VARICELLA ZOSTER RECOMBINANT HZ/su VACCINE: IMMUNOGENICITY AND SAFETY IN PLWHIV ACCORDING TO LTCD4 COUNT STRATA.

Infectious Diseases Centers

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Synopsis

Herpes zoster (HZ) is a common manifestation of Varicella-Zoster virus reactivation, occurring as a painful unilateral vesicular rash. Long lasting neuropathic pain (post herpetic neuralgia, PHN) can persist after rash resolution in up to 20% of patients. As cell mediated immunity is the main defense against herpes zoster, any condition associated with its impairment, including HIV infection, can lead to higher risk of VZV reactivation. A live attenuated vaccine showed only modest efficacy and was contraindicated in immunosuppressed patients. A novel subunit recombinant vaccine (HZ/su) containing glycoprotein E antigen and adjuvant system AS01B named Shingrix achieved outstanding results in preventing both HZ and PHN, with high response rates observed in studies investigating immunological aspects. This HZ/su vaccine has been employed in diverse immunocompromised categories, with encouraging results. To date data regarding HZ/su vaccine use in people living with HIV (PLWHIV) are very limited, but still promising.

The present study will investigate immunologic response and safety of HZ/su vaccine in PLWHIV on effective antriretroviral treatment, according to immunological situation.

Three Infectious Disease centers in Italy will participate the study, prospectively enrolling a total of 500 PLWHIV (250 with current CD4+ T-lymphocyte (LTCD4) counts < 350 cell/mm3 and 250 with current LTCD4 ≥ 350 cells/mm3).

A two-dose schedule of Shingrix will be administered to adult PLWHIV (2 months apart), as recommended by national guidelines. Blood samples will be collected at baseline and one month after schedule completion.

Primary objective of the study: evaluation of immunogenicity assessed in terms of vaccine response rates (VRR) as in pivotal trials, according to LTCD4 count strata. For humoral response, VRR is defined as a 4-fold increase in anti gE antibodies compared to baseline by ELISA.

Secondary objectives:

- evaluation of cell-mediated response by LTCD4 count strata. VRR for cell-mediated immunity is defined as a 2-fold increase in frequency of gE specific polypositive LTCD4 compared to baseline. Interferon Gamma release assay (IGRA) and analysis of cytokine secretion in supernatants (Ella) upon stimulation with gE will be performed also.
- evaluation of immunogenicity (humoral and cell-mediated) according to LTCD4/CD8 ratio (<1 vs ≥1).
- Safety will be investigated trhough a semistructured questionnaire exploring local and systemic symptoms, occurring within one week after each of the two doses, a follow up phone call 30 days

post each dose for unsolicited AEs, and a final follow up visit six months after schedule completion. Occurrence of AEs, HZ, PHN and pIMDs will be registered.

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1 Introduction

1.2 Background

Herpes zoster (HZ) is the relatively common manifestation of Varicella Zoster virus reactivation, occurring as vesicular skin rash with typical dermatomal distribution. As the virus replicates in dorsal root ganglia, its neuropathic effect results in intense pain, possibly persisting after rash resolution. This condition, termed post herpetic neuralgia (PNH), is the most frequent complication of herpes zoster episodes, developing in up to 20% of cases.

Since almost 90% of adults have been exposed to VZV, 1/3 of the general population is expected to develop herpes zoster in their lifespan. As cell-mediated immunity plays a critical role in maintaining the virus in a latency state, any condition associated with its impairment, including ageing and HIV infection, is associated with higher risk of reactivation. [12]

Since 2006, a live attenuated vaccine was approved for people over the age of 50, but its effect waned over time, was only suboptimal in elderly, and because of the risk of disseminated infection it was contraindicated in immunosuppressed patients. [3]

A recombinant subunit vaccine (HZ/su) was then developed to overcome these limitations. It contains viral glycoprotein E (gE), which is the most abundant protein both on viral envelope and on the surface of infected cells and a predominant target for immune system, as specific gE response was found to correlate with anti -VZV neutralizing antibodies. To increase vaccine immunogenicity adjuvant system AS01B was included in vaccine formulation. Pivotal studies investigating immunological effect of HZ/su vaccine defined vaccine response rate (VRR). For humoral immunity VRR was intended as 4-fold increase of anti-gE specific antibodies concentration over baseline. For cell-mediated immunity it was set as 2-fold increase in frequency of gE-specific polypositive CD4+ T-lymphocytes (LTCD4) by intracellular cytokine staining assay. [4]

