatment thus alleviating the burden of cART.
The use of TriMix may activate the immune system a-specifically. This may provide target cells for HIV replication and in the case of ATI, increased viral replication.

11.5 Compensation for injury

The sponsor has liability insurance, for all subjects, which is in accordance with article 7 of the WMO for the study at all clinical sites.

The sponsor (also) has an insurance, which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects enrolled at all clinical sites through injury or death caused by the study. The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11.6 Incentives

Reimbursement of subject travel expenses and parking costs will follow on a per center base, after receiving an original receipt. Car travel expenses will be reimbursed based on a fixed price per kilometer according to local regulations.

Subjects will not receive any other payment or compensation for participation in the study.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

The study will be conducted in accordance with the International Conference on Harmonization (ICH) for Good Clinical Practice (GCP) and the appropriate regulatory requirement(s). Essential clinical documents will be maintained per study site to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study, and retained for at least 15 years after terminating the study, or longer according to regulations by local authorities. The source data has to be kept at the study site and is accessible for the study team. It is also available for the monitor and authorities on request – in order to fulfil adequate monitoring, source data verification and to perform study site audits.

OpenClinica, running in a GCP/ICH compliant environment within Erasmus MC, will be used as Clinical Data Management System (CDMS). All data will be entered coded as required by the Personal Data Protection Act (WBP in Dutch). All study procedures in OpenClinica are traceable by the build in study audit log and by using personal, non-transferable accounts. The principal investigator per site is responsible for administering the list with personal patient data and coded study numbers (hereafter: code list). Study numbers should not contain any data, which might identify a person. The code list should be locked away in such a manner that unauthorized persons cannot access it.

Blood, urine and stool samples for routine laboratory diagnostics (i.e. red blood cell count, liver function) will be labelled with non-coded patient information. All samples for non-routine laboratory diagnostic (i.e. for intracellular cytokine staining) and all samples being processed at another site will be labelled with coded information alone.

12.2 Monitoring and Quality Assurance

Monitoring will occur according to an intermediate risk profile based on monitoring guidelines of METC Erasmus MC/NFU (Dutch Federation of University Medical Centres) and in compliance with GCP/ICH guidelines.

Every monitor visit will be reported in a written report. The head of the department has the final responsibility for filing the monitoring reports for at least 15 years after terminating the study, or longer according to regulations by local authorities. Monitoring visit reports and other documents related to iHIVARNA phase IIa will be available on request of the Board of Directors and their authorised representatives.

12.3 Amendments

A ‘substantial amendment’ is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient’s last follow-up visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

12.6 Public disclosure and publication policy

The investigators, in agreement with the Coordinating Center for the study, will publish the results of the trial in internationally indexed journals. Authorship will take into account members of the study management, participating investigators and persons responsible for coordination, data analysis and article writing.

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

a. Level of knowledge about mechanism of action.
Control of HIV viral replication in the absence of cART (known as 'elite controllers), has been attributed to the ability to develop effective HIV-specific CD8+T cell responses (5,6). The HIVACAT immunogen confers this specific immunity to key HIV regions. Furthermore, the TriMix substance provides the necessary activation molecules to enhance antigen presentation by DC's and T-cell activation (mRNA sequences of CD40L, caTLR4 and CD70). The intranodal vaccination route is based on the knowledge of the strategy to ex-vivo modify DC's with HIV-particles and successful intranodal vaccination strategies with mRNA-optimized dendritic cells in melanoma patients (6,27). These methods have already proven to be both safe and immunogenic.

