PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Dynamics and Determinants of HPV Infection from the Michigan HPV and Oropharyngeal Cancer (M-HOC) Study: Study Protocol
AUTHORS	Eisenberg, Marisa; Campredon, Lora; Brouwer, Andrew; Walline, Heather; Marinelli, Brittany; Lau, Yan; Thomas, Trey; Delinger, Rachel; Sullivan, Taylor; Yost, Monica; Goudsmit, Christine; Carey, Thomas; Meza, Rafael

VERSION 1 – REVIEW

REVIEWER	Fiona Guerra Public Health Ontario, Ontario, Canada
REVIEW RETURNED	03-Mar-2018

GENERAL COMMENTS	This protocol describes an ambitious study with the potential to provide data to fill multiple evidence gaps related to HPV infection and related cancers. I don' recall reading about what determines the end of the study. There was a brief description of the study start date and goals in terms of number of participants, but unclear what determines the close of recruitment, for example. Other than recruiting 1000 participants, what other factors would end recruitment? Perhaps I missed it. I have made some comments for the authors to consider in the attached PDF file (_FG). In particular, I feel strongly that the criteria of "have female genitals" for the sub-study is problematic. This is usually phrased as having a cervix because the cervix is the site tested for HPV, not the labia, vagina or any other part of female genitalia. In addition, having the criteria be having a cervix, takes away the possibility of exclusion of those with ambiguous genitalia or trans folks who may have a cervix but do not identify as having female genitalia. I do not recall reading any reference to benefits of participation for the participants. In particular, I think it would be great for a study such as this to provide participants with information e.g. pamphlets, a card etc for cancer screening sites, Pap smear clinic hours, clinics offering HPV vaccine or info on the vaccine. For what it's worth, it would have been great for rectal cytology/"rectal pap" to be included in this study given that to my knowledge, there are so few opportunities for this type of screening given few clinicians trained to perform it, and weak data present. Thanks for the opportunity to review. I look forward to the study results!

REVIEWER	Elizabeth Stankiewicz Machado Universidade Federal do Rio de Janeiro, Brazil
REVIEW RETURNED	18-Mar-2018

GENERAL COMMENTS	The protocol addresses important questions regarding oral persistence of HPV, but there is a few points that need clarification. They are listed below: 1. What will be the definition for persistence, and how the authors will deals with different situations such as: missing visits, re-detection of HPV infection after one negative genotyping, etc.... 2. The authors could include a sample size calculation. It was not explained why their enrollment size would be 1000. What would be the power of such a sample size? Do they have an estimated of HPV prevalence among patients already enrolled? 3. An important point is related to HPV vaccine coverage among the population that will be studied and how it would impact their results. It needs to be addressed. 4. In the abstract the authors state that the goal of the study would be evaluate HPV infection and its relationship to sexual history and long-term OPSCC risk but it is not clear if 3 years of study will be enough to stablish the relationship with OPSCC, therefore it needs to be clarified. 5. In the limitations the authors should include limitations related to genotyping and use of salive samples. 6. In the introduction the authors could include references regarding prevalence of HPV in their region (if it exists) and some references about persistence of oral HPV and time of persistence and development of OPSCCs

REVIEWER	Eduardo Franco McGill University, Montreal, Canada.
REVIEW RETURNED	19-Mar-2018

GENERAL COMMENTS	This is a useful natural history investigation of oral HPV infection. It is larger than most studies up until now, has an adequate number of follow-up visits and includes specimen collection for genital infections. If brought to the target sample size and completion, this cohort investigation is likely to advance our knowledge on the fascinating topic of oral HPV epidemiology. Arguably, not many things can be said about a study design that is already established via a funded grant and approved by an IRB. However, a few items should be addressed by the authors. Strictly speaking the HerSwab sample is not a cervical specimen; it is a vaginal self-sample. A cervical sample should be called as such only for a provider-collected exfoliated sample from the ecto- and endocervix. This was not what the authors described. The title of the project as “Dynamics and Determinants of HPV Infection: The Michigan HPV and Oropharyngeal Cancer (M-HOC) Study” is not appropriate because of the low age of participants and the short duration of follow-up, which do not permit the authors to study cancer as an outcome. The repeated-measurement design will, however, allow them to study patterns of HPV acquisition, persistence, and clearance for different HPV types. It is not clear how the authors will use the collected data and findings to inform their simulation models of transmission. Why are they taking precautions to preserve RNA in the collected samples? What plans do they have?