This novel recombinant vaccine showed high VRR and efficacy in preventing both HZ and PNH in immunocompetent subjects, maintained across age groups and over time. [5] [6][7] Recently, HZ/su vaccine efficacy, safety and immunogenicity were confirmed in various immunosuppressed categories, including stem cells and solid organ transplant receivers. [8–10][11]

However, very scarce data are nowadays available about people living with HIV (PLWHIV), with only one small phase 1/2 trial reporting acceptable safety and immunogenicity in this particular population, including both subjects on-ART and not.[12] Thus robust data about safety, immunological response and efficacy of recombinant herpes zoster vaccine in PLWHIV are lacking; moreover, PLWHIV with low CD4+ T lymphocyte count could be significantly less likely able to generate a strong antibody and cellular immune response to vaccination. Therefore HZ/su vaccine immunogenicity needs to be evaluated among the subgroup of subjects with low LTCD4.

1.2 Rationale

The present study will evaluate immunogenicity and safety of HZ/su vaccine in PLWHIV aged 18 years and older, according to current LTCD4 count (cells/mm3). Two strata will be defined based on immunological status, with threshold set at 350 cells/mm3, suggestive of substantial immunodepression.

A two-dose vaccine schedule, as recommended by national guidelines, will be administered.

1.3 Hypothesis

1. HZ/su recombinant vaccine is immunogenic in PLWHIV eliciting both humoral and cell mediated specific immunity with high vaccine response rate (VRR) in the group of patients on-ART with high LTCD4 cell count, but poorer in those with lower LTCD4.
2. HZ/su recombinant vaccine is safe and has acceptable reactogenicity profile in PLWHIV in real life scenario, with a potential different grading according to LTCD4 count.

2 Objectives

2.1 Primary Objectives

To evaluate HZ/su vaccine immunogenicity through the analysis of humoral immunity and vaccine response rate (VRR) in PLWHIV according to current LTCD4 absolute count (cells/mm3).

