EVALUATION OF CARRAGEENAN AS AN INTERVENTION TO PREVENT HUMAN PAPILLOMAVIRUS ACQUISITION

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ABSTRACT

Purpose

Measures to prevent human papillomavirus (HPV) are needed, particularly in populations at high risk of HPV acquisition. Previous research demonstrated that carrageenan, a polysaccharide extracted from red seaweed, has potent HPV inhibitory activity. In this thesis I evaluated this research topic by 1) summarizing the existing literature on carrageenan's anti-HPV activity and 2) assessing carrageenan's efficacy in an ongoing randomized controlled trial among gay, bisexual, and other men who have sex with men (gbMSM).

Methods

In the narrative review (manuscript #1), I searched PubMed for primary research articles reporting on carrageenan's inhibitory effect. Ongoing and future studies were identified by searching Clinicaltrials.gov. Eligible records were summarized qualitatively. The Lubricant Investigation in Men to Inhibit Transmission of HPV Infection (LIMIT-HPV) study protocol (manuscript #2) reported on the design and methods of a study evaluating the efficacy of a carrageenan gel among gbMSM. The interim analysis (manuscript #3) reports the results of the primary objective: efficacy of a carrageenan gel against incident anal HPV infection. From February 2016 to December 2019, 255 gbMSM (of the target sample size of 380) were randomized 1:1 to receive either a carrageenan or placebo gel. Anal samples were collected at baseline and each subsequent visit (months 1, 2, 3, 6, 9, 12), and tested for 36 HPV types (linear array assay). The primary outcome is incidence of type-specific anal HPV infection(s) undetected at baseline. The analyses were performed according to the intention-to-treat principle.

Results

The narrative review (manuscript #1) identified 8 eligible records from PubMed and Clinicaltrials.gov. These articles consist of two *ex vivo* and three clinical studies evaluating carrageenan's anti-HPV activity. Three trial protocols were identified. All articles demonstrated carrageenan's anti-HPV activity, supporting the initiation of clinical trials to test carrageenan's efficacy. The study protocol (manuscript #2) provided the framework for the conduct and analysis of the interim analysis of the LIMIT study. Finally, the interim analysis (manuscript #3) of the LIMIT study did not find a significant difference in time to incident anal HPV infection between the intervention and placebo groups (hazard ratio=1.21, 95% confidence interval=0.86-

1.70), suggesting carrageenan does not reduce the risk of incident anal HPV infections in gbMSM. These results did not vary by HIV status. There were significantly more adverse events reported in the carrageenan (59.8%) compared to the placebo (39.8%) arm.

Conclusions

Carrageenan demonstrates *in vitro*, *in vivo*, and clinical anti-HPV activity, which supported the initiation of further clinical testing. A study was designed and conducted to assess the efficacy of a carrageenan gel against incident anal HPV infections. Based on the interim analysis, use of a carrageenan gel does not seem to protect against incident anal HPV infection in the gbMSM population.

RÉSUMÉ

Objectif

Les mesures de prévention du papillomavirus humain (VPH) sont nécessaires, particulièrement dans les populations à haut risque d'acquisition des infections par le VPH. Des études antérieures ont révélé que le carraghénane, un polysaccharide extrait d'algues rouges, est un puissant inhibiteur du VPH. Dans le cadre de mon sujet de thèse, j'ai donc 1) résumé la littérature existante sur l'effet anti-VPH du carraghénane et 2) évalué l'efficacité de la carraghénane dans le cadre d'une étude menée à double insu, avec répartition au hasard, chez les hommes homosexuels, bisexuels et autres ayant des relations sexuelles avec des hommes (HRSHgb).

