Title: Protocol of a randomized controlled trial characterising the immune responses induced by varicella-zoster virus (VZV) vaccination in healthy Kenyan women: setting the stage for a potential VZV-based HIV vaccine

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SUPPLEMENTARY MATERIAL

Table 1S. KAVI-VZV-001 Laboratory Screening Tests

Test for	Type of Sample	Method
HIV-1 and HIV-2	Blood (serum)	HIV RAPIDS (Determine and UniGold) BIOELISA HIV1+2 Ag/Ab
VZV antibodies	Blood (plasma)	VIDAS® Varicella-Zoster IgG test kit
Hepatits B	Blood (plasma)	BIOELISA HBsAg V3.0
Hepatitis C	Blood (plasma)	BIOELISA HCV V4.0
Pregnancy	Urine	hCG-Combo Test
Urinalysis	Urine	Siemens Multistix 10 SG
Syphilis	Blood (plasma)	Rapid Plasma Reagin Test for Syphilis Screening
Vaginal trichomoniasis	Vaginal Swab	Wet prep showing Trichomonas vaginalis pH>4 or in pouch TV culture system
Bacterial vaginosis	Vaginal Swab	Gram stain-score by Nugent criteria pH>4
Vaginal candidiasis	Vaginal Swab	KoH prep or Gram stain, showing yeast, low pH<4
Neisseria gonohorreae	Vaginal Swab	Multiplex PCR - Xpert® CT/NG test (Cepheid)
Chlamydis trachomatis	Vaginal Swab	Multiplex PCR - Xpert® CT/NG test (Cepheid)
Haemotology	Blood	Complete blood count (haemoglobulin, hematocrit, erythrocytes, leukocytes, platelets)
Coagulation	Blood (plasma)	INR

SAMPLE COLLECTION AND MANAGEMENT

Blood Collection and processing

BD Vacutainer® SST, sodium citrate, EDTA, or sodium heparin tubes are used for blood collection. Plasma isolated from EDTA and sodium heparin tubes are stored at -80ºC for future analysis. Peripheral Blood Mononuclear Cells (PBMCs) are isolated by gradient centrifugation using Histopaque®- 1077 Hybri-Max (Sigma-Aldrich) from blood collected with sodium heparin tubes. A fraction of the PBMCs isolated are analyzed fresh in the laboratory and another fraction is stored at -150ºC for future analysis.

Cervico-vaginal specimens collection and processing

Participants are instructed to refrain from sexual intercourse, douching, or vaginal or rectal insertion of any products for at least 48 hours prior to the collection of specimens. If a participant has not complied with the instructions above, samples are still collected and the time of last sexual activity or details about the product and time of use recorded on the Case Report Form (CRF) and accounted for during analysis. If a participant is experiencing cervical bleeding, the sample collections are re-scheduled and the cause of bleeding assessed and manage appropriately by the trial physician. Cervico-vaginal secretions are collected using a flexible plastic cup (Softcup™, Instead) which is inserted into the vagina and removed after 20 minutes. The secretions collected in the softcup are diluted six fold using a protease inhibitor solution (Calbiochem) and stored at -80ºC. Cervical cells are collected using a cytobrush (Digene®, Qiagen) which is gently inserted in the cervical canal and turned 360º one time to collect the cells. Two cytobrushes are collected and placed in a tube containing supplemented R10 (RPMI medium [Sigma] containing 10^5 units/L penicillin [Sigma], 100 mg/L streptomycin [Sigma], 1.25 mg/L amphotericin [Sigma], 50 mg/L

gentamycin [Sigma] and enriched with 10% Fetal Bovine Serum [Sigma]). Cervical cells isolated from the cytobrushes are used fresh for cytometric analysis.

Rectal Specimens collection and processing

Rectal secretions and rectal biopsies are collected with a proctoscope in place. Rectal secretions are collected using two sponges (Merocel®, Medtronic) pre-moistened with 50 µL of PBS which are inserted in the rectal cavity (one at the time), left in contact to the mucosal surface for 5 minutes and then transferred to the top chamber of the spin-X tube filter 0.22 µm (Costar) containing 300 µL of protease inhibitor solution (Calbiochem). Rectal secretions are extracted from the chamber by centrifugation and stored at -80ºC. Rectal biopsies (10 punches) are collected using a Sarrat disposable forceps (STE1500, Stericom) and placed in a tube containing supplemented R10. Nine out of 10 rectal punch biopsies are digested using collagenase type II (Sigma) and used fresh for cytometric analysis. One punch biopsy is stored in Cryomatrix (ThermoScientific) at -150ºC for future analysis.

Saliva collection and processing

Participants are instructed to refrain from eating for 2h and drinking for 30 min prior to saliva collection. Two sponges (Salivette®, Sarstedt) (one at the time) are inserted at one side of the mouth (close to the parotid duct gland) for 5 min and then removed, placed into a tube and kept on ice. Saliva is extracted from the sponges by centrifugation, diluted two fold using protease inhibitor solution (Calbiochem) and stored at -80ºC.