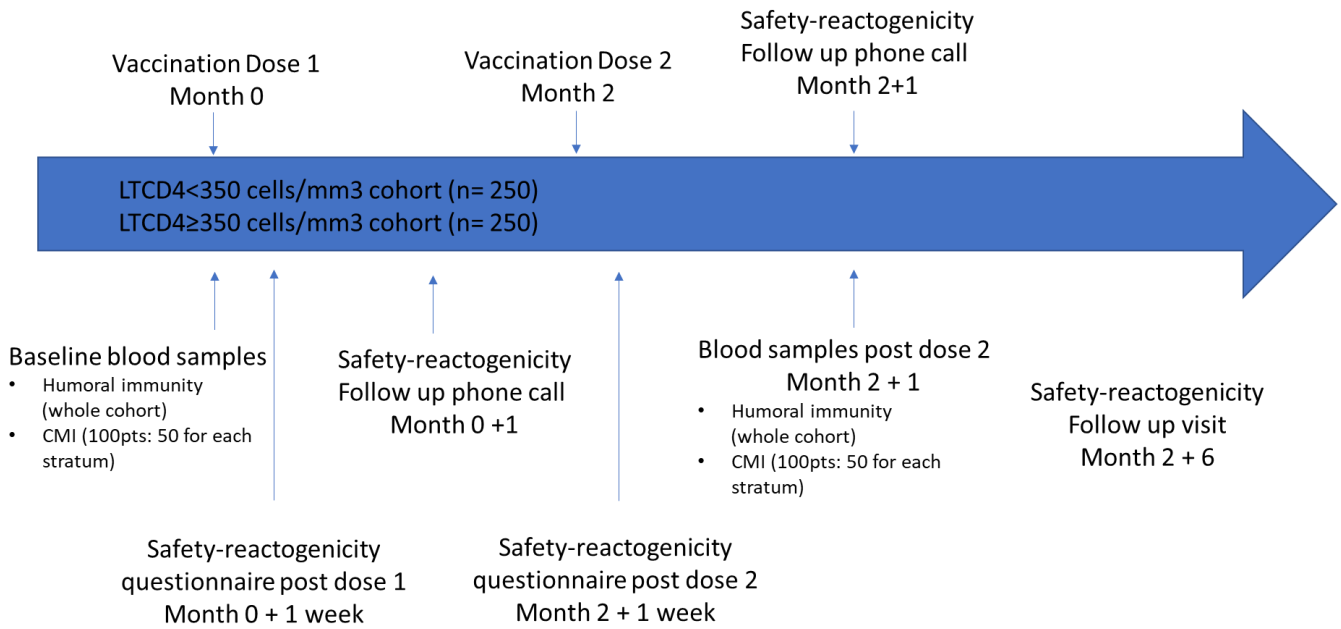
2.2 Key secondary objectives

- To verify HZ/su reactogenicity and safety profile after each of the two scheduled doses in PLWHIV according to current LTCD4 absolute count (cells/mm3).
- To evaluate HZ/su vaccine immunogenicity through analysis of cell-mediated immunity and vaccine response rate (VRR) in PLWHIV according to current LTCD4 absolute count (cells/mm3).
- To verify HZ/su vaccine immunogenicity through analysis of humoral and cell-mediated immunity and vaccine response rate (VRR) in PLWHIV according to LTCD4/CD8 ratio.

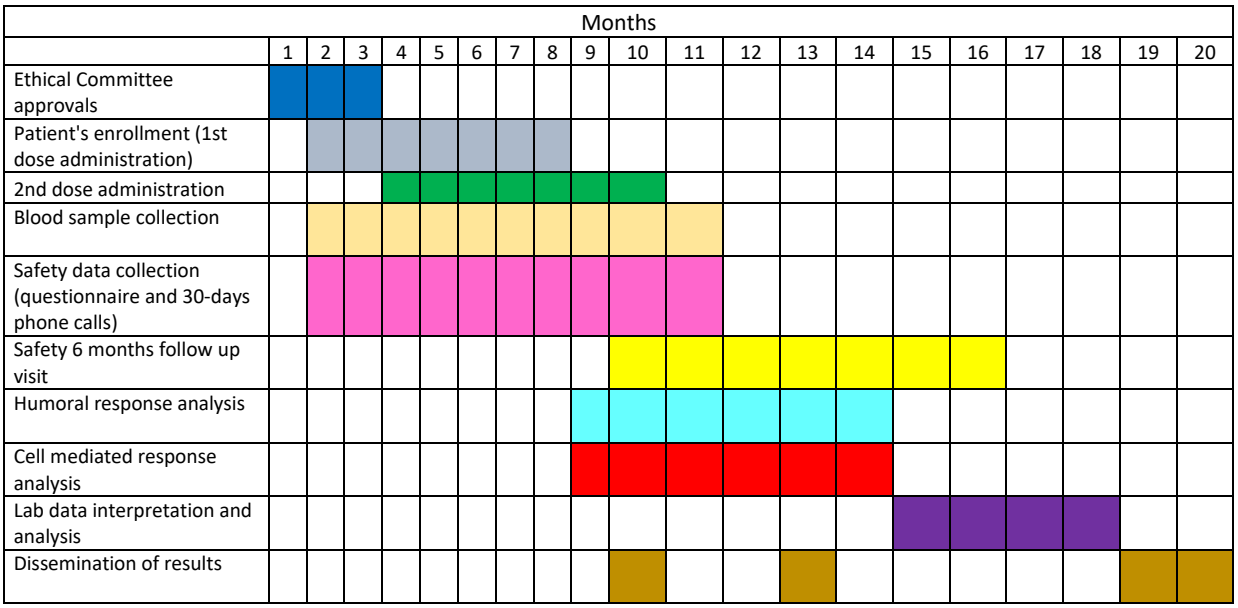
3 Study design

- Experimental design: multicentric observational prospective study, including two cohort of on-ART and virally suppressed PLWHIV, based on immunological situation (LTCD4 <350/≥350 cells/mm3).
- Vaccination schedule: two-doses schedule, administered at month 0 and 2.
- Blood sampling schedule: blood samples will be collected at baseline (M0) and one month after second dose administration (M2 +1) for humoral and cell-mediated immunity analyses.
- Data collection: data will be collected via Electronic Case Report Form (eCRF).
- Duration of the study: 20 months including 6-months safety follow up visit (M2 +6).