Méthodes

Dans la revue narrative (manuscrit n°1), j'ai utilisé la plateforme PubMed afin d'extraire les articles scientifiques traitant de l'effet inhibiteur de la carraghénane. Les études actuelles et futures ont été identifiées à l'aide du site internet Clinicaltrials.gov. Les articles admissibles ont été résumés de manière qualitative. Le protocole de l'étude LIMIT-HPV (*Lubricant Investigation in Men to Inhibit Transmission of HPV Infection,* manuscrit n°2), fait état des conceptions et des méthodes destinées à l'étude empirique de l'efficacité d'un gel à la carraghénane, chez les HRSHgb. L'analyse intermédiaire (manuscrit n°3) présente les résultats visés par l'objectif principal, soit l'efficacité du gel de carraghénane contre les infections anales par le VPH. De février 2016 à décembre 2019, 255 HRSHgb (sur un échantillon cible de 380) se sont vu attribuer de manière randomisée soit, le gel de carraghénane, soit un placebo. Des échantillons anaux ont été prélevés au début de l'étude ainsi qu'à chaque visite ultérieure (mois 1, 2, 3, 6, 9, 12). Ces derniers ont été testés pour 36 types de VPH (test à matrice linéaire). Le résultat principal de cette étude correspond à une réduction de l'incidence des infections anales par le VPH de type spécifique, non détectées au début de l'étude. Les analyses ont été effectuées selon le principe de l'intention de traiter.

Résultats

L'examen narratif (manuscrit n°1) a permis d'identifier 8 dossiers admissibles provenant respectivement de PubMed et de Clinicaltrials.gov. Ces articles portent sur deux études *ex-vivo* et trois études cliniques évaluant l'effet anti-VPH de la carraghénane. Trois protocoles d'essai cliniques ont été identifiés. Tous ces articles semblent témoigner de l'effet anti-VPH du carraghénane, supportant le lancement d'essais cliniques évaluant l'efficacité de la carraghénane.

Le protocole d'étude (manuscrit n°2) a par ailleurs fourni un cadre permettant la réalisation et l'évaluation de l'analyse intermédiaire de l'étude LIMIT. Ceci étant dit, l'analyse intermédiaire (manuscrit #3) de l'étude LIMIT n'a pas démontré de différence significative dans le temps avant l'infection par le VPH-anal entre le groupe expérimental et le groupe contrôle (hazard ratio=1,21, intervalle de confiance à 95% =0,86-1,70), suggérant que le carraghénane ne réduit pas le risque d'infections anales par le VPH chez les HRSHgb. Ces résultats ne varient pas en fonction du statut VIH. Le nombre d'effets indésirables signalés dans le groupe carraghénane (59,8 %) était nettement plus élevé que dans le groupe placebo (39,8 %).

Conclusions

Le carraghénane présente un effet anti-VPH *in vitro, in vivo* et clinique, ayant permis de lancer d'autres essais cliniques. Conséquemment, une étude a été menée pour évaluer l'efficacité d'un gel de carraghénane contre les infections anales par le VPH. Compte tenu de l'analyse provisoire, l'utilisation d'un gel de carraghénane ne semble pas protéger la population des HRSHgb contre les infections anales accidentelles par le VPH.

PREFACE AND CONTRIBUTION OF AUTHORS

This thesis consists of a literature view and three manuscripts with bridging text, followed by a discussion of the work and final conclusions. Details of the manuscript and author contributions are provided below.

Manuscript 1:

Laurie C, El-Zein M, Coutlée F, de Pokomandy A, and Franco EL. Carrageenan as a preventive agent against human papillomavirus infection: a narrative review. This manuscript is formatted for submission to *Clinical Microbiology and Infection.* The narrative review manuscript was designed, conducted, and written by CL, and critically reviewed by MZ, AdP, CF, and ELF.

Manuscript 2:

Laurie C, El-Zein M, Tota J, Tellier PP, Coutlée F, Franco EL, de Pokomandy A. Lubricant Investigation in Men to Inhibit Transmission of HPV Infection (LIMIT-HPV): design and methods for a randomised controlled trial. Laurie C, El-Zein M, Tota J LIMIT-HPV study group*, et al*. *BMJ Open* **10**, e035113 (2020). The protocol was conceived and designed by ELF, AdP, FC, and PPT, and JT contributed to grant writing. The study was managed by MZ. CL drafted the manuscript with input from ELF, AdP, and MZ. All authors reviewed the manuscript and approved the final version.

Manuscript 3:

Laurie C, El-Zein M, Tellier PP, Coutlée F, de Pokomandy A, Franco EL. Lubricant Investigation in Men to Inhibit Transmission of HPV Infection (LIMIT-HPV): efficacy of a carrageenan gel in an interim analysis of a phase II, placebo-controlled randomized controlled trial. This manuscript is formatted for the *Journal of Infectious Diseases*. The interim analysis manuscript was written by CL, who also performed the data management while an MSc student and performed all analyses. All authors (ELF, AdP, MZ, FC, and PPT) interpreted the data, critically reviewed the manuscript, and approved the final version.