	How will they define clearance of an infection? Will they use one or two consecutive visits free of HPV or free of an HPV type that is the target of a specific outcome analysis?

VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 1

This protocol describes an ambitious study with the potential to provide data to fill multiple evidence gaps related to HPV infection and related cancers.

I don't recall reading about what determines the end of the study. There was a brief description of the study start date and goals in terms of number of participants, but unclear what determines the close of recruitment, for example. Other than recruiting 1000 participants, what other factors would end recruitment? Perhaps I missed it.

> Thank you for pointing out this oversight. We have added the actual recruitment dates and numbers for Phase I of this study, as well as the statistical power.
>
> > "Phase I of this study recruited between April 2015 and December 2017, enrolling 395 participants. At this sample size and level of significance 0.05, we will detect the difference at baseline between two equally sized populations with HPV prevalence 10% and 20% with 80% power. Assuming each participant completes 10 visits, we will detect the difference between 10% and 13% ever HPV positive with more than 80% power. At the time of submission, 321 participants had completed at least one follow-up visit, and 1,693 baseline and follow-up visits had been completed. Follow-up visits are ongoing. Pending funding, phase II is anticipated to recruit a similar number of participants (potentially with additional study locations as well)."

I have made some comments for the authors to consider in the attached PDF file (_FG).

> Thank you for these comments and alerting us to the typos. We have corrected them.

In particular, I feel strongly that the criteria of "have female genitals" for the sub-study is problematic. This is usually phrased as having a cervix because the cervix is the site tested for HPV, not the labia, vagina or any other part of female genitalia. In addition, having the criteria be having a cervix, takes away the possibility of exclusion of those with ambiguous genitalia or trans folks who may have a cervix but do not identify as having female genitalia.

> Thank you for your helpful comments here. The phrasing "have female genitals" was an attempt to be more inclusive than having "be female" as the criterion. Unfortunately, "have a cervix" is not an accurate inclusion criterion for us, for two reasons. First, participants who have had a hysterectomy that removes their cervix are not excluded from the cervicovaginal sub-study. Second, the swab that participants use (HerSwab) is a vaginal self-swab designed to sample the vagina near the cervix (as Reviewer 3 points out). Nevertheless, we very much

appreciate your suggestion, and we have rephrased the inclusion criterion as “have a vagina,” which is accurate and, we hope, more inclusive.

I do not recall reading any reference to benefits of participation for the participants. In particular, I think it would be great for a study such as this to provide participants with information e.g. pamphlets, a card etc for cancer screening sites, Pap smear clinic hours, clinics offering HPV vaccine or info on the vaccine.

Thank you for your attention to the participants. Our staff are knowledgeable and make themselves available to discuss vaccination and screening with interested participants. Because of the nature of the sexual and behavioral questionnaire, we also have pamphlets for the UM Sexual Assault Prevention and Awareness Center available to participants. Finally, we will disseminate population-level results to participants through newsletters. We have added this information to the manuscript.

For what it's worth, it would have been great for rectal cytology/"rectal pap" to be included in this study given that to my knowledge, there are so few opportunities for this type of screening given few clinicians trained to perform it, and weak data present.

We absolutely agree. We would have liked to include an anal swab, and we are considering the logistics of including this for Phase II of this study. However, given the self-sampling nature of this study and the primary focus on oral HPV infection, we have not included that option for participants at this time.

Thanks for the opportunity to review. I look forward to the study results!

We appreciate your helpful and positive comments!

Reviewer: 2

The protocol addresses important questions regarding oral persistence of HPV, but there is a few points that need clarification. They are listed below:

1. What will be the definition for persistence, and how the authors will deals with different situations such as: missing visits, re-detection of HPV infection after one negative genotyping, etc....

