PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Protocol of a Randomized Controlled Trial Characterizing the Immune Responses Induced by Varicella Zoster Virus (VZV) Vaccination in Healthy Kenyan Females: Setting the Stage for a potential VZV-based HIV Vaccine.
AUTHORS	Perciani, Catia; Jaoko, Walter; Walmsley, Sharon; Farah, Bashir; Mahmud, Salaheddin; Ostrowski, Mario; Anzala, Omu; KAVI-ICR, Team; MacDonald, Kelly

VERSION 1 - REVIEW

REVIEWER	Salim S. Abdool Karim CAPRISA, South Africa
REVIEW RETURNED	16-May-2017

GENERAL COMMENTS	This protocol to assess the immune responses to a candidate HIV vaccine based on a VZV vector is a well written and well designed, randomized trial with 2 groups of women in Kenya (each n=22) receiving either VZV vaccine at baseline or at 12 weeks post-enrolment and followed up for 48 weeks. Major comments: 1. This is an important study for the future of VZV-vectored vaccines. The goal of this study was to assess whether VZV vaccination had an effect on the women's immune activation state both systemically and in the genital/rectal mucosa. In addition, the study set out to assess the effect of VZV vaccination on VZV-specific circulating and mucosal humoral and cellular responses. This is an important step in developing a VZV-vectored vaccine since vectored vaccines (such as Ad5) may paradoxically lead to increased risk of HIV acquisition, presumably through immune activation. With persistently replicating vectors, the potential problem of immune activation may become a long-term challenge once vaccinated. Hence, this study becomes important to assess this potential problem that may increase HIV acquisition, especially at the mucosal sites. 2. The primary endpoint is a reasonable choice even though it is not a known correlate of increased HIV risk in vaccine studies. The choice of measuring immune activation in cervical CD4+T cells through co-expression of CD38 and HLA-DR at 12 weeks post-vaccination is a reasonable and pragmatic approach. Importantly, the study does include measurement of pro-inflammatory genital cytokines, a known correlate of HIV acquisition in women. 3. The study would be strengthened by including as assessment of the vaginal microbiome as this is a major determinant of immune

	activation in the genital tract, especially in settings where sexually transmitted infections and bacterial vaginosis is common. 4. Since the study started about 2 years ago and involves just 44 women with 48 weeks of follow-up, the study may be completed or reaching completion by now. Minor comments: Line 81 – consider cutting 'effector' as duplicate Line 104 – cut 's' in 'sthe' Line 149 - The participant number should be more clearly stated upfront in the study population section, rather than at the end of the sample size justification section. Line 277 – 'thorough' not 'thoroughly' Line 282 and 284 – add 'the' RV144 trial Line 286 'in' rather than 'with' Line 364 Competing interests: 'None to declare' should be removed as the authors do mention a competing interest.

REVIEWER	***Tony Cunningham*** The Westmead Institute for Medical Research 176 Hawkesbury Road Westmead NSW 2145
REVIEW RETURNED	26-May-2017

GENERAL COMMENTS	With regard to the study design key prior publications have not been acknowledged and there are some misinterpretations and inadequate preliminary data. Essentially they wish to immunize their VZV seropositive subjects with concentrated live attenuated Oka strain varicella vaccine which is similar to the concentration used in Zostavax, intramuscularly. Indeed the protocol is similar to the Zostavax trials but in younger people. Are they using Zostavax? They do not cite or acknowledge previous papers by the Weinberg and Levin laboratory and others which define the systemic humoral and immune response to Zostavax, including data showing induction of central memory as well as effector memory CD4 T cells. They presume that the live attenuated virus will reactivate throughout life like wild type virus. Most importantly they presume that the systemically administered vaccine will induce VZV specific and/or non-specific activated CD4 T cells which will home to cervical and rectal mucosa. What is the evidence for the latter? NB In the discussion they also state that the use of such a vaccine could have a dual purpose in preventing both varicella and HIV but here they are using recall immunization with 'Zostavax' not primary varicella immunization

VERSION 1 – AUTHOR RESPONSE

Reviewer 1: Dr. Abdool Karim

- We thank Dr. Abdool Karim for his thorough revision of this manuscript. As indicated below, the manuscript has been altered to include his corrections and suggestions. We also take this opportunity to further discuss some of the points raised by him.