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CHAPTER 1: INTRODUCTION

1.1 LITERATURE REVIEW

Epidemiology of anal cancer

Each year, 20,000 new cases of anal cancer are observed globally.[1] In the United States (US), anal cancer represents 0.5% of all new cancer cases. Although anal cancer is a relatively uncommon malignancy, both the incidence and mortality of anal cancer are increasing. On average, the incidence of anal cancer increased 2.2% per year from 1975-2015, and the mortality has increased 3.1% per year over 2007-2016.[2] The incidence of anal cancer varies between subgroups. For example, among the general population, anal cancer incidence is 1.9/100,000 persons, and the incidence is higher among women compared to men, 2.2 to 1.6 per 100,000 persons, respectively.[2] Women with a previous history of HPV-related cervical disease[3] and human immunodeficiency virus (HIV) positivity are at higher risk.[4] Among HIV-negative gay, bisexual, and other men who have sex with men (gbMSM), anal cancer incidence is 5.1/100,000 person-years;[5] and among HIV-positive gbMSM, anal cancer incidence increases to 45.9/100,000 person-years.[5] Anal cancer, while rare, is an emerging public health threat, especially in high risk populations, such as HIV-positive and negative gbMSM.

Putative anal cancer precursors

The majority (85%) of anal canal cancer cases consist of squamous cell carcinoma (SCC).[6] The proposed precursor, anal squamous intraepithelial lesions (SIL), and anal cancer tend to occur adjacent to the squamocolumnar junction of the anus.[7] Different classification schemes for pre-cancerous anal lesions exist. The goal of the nomenclature is to distinguish between lesions that are likely or unlikely to progress to anal cancer.[8] Potentially precancerous changes in the anus were first described by pathologists as varying levels of dysplasia: mild, moderate, and severe.[9] When researchers made the connection between HPV and anal dysplasia, terminology similar to the Bethesda System for cervical cancer was adopted: anal intraepithelial neoplasia of grades I, II and III, which was based on histology and graded on the proportion of epithelium affected by the oncogenic changes.[10] In 2012, a unified nomenclature was proposed by the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology through the Lower Anogenital Squamous Terminology Standardization (LAST) project for HPV-associated

squamous lesions of the lower anogenital tract, including anal neoplasia. This proposal recommended a two-tiered system of low-grade and high-grade squamous intraepithelial lesions (LSIL and HSIL), where the HSIL category can be further classified with the intraepithelial neoplasia grading system.[11] This is the system in use today.

Anal HSIL was established as the precursor lesion that leads to anal cancer in a retrospective observational study by Berry et al.[12] While anal LSIL can include condyloma which will not progress to cancer, some LSIL may progress to HSIL.[129] Condyloma are typically associated with low-risk HPV infection, however, they still present an impediment to quality of life, with patients experiencing substantial psychological distress.[13,14] Further, presence of condyloma does not necessarily rule out the presence of high-risk HPV, and coinfection with other HPV types is common. For example, in a cross-sectional study among people living with HIV with low grade lesions, approximately one-third of the lesions were associated with high risk HPV types only; consequently, the authors advised against treating anal condyloma as a low risk lesion, especially in gbMSM living with HIV.[15]

RISK FACTORS

Persistent infection with oncogenic HPV is the primary risk factor for the development of anal SCC.[16–19] A population-based case-control study that included men and women, identified the following risk factors for anal cancer: receptive anal intercourse (for men and women), genital warts, number of lifetime sexual partners, and cigarette smoking.[19] Similarly, a systematic review by van der Zee and colleagues identified smoking, sexual behavior, especially receptive anal intercourse, and HIV infection as risk factors for anal cancer.[20] The major risk factors are discussed below.