LABORATORY MEASUREMENTS

Cellular Immune Activation

Multicolor flow cytometric analysis are performed on mononuclear cells isolated from blood, cervix and rectum and stained using pre-determined concentrations of the following antibodies: LIVE/DEAD Far Red Cell Stain Kit (Invitrogen); anti-CD3 APCeFluor780 (clone SK7), anti-HLA-DR FITC (clone L243), anti-CD49d PE (clone 9F10), anti-Ki67 PECy7 (clone 20Raj1) (eBioscience); anti-CD4 BV510 (clone SK3), anti-CD38 PE-CF594 (clone HIT2), anti-CCR5 BV421 (clone 2D7) (BD Horizon); anti-CD69 (clone FN50), and anti-Integrin β7 PECy5 (Clone FIB504) (BD Pharmingen). Samples are acquired on an LSRII flow cytometer driven by the DiVa software package (BD Biosciences). Analysis of the acquired data is performed using FlowJo software (Tree Star, Inc.).

VZV-specific Cellular Responses

Mononuclear cells isolated from blood, cervix and rectum are stimulated with a pool of peptides comprised of overlapping 15-mer peptides derived from VZV glycoprotein E (mixture of 124 individual peptides at 0.4 μg of individual peptide/test) and VZV Open Reading Frame 4 (ORF4) protein (mixture of 88 peptides, 0.35 μg of individual peptide/test) (Mimotopes, Victoria, Australia) in presence of 1 μg/mL of anti-CD28 and anti-CD49d (BD FastImmune). Two hundred (200) μL of ressuspended cells were used in each test. Stimulation with staphylococcal enterotoxin B (SEB, Sigma) (0.6 μg/test) or DMSO (1.6%) (Sigma) is used as positive and negative controls respectively. After 2h of incubation at 37°C with 5% CO_2, the secretion inhibitor containing monensin (GolgiStop™ BD Bioscience) is added to the samples and the

cells incubated for additional 3h. Stimulation is quenched by adding 2 mL of supplemented R10 to each tube. The cells are rested overnight at 4°C and stained in the following day with pre-determined concentrations of the following antibodies: LIVE/DEAD Far Red Cell Stain Kit (Invitrogen); anti-CD3 APCeFluor780 (clone SK7), anti-CD8 Alexa Fluor 700 (clone RPA-T8), anti-CD45RA PECy5 (clone HI100), anti-CCR7 PE (clone 3D12), anti-IFNγ eFluor450 (clone 4S.B3), anti-IL-2 FITC (MQ1-17H12) (eBioscience); anti-CD4 BV510 (clone SK3) (BD Horizon); anti-TNFα PECy7 (clone MAb11) (BD Pharmingen). Samples are acquired on an LSRII flow cytometer driven by the DiVa software package (BD Biosciences). Analysis of the acquired data is performed using FlowJo software (Tree Star, Inc.).

VZV-specific IgA and IgG detections

Antibodies to VZV are quantified by an in-house direct enzyme-linked immunosorbent assay (ELISA) using 96 well high-binding Costar® plates (Corning Incorporate Corning NY, USA) coated with VZV glycoprotein (EastCoast Bio Inc, Maine, USA). Biotin-conjugated goat anti-human IgG (KPL, Gaithersburg, MD, USA) or biotin-conjugated goat anti-human IgA (KPL, Gaithersburg, MD, USA) in combination with Horseradish peroxidase streptavidin (HRP-streptavidin) (KPL, Gaithersburg, MD, USA) and 3,3',5,5'-Tetramethylbenzidine (TMB) Liquid Substrate (Sigma-Aldrich) is used to identify the VZV-specific immunoglobulins present in the human specimens (plasma, saliva and cervicovaginal and rectal secretions). The standardization and validation of this procedure for the different specimens are ongoing (manuscript under preparation).

Measurement of Cytokines and Chemokines Concentrations

Electro-chemiluminescence U-Plex system (Meso Scale Discovery) is used to measure the concentration of 14 cytokines (IL-1α, IL-1β, IL-6, IL-8, IL-10, IL-17A, IFNγ, GM-CSF, IP-10, MIP-1α, MIP-1β, MIP-3α, TNF-α and IL-4). All samples are run in duplicate following the manufactures' instructions.

STORAGE OF BIOLOGICAL SAMPLES

Biological samples are stored at KAVI-ICR, and the University of Toronto. Ancillary studies not described at KAVI-VZV-001 protocol will be subjected to analysis by the ethics boards and performed only upon their approval and only for samples whose donors agreed to their use for future unspecified research and have declared that in the Informed Consent Form.