Study diagram



Study Gantt chart



4 Study cohort

4.1 Study population

We plan to enroll 500 PLWHIV on antiretroviral treatment, virally suppressed (HIVRNA <50 copies/ml in the last 6 months), stratified by current LTCD4 absolute count (cells/mm3) as follows:

- 250 subjects with LTCD4<350 cells/mm3
- 250 subjects with LTCD4≥350 cells/mm3

Three Italian Infectious Diseases centers will participate the study:

- San Paolo Hospital, University of Milan
- Tor Vergata Polyclinic, Tor Vergata University, Rome
- Umberto I Polyclinic, La Sapienza University, Rome

4.2 Inclusion criteria

- PLWHIV ≥18 years old, on antiretroviral treatment, virally suppressed (HIVRNA <50 copies/ml in the last 6 months)
- Able to provide informed consent
- Available CD4 cell count in the last 6-months

4.3 Exclusion criteria

- Age <18 years old
- Active herpes zoster episode at enrollment
- Acute disease/fever at enrollment
- Known allergy to vaccine component
- Other than HIV immunosuppressive condition or treatment. Prednisone < 20 mg/day, or

equivalent., is allowed. Inhaled and topical steroids are allowed

- Pregnancy
- Lactation
- Women planning to become pregnant or planning to discontinue contraceptive methods

5.Conduct of the study

5.1 Ethical considerations and Informed consent

The study will be conducted in accordance with the International Conference for Harmonization Good Clinical Practice (ICH-GCP) regulations and guidelines and the current revision of the Declaration of Helsinki. The study and any subsequent amendment will be submitted to the centralized Ethics Committee and notified in each Ethics Committee of the centers involved. HZ/su vaccine will be administered as part of good clinical practice, as recommended by national guidelines. No other intervention besides peripheral blood sample withdrawal will be introduced. Subjects will provide their free and written informed consent for study participation, including consent for blood withdrawals and for the personal data processing.

5.2 Data collection

Data will be collected in eCRF, assigning unique numerical code to each participant.
General demographic as well as HIV- and VZV-related data will be collected at baseline (M0).
Safety and reactogenicity data will be investigated through a semistructured questionnaire after each of the two scheduled vaccine doses (within one week from doses administration, M0+1W, M2 +1W).
Unsolicited adverse events will be assessed by follow up phone calls, 30 days after each dose (M0 +1, M2 +1). A final follow up visit 6 months after schedule completion (M2 +6) will be performed, assessing any other adverse event occurred later on.

5.3 Biological samples

Blood samples will be taken at baseline (first dose, M0) and one month after the second dose (M2 +1), to estimate:
- gE specific humoral response
-cell-mediated response as intracellular cytokine staining (ICS) assay for polypositive LTCD4 frequency, Interferon Gamma Release Assay (IGRA) upon gE stimulation, and plasma cytokines production through supernatants analysis by Ella assay.
Samples will not be labeled with any information directly referable to patients, as unique numerical code attributed to each subject will be used.

In detail the following samples will be collected at the two time points:

- 5ml clot activator whole blood for ELISA
- 30ml EDTA whole blood for ICS
- 6ml heparin whole blood for IGRA and Ella

Humoral response will be assessed in the whole 500 participants, while cell-mediated immunity through ICS, IGRA, and Ella assays will be performed in 100 patients, 50 from each LTCD4 strata.

6 Study procedures

6.1 Baseline data collection

At baseline, presence of inclusion criteria and absence of exclusion criteria will be verified. Eligible patients will be offered to participate the study. After informed consent signature, the following data will be collected at baseline (M0) during first HZ/su dose administration from review of clinical records:

- demographic data including age, sex, ethnicity, mode of HIV transmission
- presence of comorbidities, HBV and HCV serologic status
- HIV related data including previous or current AIDS diagnosis, LTCD4 nadir, current LTCD4 cell count and percentage, current LTCD4/CD8 ratio, time on ART, time on viral suppression, current ART regimen.
- VZV related features: VZV Ab, previous HZ episodes, previous HZ live attenuated vaccine.

Data regarding comorbidities with special regard to new onset immunosuppressive conditions or immunomodulatory treatments, as well as other vaccines administration within 30days from HZ/su, will be reassessed at visit two (M2).

6.2 Vaccination

Vaccines will be stored in temperature-controlled refrigerators located in each study center, as appropriate according to manufacturer indications.

In absence of any contraindication, patients will receive a 0.5ml intramuscular injection, preferably at non-dominant arm deltoid, containing 50µg of VZV glycoprotein E antigen reconstituted with AS01B adjuvant system.