Human papillomavirus

HPV is an established risk factor for 8 cancers (cervix, anus, vagina, vulva, penis, oral cavity, oropharynx, and larynx). Of the more than 200 distinct HPV types identified, over 40 types are associated with anogenital infection.[21] The HPV types that infect the anogenital region are classified based on their association with cancer risk: high-, medium-, and low-risk. [22] Infection with low-risk types, such as HPV6 and HPV11, are associated with genital warts, whereas high-

risk types, such as HPV16 and HPV18, are associated with anogenital cancers. Based on oncogenicity and tissue tropism, the alphapapillomaviruses can be divided into three categories: subgenus 1 consists of low oncogenic risk types (HPVs 6, 11, 40, 42, 44, 54), subgenus 2 consists of high oncogenic risk types (HPVs 16, 18, 26, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, 82), and subgenus 3 consists of mostly commensal types (HPVs 61, 62, 71, 72, 81, 83, 84, 89).[23–26]

SCC is strongly linked to HPV infection, where HPV16 is the "most carcinogenic HPV type in the anus".[27] In the late 1980s to early 1990s, researchers made the link between HPV and anal cancer.[28–30] In a case-control study (417 cases) conducted in Denmark and Sweden from 1991-1994, high-risk HPV types were observed in most (84%) anal cancer specimens.[31] An estimated 88% of anal cancers are attributable to infection with high-risk HPV.[32] In a recent systematic review and meta-analysis, the prevalence of HPV16 in anal HSIL among HIV-positive and negative men was 51% and 47%, respectively, and in anal cancers 67% and 82%, respectively.[27] In a cohort study of gbMSM (n=357), prevalence of any HPV type in individuals diagnosed with HSIL was 98%.[33] A cohort study of gbMSM living with HIV in Montréal, Québec identified that 64% of participants with HPV16 at baseline went on to develop anal HSIL within two years of follow-up.[34] A retrospective study of anal cancer in Québec reported an HPV prevalence of 86% in anal cancer biopsies from gbMSM.[35]

High risk sexual behavior

Anal HPV is acquired primarily via receptive anal intercourse in men.[19,36,37] In men, high-risk sexual behavior such as receptive anal intercourse and having multiple sexual partners, in particular, is strongly associated with anal HPV[36,37] and anal cancer.[19] In contrast to the prevalence of cervical HPV infection, which declines after the age of 30, anal HPV infection is stable across most age groups in HIV-negative and positive gbMSM.[38] The stability of prevalent anal HPV infection in men could be due to differences in sexual behavior between women and gbMSM, e.g., gbMSM between 50 and 60 years of age continue to report multiple sexual partners.[39,40] This is consistent with the finding that among HIV-negative gbMSM, having multiple sexual partners was identified as an independent predictor of anal SIL.[36]

Human immunodeficiency virus

According to a systematic review and meta-analysis of multi-national data, there is a clear increase in the yearly anal cancer incidence among HIV-positive gbMSM when comparing the pre and post combined antiretroviral therapy (cART) eras (78 vs. 22 per 100,000 person-years, respectively).[5] While Acquired Immune Deficiency Syndrome (AIDS)-defining cancers have decreased in incidence, anal cancer incidence has increased.[41] In the US, anal cancer is the fourth most common cancer in HIV infected individuals, after non-Hodgkin lymphoma, Kaposi sarcoma, and lung cancer.[42] Results from a population-based study in the US identified that the increase in anal cancer cases over 1980-2005 was strongly influenced by the increase in HIV cancer cases among men, but not women.[43]

A recent meta-analysis reported overall HPV prevalence stratified by HIV status and sex. The goal of the meta-analysis was to quantify the different HPV types present in normal cytology, anal cancer precursors, and finally anal cancer. Overall HPV prevalence was 100% in HIV-positive anal cancer, and 90% in HIV-negative anal cancer for both sexes.[27] Amongst those with normal anal cytology, HPV prevalence was 57% in HIV-negative men and 76% in HIV-positive men.[27] These results are consistent with observations from a multi-cohort study from North America where anal cancer incidence rates were evaluated among HIV-positive and negative gbMSM, other men, and women. Compared to HIV-negative men, the rate ratio for anal cancer among HIV-positive gbMSM was 80.3 (95% CI: 42.7-151.1) and 26.7 (95% CI: 11.5-61.7) for HIV-positive men (after demographic adjustment).[44] Prevalence of HPV is higher in HIV-positive compared to HIV-negative men, as is the risk of anal cancer.[45] Further, the risk of persistent HPV infection is seven times higher in HIV-positive individuals.[46,47] A cross-sectional study (n=1021) of HIV-positive and negative gbMSM found HSIL affected HIV-positive gbMSM at younger ages and more frequently.[48] In a small study (n=62) of Japanese gbMSM and men who have sex with women (MSW), high-risk HPV was more prevalent in HIV-positive than HIV-negative gbMSM, and HSIL was detected more frequently in condyloma in HIV-positive gbMSM than in HIV-negative MSW.[49] In Montréal, Quebec, the HIPVIRG (Human Immunodeficiency and Papilloma Virus Research group) cohort study of gbMSM living with HIV identified 97.9% anal HPV prevalence at baseline, with HPV16 being the most prevalent