Thank you for your comments—we have made the definition of our outcomes more explicit:

> “Outcomes of interest include, but are not limited to, HPV prevalence (detection of HPV, or detection of a specific HPV genotype), incidence (detection of HPV in a previously uninfected person, or detection of a specific HPV genotype in a person who previously tested negative for that genotype), persistence (detection of HPV at subsequent study visits, or detection of specific genotypes at subsequent study visits), and clearance (non-detection of HPV in a previously infected person, or non-detection of a specific HPV genotype in a person previously infected by that genotype). Other patterns of HPV detection, such as patterns of intermittent detection of the same genotype or detection of an HPV genotype at the oral site after previous detection at the genital site (or vice versa), will be considered.”

We will handle missing visits by adjust for the time between visits as appropriate. Other patterns of detection, like the one you mention, are of interest to us (see excerpt above), and our treatment of them will necessarily depend on their frequency of occurrence in the study.

2. The authors could include a sample size calculation. It was not explained why their enrollment size would be 1000. What would be the power of such a sample size? Do they have an estimated of HPV prevalence among patients already enrolled?

Thank you for pointing out this oversight. We have added the actual recruitment dates and numbers for Phase I of this study, as well as the statistical power.

> "Phase I of this study recruited between April 2015 and December 2017, enrolling 395 participants. At this sample size and level of significance 0.05, we will detect the difference at baseline between two equally sized populations with HPV prevalence 10% and 20% with 80% power. Assuming each participant completes 10 visits, we will detect the difference between 10% and 13% ever HPV positive with more than 80% power. At the time of submission, 321 participants had completed at least one follow-up visit, and 1,693 baseline and follow-up visits had been completed. Follow-up visits are ongoing. Pending funding, phase II is anticipated to recruit a similar number of participants (potentially with additional study locations as well)."

3. An important point is related to HPV vaccine coverage among the population that will be studied and how it would impact their results. It needs to be addressed.

Yes, HPV vaccination status is a very important covariate, and we are sorry that we were not clear that participants self-report their HPV vaccination status. We have highlighted the inclusion of HPV vaccination status in the Social and Sexual Behavior Survey, and it is listed it as a covariate in the statistical analysis. We will also be looking at the demographic and behavioral determinants of HPV vaccination.

4. In the abstract the authors state that the goal of the study would be evaluate HPV infection and its relationship to sexual history and long-term OPSCC risk but it is not clear if 3 years of study will be enough to stablish the relationship with OPSCC, therefore it needs to be clarified.

Yes, we were not sufficiently clear that, while the overall MHOC study is broadly investigating HPV and OPSCCs, the epidemiological study we are describing in this protocol paper is only designed to evaluate the association of sexual, behavioral, and demographic covariates with HPV incidence, clearance, and persistence. We are not evaluating OPSCC risk within the epidemiological component of our study. A separate component is developing models to predict and simulate OPSCC at the population level (see eg Brouwer et al, PLOS One 2016). We have edited the title, abstract, and introduction accordingly.

5. In the limitations the authors should include limitations related to genotyping and use of saliva samples.

We have added the following limitations to our discussion:

> "The quality of our saliva and oral rinse specimens may depend on the saliva production and swishing efficacy of each participant, although this is mitigated by the sensitivity of the PCR analysis. Finally, we only test for 18 genotypes, which, although we cover all high-risk types, may not give as complete a picture of patterns of mucosal HPV infection*."*

6. In the introduction the authors could include references regarding prevalence of HPV in their region (if it exists) and some references about persistence of oral HPV and time of persistence and development of OPSCCs.

> Thank you for the suggestion. Unfortunately, there is no published data on the prevalence of HPV in our population. However, we have included the following references for persistence of oral and genital HPV and for the time to the development of OPSCC.
>
> > "Estimates from the National Health and Nutrition Examination survey (NHANES) suggest that oral HPV prevalence in the U.S. is about 11–12% in men and 3–4 % in women [11–14]. However, while oral HPV infections may clear relatively quickly (the HPV in Men trial estimated a mean clearance time of approximately 7 months [15]), persistent infections may lead to cancer after several decades (although there are no precise estimates of time-to-cancer for OPSCCs, cervical lesions are estimated to progress to cancer in 10–30 years [16])."