OUTCOMES FOR KAVI-VZV-001 TRIAL

Table 2S: Outcomes for KAVI-VZV-001 trial

Outcome	Hypothesis	Outcome measure
Primary: Cervical mucosal immune activation after VZV-vaccination	Vaccination against varicella does not result in elevated immune activation after 12 weeks of immunization	Change in frequency of cervical $CD38^{+}HLA\text{-}DR^{+}CD4^{+}$T cells 12 weeks after VZV vaccination compared to baseline
Secondary:		
a) Effector VZV-specific $CD8^{+}$T cells at the rectal mucosa after VZV vaccination	Vaccination against varicella boosts effector VZV-specific $CD8^{+}$ T cells response at rectal mucosa	Change in frequency of rectal VZV-specific IFNγ-producing $CD8^{+}T_{EM}$ cells 12 weeks after VZV vaccination compared to baseline
b) Pro-inflammatory cytokines/chemokines secretion after VZV-vaccination	Vaccination against varicella does not result in elevated pro-inflammatory signature after 12 weeks of immunization	Change in level of pro-inflammatory cytokines/chemokines in blood, cervix and rectum measured at 12 weeks after VZV vaccination compared to baseline
Tertiary:		
a) VZV-specific effector $CD8^{+}$ T cells in blood after VZV vaccination	Vaccination against varicella boosts effector $CD8^{+}$T cells response in blood	Change in frequency of IFNγ-producing $CD8^{+}T_{EM}$ cells in blood 4 weeks after VZV vaccination compared to baseline

Outcome	Hypothesis	Outcome measure
b) Memory phenotype and VZV-specific T cell function in blood and rectum	The levels of VZV-specific cellular responses vary during the study due to vaccination or VZV subclinical reactivation	Memory phenotype and VZV-specific function in $CD8^+$ and $CD4^+$ T cells in blood and rectum longitudinally throughout the study.
c) Longitudinal immune activation profile in blood, cervix and rectum	Immune activation is enhanced after vaccination against varicella, but it returns to basal levels within 12 weeks post-vaccination	Expression of activation markers such as CD38, CD69, Ki67 and HLA-DR, HIV co-receptor CCR5 and mucosal homing markers in $CD4^+$T cells in blood, cervix and rectum longitudinally throughout the study.
d) Mucosal VZV-specific IgG and IgA antibodies after VZV vaccination	Vaccination against varicella boosts mucosal VZV-specific humoral immune responses	Change in concentration of VZV-specific IgG and IgA in saliva, cervico-vaginal and rectal secretions 4 weeks after VZV vaccination compared to baseline
e) VZV-specific IgG and IgA antibodies in blood after VZV vaccination	Vaccination against varicella boosts VZV-specific humoral immune responses in response in blood	Change in concentration of VZV-specific IgG and IgA in blood 4 weeks after VZV vaccination compared to baseline
f) Longitudinal VZV-specific humoral responses in blood and mucosal secretions	The levels of VZV-specific humoral responses vary during the study due to vaccination or VZV subclinical reactivation	VZV- specific IgG and IgA concentrations in plasma, saliva, cervico-vaginal and rectal secretions longitudinally throughout the study.
Exploratory:		
a) Immune correlations between different sampling sites (blood, cervix and rectum)	Some of the immune responses assessed are tightly correlated between sites, allowing for them to be used as surrogate markers in future clinical studies	Correlation between immune responses assessed in different tissues including expression of activation markers such as CD38, CD69, Ki67 and HLA-DR, HIV co-receptor CCR5, mucosal homing markers in $CD4^+$T cells, cellular and humoral VZV-specific responses
b) Herpesviruses prevalence at KAVI-VZV-001 cohort	Epidemiological data on herpesviruses will shed some light on their prevalence in Africa	Varicella-Zoster Virus (VZV), Herpes Virus-2 (HSV-2), Cytomagalovirus (CMV), Epstein Barr (EBV) at KAVI-VZV-001 cohort
c) Acceptability and feasibility of mucosal sampling at KAVI-VZV-001 cohort	Pre-screening sections adopted during recruitment phase promotes participants' awarness about the study objectives, procedures and their rights reducing the stress associated with some of the sampling procedures and promoting a good retention rate in the study	Acceptability and feasibility of mucosal sampling assessed by questionnaire and participant compliance to the study visits
d) Association between Nugent scoring (used for bacterial vaginosis - BV - diagnostic), pro-inflammatory cytokine signature, cellular immune activation and microbiome	A portion of our study cohort changes their BV status during the study and we hypothesize that this change is associated with changes in pro-inflammatory cytokine, cellular immune activation and microbiome signatures	Comparison between changes in BV nugent score and changes in pro-inflammatory cytokine, cellular immune activation and microbiome signatures (such as *Gardnerella, Prevotella, Mobiluncus, Sneathia sp and Lactobacillus sp*)

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