type (38.2%).[50] In Toronto, Ontario, a clinic-based cross-sectional study of gbMSM living with and without HIV identified a 67.7% and 51.7% anal high-risk HPV prevalence, respectively.[51] While there is little evidence to suggest HIV is independently associated with HPV-associated cancers, because HIV may suppress immune function related to HPV clearance, this allows HPV-associated lesions to persist and provides time for carcinogenic genetic changes to accumulate.[52]

NATURAL HISTORY

Risk factors that are predictive for the progression of LSIL to HSIL are being immunosuppressed as a result of HIV infection with a low CD4 count, presence of anal HPV infection, and coinfection with multiple HPV types, especially high-risk HPV types.[34,47,54–58]

Since no studies evaluated regression rates of anal SIL prior to 2003, Tong and colleagues performed a retrospective cohort study to estimate regression rates of HSIL. While Tong et al found LSIL progresses to HSIL at a rate of 12.6%, and HSIL progressed to anal cancer at a rate of 1.2% per year, the lesions commonly and spontaneously regress at a rate of 23.5% per person-year.[53] These findings have important implications for current practice, as some believe any HSIL should be treated.[59]

There is insufficient data on the progression of anal precursor lesions to anal cancer.[60] Roberts et al reviewed progression rates of SIL to SCC in 2017.[9] The summarized evidence suggests SIL does progress to SCC; of the six studies reviewed, the rate of progression was 11% over 42 months; 8.6% over 53 months; 12.6% over 18 months; 24.5% over 36 months; and 19.6% over 57 months for prevalent HSIL (or 64 months for incident HSIL). Although the start and end points differed for each study, it appears that the rate of progression is relatively low.[12,53,58,61,62]
In a retrospective observational study by Berry et al, the average progression time to anal cancer among HIV-positive gbMSM with prevalent HSIL was 57 months, whereas for men with incident HSIL, the average progression time was 64 months.[12] This was the first study to demonstrate that HSIL progresses directly to anal cancer. Similarly, two small studies in the early 2000s demonstrated progression from HSIL to anal cancer; however, neither study reported whether the original lesion was prevalent or incident. In the first study, after a median of 5 years,

three of the 35 HIV-uninfected patients developed SCC from multifocal HSIL.[62] In a subsequent study of 446 HIV-positive gbMSM, five men refused treatment for HSIL and developed SCC a median of 8.6 months after their HSIL diagnosis.[63] While these studies demonstrate HSIL can progress to anal cancer, prospective studies are needed to better characterize the rate of SIL progression and regression, in addition to the determinants associated with these changes.[64]

CURRENT PREVENTION STRATEGIES

Male condom

Condoms are moderately effective in reducing HPV transmission.[65,66] They have been shown to only partially protect from HPV transmission in MSW.[67] Among gbMSM, participants who never used condoms were more likely to be infected with any HPV type compared to those who always used condoms, however, this association was not significant for high-oncogenic risk types.[68] Additionally, data from a cross-sectional study of 5371 HIV-positive and 30457 HIV-negative gbMSM in the US (in 2005, 2008, 2011, and 2014) suggests a decline in condom use.[69] Given the high prevalence of anal HPV infection in gbMSM regardless of HIV serostatus, condoms either do not appear to substantially reduce HPV transmission, or few gbMSM always use condoms during sexual encounters.[70] However, clinical evidence suggests that the best protection from HPV infection is obtained by the combination of HPV vaccination and condom use,[71] suggesting condoms offer some protection.