The second dose will be administered 2 months (±14 days) after the first one, in line with manufacturer indications.

A standard 15-minutes observation period for rapid reactions prevention will be applied, prolonged to 30 minutes for patients with history of previous reaction to other drugs/vaccines.

Vaccine schedule will be interrupted in case of:

- severe allergic reaction following the first dose
- severe adverse event following the first dose, deemed related to vaccination by the investigator
- pregnancy

Dose two administration will be postponed (up to six months after dose one) in case of acute illness at the scheduled appointment.

6.3 Humoral immunity analysis

Anti-glycoprotein E (gE) antibodies concentration will be assessed for the whole population at baseline (M0) and one month (± 14 days) after the second Hz/su vaccine dose (M2+1).

ELISA

The magnitude of the anti-glycoprotein E (gE) antibody response is determined by using an in-house ELISA assay. Briefly, high-binding 96-well plates (Greiner Bio-One, Austria) are coated with 3µg/ml of recombinant VZV gE protein in coating buffer (carbonate-bicarbonate 0.5mM pH 9.6 in H2O) and incubated overnight at 4°C. Plates are washed with PBS-0.05% Tween-20 and blocked for 1 h with PBS-2% BSA at 37°C. Plasma samples are diluted in PBS-1% BSA in triplicates, added to plates, and incubated for 2 hours at 37°C.

The anti-varicella zoster virus (VZV) 90/690 reference (National Institute for Biological Standards and Control) will be used as reference standard curve. To detect total gE-specific IgG antibodies the biotinylated goat anti-human IgG is used at 1:2500 (ThermoFischer Scientific). Plates are incubated with the secondary antibody and avidin-HRP diluted at 1:2000 (ThermoFischer Scientific) for 30min at RT in the dark and mild agitation. The detection was carried out with 1X3,3',5,5'-Tetramethylbenzidine (TMB) (Invitrogen) and

quenched with 1M H2SO4. Sample optical density (OD) is measured by using Tecan SunriseTM at 450nm and 620nm, and results expressed as IU/ml.

6.4 Cell-mediated immunity analysis

In a subgroup of 100 patients (50 for each LTCD4 stratum), cell-mediated immunity will be investigated at baseline (M0) and one month (+/- 14 days) after the second HZ/su vaccine dose (M2 +1).

Intracellular cytokine staining (ICS) assay

Thawed PBMCs are left resting for 3 hours at 37°C and stimulated with a pool of 15-mer sequences with 11 amino acids overlap, covering the complete sequence of the VZV gE protein. Staphylococcal Enterotoxin B (SEB) (1µg/ml) is used as positive control, whereas negative controls are left untreated. Brefeldin A (1 mg/ml, Sigma Aldrich) is added after 2 hours of stimulation. After 18 hours, cells are harvested and stained for surface markers 20 min at 4°C in the dark; after paraformaldehyde fixation (1%, Sigma-Aldrich), cells are permeabilized with Saponin 0.2% (Sigma-Aldrich) and stained for intracellular cytokines for 30 min at room temperature (RT). Antibodies used are: CD4-APC-Vio770, CD8-PerCP-Vio700, CD3- PE, IFN-γ-VioBlue, IL-2-APC, TNF-A-PE-Vio770, and CD40L-FITC (Miltenyi Biotec, Bergisch Gladbach, Germany). Dead cells are labeled using Viobility Fixable Dye (Miltenyi Biotec, Bergisch Gladbach, Germany). Samples are acquired with FACSVerse (BD Biosciences), and data analyzed by using FlowJo software 10.7.2 (BD Biosciences). Unspecific activations in unstimulated controls are subtracted from stimulated samples to account for specific activation.

IFN-γ release assay (IGRA) and extracellular cytokine profile by Ella

To assess T-lymphocyte specific response, 500 µl of fresh heparinized blood will be incubated overnight at 37◦C and 5% CO2, with pools of lyophilized peptides, consisting mainly of 15-mer sequences with 11 amino acids overlap, covering the complete sequence of the VZV gE protein at a final concentration of 1µg/ml. For each stimulation a negative and positive (SEB, 1µg/ml final concentration) condition will be included. After 18 hours (16-20 hours) of incubation, supernatants will be harvested and stored at -80◦C. IFN-γ production will be assessed with a Simple PlexTM Ella Assay (ProteinSimple, CA, USA) on EllaTM microfluidic system (Bio-Techne, Minneapolis, USA), following manufacturer's instructions. IFN-γ response will be defined as peptide or SEB stimulated condition minus unstimulated condition. Furthermore, supernatants will be also analyzed for the production of different other cytokines (such as IL-2, TNF-α, IL-4, IL-10) using the EllaTM microfluidic system (Bio-Techne, Minneapolis, USA), to assess the polyfunctional profile of stimulated T-Lymphocytes. The development of an IGRA test, would represent a valuable tool for assessing cell-mediated response to VZV gE peptide, easily applicable to clinical settings.