Major comments:

1. This is an important study for the future of VZV-vectored vaccines. The goal of this study was to assess whether VZV vaccination had an effect on the women's immune activation state both systemically and in the genital/rectal mucosa. In addition, the study set out to assess the effect of VZV vaccination on VZV-specific circulating and mucosal humoral and cellular responses. This is an important step in developing a VZV-vectored vaccine since vectored vaccines (such as Ad5) may paradoxically lead to increased risk of HIV acquisition, presumably through immune activation. With persistently replicating vectors, the potential problem of immune activation may become a long-term challenge once vaccinated. Hence, this study becomes important to assess this potential problem that may increase HIV acquisition, especially at the mucosal sites.

2. The primary endpoint is a reasonable choice even though it is not a known correlate of increased HIV risk in vaccine studies. The choice of measuring immune activation in cervical CD4+T cells through co-expression of CD38 and HLA-DR at 12 weeks post-vaccination is a reasonable and pragmatic approach. Importantly, the study does include measurement of pro-inflammatory genital cytokines, a known correlate of HIV acquisition in women.

3. The study would be strengthened by including as assessment of the vaginal microbiome as this is a major determinant of immune activation in the genital tract, especially in settings where sexually transmitted infections and bacterial vaginosis is common.

- We agree with Dr. Abdool Karim about the importance of incorporating a thorough analysis of the vaginal microbiome in this cohort.
During the course of this study (approximately 48 weeks), changes in the bacterial vaginosis (BV) status in a subset of our cohort is expected. Here we have the opportunity to characterize the vaginal microbiome before BV onset, during infection and after BV treatment and correlate changes in the microbiome to the pro-inflammatory cytokine signature and markers of cellular immune activation. This analysis has been placed as an exploratory outcome in the Supplementary Material (Table 2 S, Page 7).

4. Since the study started about 2 years ago and involves just 44 women with 48 weeks of follow-up, the study may be completed or reaching completion by now.

- The study is currently ongoing. We expect to complete sample collection within the calendar year. The longitudinal and mucosal-based nature of KAVI-VZV-001 and its stringent eligibility criteria required an elaborated recruitment strategy. This strategy constituted of multiple informative sections (4 up to 6 sections), creating several opportunities for the volunteers to discuss the objectives and requirements of this study with the study staff and with external people they wished at the same time that the study staff could evaluate some of the eligibility criteria and assess participants compliance to the sections. This design, despite a prolonging recruitment phase, successfully allowed for the enrollment of highly motivated and committed volunteers.

Minor comments:

Line 149 - The participant number should be more clearly stated upfront in the study population section, rather than at the end of the sample size justification section.

- The reviewer's observation is very pertinent. We have included a sentence in the Study Design section stating the number of participants to be enrolled in the study (Page 7, Lines 146).

a. Line 81 – consider cutting 'effector' as duplicate
b. Line 104 – cut 's' in 'sthe'
c. Line 277 – 'thorough' not 'thoroughly'
d. Line 282 and 284 – add 'the' RV144 trial
e. Line 286 'in' rather than 'with'
f. Line 364 Competing interests: 'None to declare' should be removed as the authors do mention a competing interest.

- The corrections (a to f) were done as suggested.

Reviewer 2: Dr. Cunningham

- We thank Dr. Cunningham for his revision of this manuscript and insightful comments and take this opportunity to address the questions raised by him.

1. With regard to the study design key prior publications have not been acknowledged and there are some misinterpretations and inadequate preliminary data. Essentially they wish to immunize their VZV seropositive subjects with concentrated live attenuated Oka strain varicella vaccine which is similar to the concentration used in Zostavax, intramuscularly. Indeed the protocol is similar to the Zostavax trials but in younger people. Are they using Zostavax? They do not cite or acknowledge previous papers by the Weinberg and Levin laboratory and others which define the systemic humoral and immune response to Zostavax, including data showing induction of central memory as well as effector memory CD4 T cells.