Reviewer: 3

This is a useful natural history investigation of oral HPV infection. It is larger than most studies up until now, has an adequate number of follow-up visits and includes specimen collection for genital infections. If brought to the target sample size and completion, this cohort investigation is likely to advance our knowledge on the fascinating topic of oral HPV epidemiology. Arguably, not many things can be said about a study design that is already established via a funded grant and approved by an IRB. However, a few items should be addressed by the authors.

Strictly speaking the HerSwab sample is not a cervical specimen; it is a vaginal self-sample. A cervical sample should be called as such only for a provider-collected exfoliated sample from the ecto- and endocervix. This was not what the authors described.

> Thank you for pointing this out. Our language choice of language was based on our team's shorthand for our sub-study, but both the swab and the sub-study are better described as "cervicovaginal." We have changed this throughout and made clear that the HerSwab is a self-collection vaginal swab designed to sample near the cervix.
>
> > "Participants fulfilling the eligibility criteria are given the option at each study visit to provide a self-collected cervicovaginal swab sample using a HerSwab in addition to the oral specimen. The HerSwab is a vaginal swab designed to sample near the cervix."

The title of the project as "Dynamics and Determinants of HPV Infection: The Michigan HPV and Oropharyngeal Cancer (M-HOC) Study" is not appropriate because of the low age of participants and the short duration of follow-up, which do not permit the authors to study cancer as an outcome. The

repeated-measurement design will, however, allow them to study patterns of HPV acquisition, persistence, and clearance for different HPV types.

Yes, we were not sufficiently clear that the study we are describing in this protocol is only one part of the overall MHOC study, which is a multi-aim study broadly investigating HPC and OPSCCs. The epidemiological study described here is designed, as you say, to evaluate the association of sexual, behavioral, and demographic covariates with HPV incidence, clearance, and persistence; we are not evaluating OPSCC risk. We have edited the title, abstract, and introduction accordingly.

"Dynamics and Determinants of HPV Infection from the Michigan HPV and Oropharyngeal Cancer (M-HOC) Study"

It is not clear how the authors will use the collected data and findings to inform their simulation models of transmission.

We have updated the manuscript (see edits in the Modeling analysis section) to clarify how we plan to use the collected data to inform transmission models. The sexual behavior questionnaires will be used to create realistic sexual networks that capture attributes like partner degree, rates of partner acquisition, and type of sexual contact. This will be accomplished by drawing each simulated individual from the measured distributions and correlation patterns of these variables in our population to generate simulated populations with sexual behavior patterns similar to those measured in our study (e.g. using approaches based on configuration model methods [27 Newman] to connect our simulated sexual network based on partner history data). We will simulate HPV transmission on realizations of these networks, parameterizing the transmission so that the results are consistent with the observed patterns of prevalence, incidence, and clearance.

Why are they taking precautions to preserve RNA in the collected samples? What plans do they have?

That is a great question. That omission was an oversight. We are attempting to preserve RNA to distinguish between active and latent HPV infections.

"Presence of RNA in addition to DNA will be used to distinguish between active and latent infections."

How will they define clearance of an infection? Will they use one or two consecutive visits free of HPV or free of an HPV type that is the target of a specific outcome analysis?

We have updated the manuscript to be more specific about the HPV-related outcomes (see response to Reviewer 2's similar question). We distinguish between clearance of all HPV genotypes and genotype-specific clearance. We define clearance as a visit with a positive HPV sample followed by a visit with a negative HPV sample. However, we may exclude participants with alternating positive/negative samples if that seems appropriate. All definitions and statistical analyses will be fully explained in publications presenting the data analysis.

VERSION 2 – REVIEW

REVIEWER	Fiona Guerra Public Health Ontario, Ontario, Canada
REVIEW RETURNED	15-Jun-2018

GENERAL COMMENTS	Thank you for your thoughtful and clear responses to my comments. I think the reviewers as a group raised a number of relevant issues, and your responses have resulted in a much stronger manuscript. All the best!

REVIEWER	Eduardo Franco McGill University, Canada
REVIEW RETURNED	27-May-2018

GENERAL COMMENTS	The authors have appropriately addressed the reviewers' concerns and suggestions, including mine. Best wishes.