6.5 Safety profile evaluation

Adverse events occurring from first dose administration (M0) to end of follow up (M2 + 6) will be recorded. One week after both first and the second dose (M0 +1w, M2 +1w) patients will be given a semi-structured questionnaire investigating local and systemic symptoms, similar to the one used in previous studies by our working group [14] . The questionnaire will address the occurrence and grading of solicited adverse events, both local and systemic, after vaccine administrations. Solicited adverse events will include pain, redness or swelling/induration of injection site, fever, fatigue, headache, gastrointestinal symptoms, myalgia.
The questionnaire may be filled in by the subject via a dedicated online form, or by the clinician during telephone interview, according to patients' preference.
Unsolicited adverse events will be assessed through follow up phone calls, that will be scheduled 30 days (±7 days) after each dose.

Subjects will be instructed to contact the study site in case of adverse events at any moment during follow up. Severity of the symptoms will be assessed referring to FDA's Toxicity Grading Scale [15], in particular whether these events interfere or prevent daily activity (Grade 3), require ER visit or hospitalization (Grade 4) or interruption of the vaccine schedule.
The investigator will establish relationship with vaccine administration after excluding other possible causes, according to clinical judgment.
A follow up visit assessing possible further adverse events and/or HZ episodes will be scheduled six months (±1 month) after HZ/su vaccine dose 2 (M2 +6).

As adjuvanted vaccines are considered at potential risk for triggering autoimmune conditions, potential immune-mediated diseases (pIMDs), will be assessed throughout study period as adverse events of special interest (AESI) together with HZ and PHN cases, and reported to manufacturer as appropriate.
In detail, pIMDs are inflammatory and/or neurological conditions possibly due to autoimmune disordes[16].
Suspected herpes zoster episodes during study period will be investigated at their occurrence, and, in case of clinical confirmation, vaccine administration will be suspended. A new-onset cutaneous rash will be clinically confirmed as herpes zoster if unilateral and painful, especially if accompanied by vesicular lesions, in absence of any alternative diagnosis.
Subjects participating the study will be informed about signs and symptoms suggestive of herpes zoster and will be warned to contact the study site in case of suspect episodes.

Pregnancy cases will be recorded and reported to manufacturer as appropriate and vaccine schedule will be interrupted, as HZ/su vaccine is not currently approved for administration in pregnant women.

Local Symptoms Grading according to FDA's Toxicity Grading Scale [15]

	Grade 1 - Mild	Grade 2 - Moderate	Grade 3 - Severe	Grade 4 – Potentially life threatening
Pain	Does not interfere with activity	Repeated use of nonnarcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Erythema	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis

Systemic symptoms grading according to FDA's Toxicity Grading Scale [15]

	Grade 1 - Mild	Grade 2 - Moderate	Grade 3 - Severe	Grade 4 – Potentially life threatening
Fever (C°)	38.0-38.4	38.5-38.9	39.0-40.0	>40.0

Nausea/vomiting	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhea	2 – 3 loose stools or < 400 gms/24 hours	4 – 5 stools or 400 – 800 gms/24 hours	6 or more watery stools or > 800gms/24 hours or requires outpatient IV hydration	ER visit or hospitalization
Headache	No interference with activity	Repeated use of nonnarcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization

7. Data evaluation

7.1 Primary endpoints

Humoral vaccine response rate (VRR), according to LTCD4 cell count strata, defined as 4-fold increase over baseline (prior to vaccination) anti-gE concentration (mUI/ml) by ELISA