- As stated under section "Study Agent and Randomization" (Page 9, Lines 168-169), we utilize here the commercial live attenuated VZV vaccine (Zostavax®, Merck). It is not a coincidence that the study holds some similarities to the Zostavax® clinical trials: we deliberately administered the Zostavax® vaccine following the dose and route of administration recommended by the manufacturer, since we built this trial on the long-standing use of live-attenuated VZVOka to prevent varicella and zoster infections and their well-described safety and protective profiles(1-6). Moreover, it is based on the premise that VZV vaccination induces protective cellular and humoral responses against the virus, including effector memory T cells - as correctly stated by Dr. Cunningham - that this study has been conceptualized7-9. However, in order to explore VZV as a potential vector in an HIV vaccine, we need to further characterize the type of immune responses induced at the genital and gastrointestinal mucosa, sites of primordial importance for HIV infection. Thus, our aim is in contrast to previous studies involving VZVOka vaccine, including Zostavax® trials. We aim to characterize the immune responses at the cervical and rectal mucosa of individuals rather than focusing on systemic immunity because in the context of HIV protection, there is evidence that mucosal effector responses are particularly important. Additionally, we propose to determine important aspects of immune activation in both mucosa and blood, which have not been elucidated in the context of VZV yet. We have also focused first on the VZV pre-immune population because the risk of excessive immune activation in the CD4+ T cell population (whether mucosal or systemic) could potentially increase risk of HIV acquisition.
We have amended the manuscript to include a more detailed description of the prior findings involving VZVOka vaccines, which set the ground for this work.

2. They presume that the live attenuated virus will reactivate throughout life like wild type virus. Most importantly they presume that the systemically administered vaccine will induce VZV specific and/or non-specific activated CD4 T cells which will home to cervical and rectal mucosa. What is the evidence for the latter?

- Live-attenuated VZV vaccines have been shown to establish latency in neural ganglia and the immune responses induced against the virus to boost either by endogenous or exogenous re-exposures(7,10). There is also evidence that VZV can establish latency in enteric neurons and undergo reactivation in the gut(11,12). Furthermore, it has been recently described that VZV can alter cell signaling to augment T cell skin trafficking(13). Therefore it is plausible to hypothesize that VZV-specific cells could also be found at the mucosa, which will be tested in this trial. Here we also seek to characterize the level of immune activation prior and post VZV vaccination to determine whether vaccination could enhance the influx of activated CD4 T cells, i.e. targets for HIV infection, to the mucosa.

3. In the discussion they also state that the use of such a vaccine could have a dual purpose in preventing both varicella and HIV but here they are using recall immunization with 'Zostavax' not primary varicella immunization

- In our discussion (Page 15, Lines 300-303) we suggest a possible scenario where a VZV-based HIV vaccine could serve a dual role preventing both VZV and HIV infections. We would like to clarify that this statement was to merely display another potential advantages of adopting VZV as a vector for HIV antigens, but that we do not claim that this study will test it. For that concept to be actualized, we hypothesize that VZV negative children would need to receive a combination VZV-HIV in early childhood and be boosted at puberty. The aims of this trial remain to assess the effects of VZV vaccination in the mucosal immune responses in individuals with pre-immunity to VZV.

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VERSION 2 – REVIEW

REVIEWER	Salim S. Abdool Karim CAPRISA, South Africa
REVIEW RETURNED	07-Jun-2017

GENERAL COMMENTS	None. All my comments have been addressed

REVIEWER	Anthony L Cunningham The Westmead Institute for Medical Research and University of Sydney
REVIEW RETURNED	18-Jun-2017

GENERAL COMMENTS	The trial proposal is improved by the Zostavax references. It is still a doubtful proposition that Zostavax will induce specific memory T cells that persist in the genital mucosa, despite the references from the Gershons about gut mucosa. I would have preferred a pilot study addressing this issue before the fully fledged trial but accept that this will now be done in the context of the trial.