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism.
To date, clinical experience with HIVACAT and TriMix in the iHIVARNA phase I trial showed that the vaccination is well tolerated with limited adverse events obviously or possibly related to the product. TriMix has been used with tumor-associated antigens in mRNA-based antitumor therapy studies and has proven to be safe (6).

c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?
Pre-clinical studies in mice have shown a strong induction of vaccine specific immune responses (ELISPOT) after administration of the TriMix HIVACAT combination. Ex vivo stimulation of PBMC with electroporated DC from HIV-1 infected patients showed a strong induction of vaccine specific responses (see IMPD document 4_iHIVARNA_NonClinical_ASP_01).

d. Selectivity of the mechanism to target tissue in animals and/or human beings.
Targeting of DC’s by intranodal administration was shown in mice (section 2.2.4..8.1 of MPD doc 4). eGFP localizes in CD11c positive cells. Luciferase activity was eliminated by depletion of DC by diphtheria toxin in CD11c-DTR transgenic mice. Bioluminescence was shown after intranodal administration of luciferase mRNA in mice (section 2.2..3.2.3.d of IMPD doc 4) (6)

e. Analysis of potential effect.

Intranodal vaccination experiments with murine TriMix and HIVACAT in C57BL/6 mice showed a strong induction of cellular immune responses as measured by IFNγ ELISPOT and in vivo CTL.

f. Pharmacokinetic considerations

According to the Note for guidance on preclinical pharmacological and toxicological testing of vaccines (CPMP/SWP/465/96), pharmacokinetic studies are normally not needed. The need for specific studies should be considered in a case-by-case basis and may include considerations such as local deposition studies, which would assess the retention at the site of injection and its further distribution; histopathological studies of the draining lymph nodes, which might illustrate depot characteristics of the vaccine. Distribution studies should be considered in case of new formulations, novel adjuvants or alternative routes of administration (eg. oral or intranasal).

Local deposition of intranodally administered mRNA is supported by studies conducted by the Applicant with Fluc mRNA and GFP mRNA (2.2.4.8.1 Local deposition studies). As demonstrated in these studies, intranodal injection of mRNA allows for its uptake and translation by resident DCs in mice. mRNA is stable up to 12 hours in lymph nodes and functional protein can be observed up to 9 days. The only cell type observed to express functional protein derived from administered mRNA was resident DCs (see study details and results in section 2.2.4.8.1 Local deposition studies).

g. Study population

Only patients who fulfill their regular outpatient clinic visits with their treating physician will be selected. All patients are on stable cART treatment and CD4 counts should be above 450/ml, with an undetectable PVL. Women of child-bearing potential are tested for pregnancy and are required to use contraceptives. Regular pregnancy testing is included in the protocol.

h. Interaction with other products

Vaccination is topical and pharmaco-active compounds are however not included. Therefore no interactions with cART or any other medication and products or other treatment is expected.

i. Predictability of effect

Biomarkers for measuring the effectiveness of HIVACAT/TriMix are T cell immune responses as measured with two assays. Indirectly PVL may reflect effectiveness of vaccination.

j. Can effects be managed?

Direct management of adverse immune responses is not foreseen, however, immune suppressive medication (prednisone) may be administered at the discretion of the physician. Any unforeseen allergic/anaphylactic reactions can be managed with standard treatment (e.g. with epinephrine, steroids and antihistamine – according to local guidelines). During antiretroviral treatment interruption (ATI) frequent monitoring allows to follow the viral rebound. Criteria for re-start of cART are defined in the study protocol.

13.2 Synthesis

Risks on vaccination as strategy in preventing infectious diseases (e.g. influenza) are well known and generally regarded as safe. Recent studies on intranodal vaccination and the usage of TriMix proved the procedures and product being safe and well tolerated in general. Knowledge of intranodal vaccination with TriMix and HIVACAT is limited to iHIVARNA Phase I trial and showed that the vaccine was well tolerated, with few non-serious AE obviously or possible related to the vaccination. Lastly, ATI is used regularly in clinical studies. To further limit the risks; patients are followed up until one hour after vaccination, regular telephone follow-ups are scheduled and multiple visits including safety laboratory analysis are planned (see: chapter 8.3, chapter 9). Furthermore, the ATI is limited to a period of 12 weeks; while PVL monitoring takes place on a regular base in order to detect HIV replication. The investigator will review the physical examination, safety laboratory results and the patient diary before continuing any study activities. Lastly, a DSMB is appointed.

14. REFERENCES

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