7.2 Secondary endpoints

- Safety profile/reactogenicity, according to LTCD4 count:
    - Discontinuation of scheduled vaccine administration due to severe adverse events
    - Occurrence and relationship to vaccination of severe (Grade 4) local and systemic, solicited and unsolicited symptoms from dose 1 administration to end of follow up (M2 + 6).
    - Occurrence and relationship to vaccination of moderate (Grade 3) local and systemic, solicited and unsolicited symptoms from dose 1 administration to end of follow up (M2 + 6).
    - Occurrence and relationship to vaccination of AESI (pMID, HZ and PHN) from dose 1 administration to end of follow up (M2 + 6).
- Cell- mediated vaccine response rate (VRR), according to LTCD4 cell count strata, defined as:
    - ICS: 2-fold increase over baseline (prior to vaccination) for frequency of gE-specific polypositive LTCD4, expressing at least 2 activation markers among IFN-γ, IL-2, TNF-α, and/or CD40L by intracellular cytokine staining assay;
    - IGRA: 2-fold increase over baseline (prior to vaccination) for IFN-γ production upon gE stimulation.
- Vaccine response rate (VRR) according to LTCD4/CD8 ratio (< vs ≥1), defined as:

    - Humoral response: 4-fold increase over baseline (prior to vaccination) anti-gE concentration (mUI/ml) by ELISA
    - Cell-mediated response:
        - ICS: 2-fold increase over baseline (prior to vaccination) for frequency of gE-specific polypositive LTCD4, expressing at least 2 activation markers among IFN-γ, IL-2, TNF-α, and/or CD40L by intracellular cytokine staining assay;
        - IGRA: 2-fold increase over baseline (prior to vaccination) for IFN-γ production upon gE stimulation.
- Evaluation of polyfunctional profile of stimulated T-Lymphocytes through analysis of extracellular cytokine (IL-2, TNF-α, IL-4 and IL-10) production in supernatants

7.3 Statistical plan and data analysis

Patient characteristics according to LTCD4 cell count strata (<350, ≥ 350 cells/mm3), will be described with median and IQR for continuous variables, absolute and relative frequencies for categorical ones. Kruskal-Wallis and Chi-square test will be used for the comparisons, as appropriate.
For the primary endpoint of vaccine response rate (VRR), a binary endpoint will be constructed to evaluate the humoral response in PLWH and compare the two groups (CD4<350 vs CD≥ 350 cells/mm3):
4-fold increase at 1-month after 2nd dose compared to baseline anti-gE concentration (mUI/ml)

As secondary endpoints two other binary endpoints will be evaluated:

- cell-mediated response (Intracellular Cytokine staining): 2-fold increase at 1-month after 2nd dose compared to baseline in the frequency of gE-specific LTCD4 cells, expressing at least 2 activation markers among IFN-γ, IL-2, TNF-α, and/or CD40L.
- cell-mediated response (IFN-γ release assay): 2-fold increase at 1-month after 2nd dose compared to baseline (prior to vaccination) in the IFN-γ production upon gE stimulation.

Accordingly, 3 separate logistic crude and adjusted regression models for primary and secondary endpoints will be fitted, to estimate the effect of LTCD4 cell count strata at vaccination and on vaccine response rate. Age, CD4 at nadir, hepatitis co-infections, ethnicity and concomitant administration of other vaccines will be considered confounders.
In addition, the vaccine response rate will be evaluated with a second exposure of interest, LTCD4/CD8 ratio; ratio will be used as continuous variable or stratified in two classes (<1 vs ≥1). Unadjusted and adjusted regression models, with the same set of covariates previously described, will be used to investigate this association.
Anti gE-Ab concentration at 1-month after 2nd dose will be evaluated as continuous variable by calculating the mean difference (with 95% confidence interval) in concentration according to LTCD4 strata and compared by univariable and multivariable linear regression models.
IL-2, TNF-α, IL-4 and IL-10 levels in stimulated T-Lyphocytes before and after vaccination, will be quantified to evaluate the difference in cytokine profile production in the two LTCD4 strata. Mean difference from baseline to 1 month after completion of the primary cycle will be compared using ANOVA and linear regression models according to LTCD4.
Local and systemic symptoms/adverse events will be presented as counts, percentages, and associated 95% confidence intervals. Univariate and multivariable logistic regression models will be used to evaluate the association between LTCD4 strata and the occurrence of moderate and severe symptoms after each dose.
Despite the observational approach, we do not expect a huge amount of missing information for key variables, particularly for the main exposure of interest, as availability of LTCD4 in the last 6 months is among inclusion criteria. For the primary outcome, as reported below, we expect a drop-out rate <10% that will not have a negative impact on the power of the study. In case of an increase of the proportion of drop-out (which means missing vaccine response data post second dose) over 10 % we will consider to increase

the recruitment of more than 500 patients in the study, and eventually the informative censoring will be controlled, using inverse probability of censoring weights (IPCW). We do not expect substantial number missing data also for the main covariates listed above, if one of these showed a significant proportion of missing information (e.g. nadir CD4) a multiple imputation approach could be used to address this issue. Analyses will be conducted using Stata version 14 (StataCorp LLC College Station, TX).

7.4 Sample size

For the primary endpoint humoral VRR, we consider the overall VRR of 98.5% from the general population, reported by Lal H. et al. and a proportion of VRR ranging from 92.3%-98.1% for the HIV population[12].
A VRR difference of 10% or more between the two immunological strata (LTCD4 < 350 cells/mmc vs LTCD4 ≥350 cells/mmc) would be significant in clinical practice.
Using the upper estimate as reference value for the LTCD4 ≥350 cells/mmc group (98.1%), with a total of 500 subjects enrolled (1:1 ratio in the 2 groups), an alpha=0.05 we will have a power ≥80% to detect a risk difference in VRR of 5.4% or more in the < 350 cells/mmc group vs ≥350 cells/mmc, if the dropout rate during the study remains ≤10%..
Using the same setting but considering the lower estimate (92.3%) for the LTCD4 ≥350 cells/mmc group, we will be able to detect a risk difference of 8.6% or more. Considering as a possible scenario the midpoint 95.2% of VRR for LTCD4 ≥350 cells/mmc group, we will be able to detect a risk difference of 7.4% or more with a power ≥80%. Power analyses are performed with the 'two-sample proportions test Pearson's chi-squared test' using Stata version 14.0.
Drop-out rate was estimated based on previous experience, as during SARSCoV-2 vaccine related immunological study, that required serial blood withdrawals, a drop-out rate close to 20% was observed. Since the present Hz/su vaccine protocol only requires one extra access to clinics for blood sample withdrawal (M2+ 1), and one final follow up visit (M2 +6), we expect a lower drop-out rate, further reduced by frequent contact with healthcare personnel (safety questionnaires at M0+7, M2+7, follow up phone calls for unsolicited events at M0+ 1, M2 + 1).

		VRR			
N.patients/group	**dropout**	**LTCD4≥ 350**	**LTCD4< 350**	**Risk difference**	**power**
250	10%	98.1%	93.1%	-5.0%	73.6%
250	10%	98.1%	92.5%	-5.4%	80.3%
250	10%	98.1%	92.0%	-6.0%	84.1%
250	10%	98.1%	91.0%	-7.0%	91.0%
250	10%	95.2%	88.2%	-7.0%	76.9%
250	10%	95.2%	88.2%	-7.4%	80.6%
250	10%	95.2%	87.2%	-8.0%	85.2%
250	10%	95.2%	86.2%	-9.0%	91.0%
250	10%	92.3%	84.3%	-8.0%	75.3%
250	10%	92.3%	83.7%	-8.6%	80.3%
250	10%	92.3%	83.3%	-9.0%	83.3%
250	10%	92.3%	82.3%	-10.0%	89.2%

8 Limitations of the study

Patients' hesitancy towards a relatively new vaccine as HZ/su could negatively affect enrollment process. However, most of the selected sites serve as vaccination centers for subjects who are in active follow up. Therefore, expert personnel are involved in vaccine proposal and generally achieve high acceptance rates, as recently shown with largely successful anti SARSCoV-2 vaccination campaign.
Moreover, data regarding cell-mediated immunological response obtained from a restricted subgroup of subjects may underrepresent the whole enrolled population.
We expect to partially overcome this limitation by selecting those patients from the different LTCD4 categories whose basal characteristics are representative of each stratum.
Since our sample excludes subjects not currently taking antiretrovirals or with residual HIV replication, no conclusion can be drawn regarding Hz/su administration, immunogenicity and safety in these categories. Our choice was to try to overcome possible confounding effects played by active viral replication on immunological dynamics after HZ/su vaccine administration.

9. Publication plans

- EACS abstract 07/24: Reactogenicity
- CROI abstract 09/24: Preliminary immunological response
- SIMIT abstract 10/24: Preliminary immunological response
- Journal of infectious Diseases/AIDS paper 06/25: Complete results

10. References

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