Prophylactic HPV vaccine

Three commercially available prophylactic HPV vaccines are currently on the market. Cervarix®, a bivalent vaccine, protects against HPV16 and 18;[72] Gardasil®, a quadrivalent vaccine, protects again HPV16 and 18, as well as HPV6 and 11,[73] and Gardasil 9® protects against HPV16, 18, 6, and 11, as well as types 31, 33, 45, 52, and 58.[74] These vaccines offer protection against cervical and anal SIL and cancer in women. In 2012, Canada's National Advisory Committee recommended vaccination of boys.[75,76] The committee recommends both Gardasil® and Gardasil®9 for males 9-26 years old, in order to protect them from anogenital warts, pre-cancerous lesions, and anal cancer.[76] Additionally, the vaccines are recommended for immunocompetent individuals infected with HIV of any age.[75] While in Canada, the vaccine is only licensed for men aged 9 to 26, because of the recent change in indication for vaccination in

the US, researchers have suggested that soon the Canadian monograph will be updated to include gbMSM up to 45 years of age.[77]

In a randomized controlled trial (RCT) conducted from 2004 and 2008, the efficacy of the quadrivalent HPV was tested in young males, including heterosexual men and gbMSM who had between 1 and 5 sexual partners, with the primary endpoint of a reduction in incidence of external lesions related to the HPV-vaccine types: HPV6, 11, 16, 18. The vaccine demonstrated a 90% effectiveness at preventing external lesions associated with HPV6, 11, 16, and 18. Additionally, the vaccine was 100% effective at preventing HPV 16 and HPV 18 infection. The vaccine appears to be more effective among heterosexual men than gbMSM; however, this finding was non-significant.[78] In a substudy of the previously mentioned RCT, Gardasil® was shown to reduce the rate of HSIL among gbMSM.[79] Out of the 602 healthy gbMSM, 24 in the placebo group developed HSIL, whereas only five in the vaccine group developed HSIL. These results are promising. However, the benefits of HPV vaccination in older gbMSM are limited, as the vaccine is exclusively prophylactic, and gbMSM could already be infected with vaccine-type HPV.[80] For men previously exposed to HPV, vaccination is moderately effective, and very effective in the HPV-naïve.[81]

Groups at a high risk for anal HPV infection, such as HIV-positive and negative gbMSM, are unlikely to benefit from herd effects that result from vaccinating only women.[5,82] Additionally, the vaccine does not protect against all HPV types. Canada transitioned to gender-neutral vaccination between 2013 and 2016, with six provinces including boys in their vaccination programs,[83] and currently, all Canadian provinces and territories include boys in school-based HPV vaccination programs.[84] Because of the delay in vaccinating boys, several older men are unlikely to be vaccinated, and around half the men who are eligible to be vaccinated are not vaccinated.[85] In Toronto, Ontario, a small qualitative study of gbMSM living with HIV reported most participants had not considered HPV vaccination and believed HPV primarily affected girls or women.[86] Low vaccine uptake and high HPV prevalence indicate the current population of gbMSM are largely unprotected from HPV infection and associated diseases. There is then an urgent need for additional primary prevention measures.

Screening for anal cancer precursors

Among the general US population from 2008-2014, the five-year survival for anal cancer was 81.7% if the cancer was detected early when it was still localized. An anal cancer screening program could be beneficial for detecting anal cancer earlier, as anal cancer survival decreases at later stages of progression.[2] Screening of anal lesions is performed by anal cytology, then high-resolution anoscopy (HRA). An anal screening program is controversial because there is no current evidence of benefit from randomized prospective studies.[87] In 2017, Wasserman et al. reviewed whether screening for and treatment of SIL in HIV-positive gbMSM is justified. He concluded that until there are clinical trials demonstrating a decrease in the incidence of anal cancer, as well as a reasonable morbidity for the repeated treatment of SIL, it should not be performed.[60] Similarly, authors of a systematic review and meta-analysis concluded that until there are clinical trials showing screening and treatment of SIL is beneficial, it should be performed exclusively in a research setting.[5] Some researchers, such as Palefsky, assert that until there is concrete evidence that screening and treatment of SIL does not reduce the incidence and mortality of anal cancer, it would be unethical to withhold treatment;[59] the Anal Cancer/HSIL Outcomes Research (ANCHOR) study aims to answer this question (discussed below).[88] Indeed, no randomized studies were conducted to support screening and removal of cervical lesions to prevent cervical cancer, but it would be unethical to conduct such trials now, given the substantial epidemiological evidence demonstrating that treatment of cervical lesions decreases cervical cancer incidence.[89] Implementation of anal cancer screening and prevention measures is important, especially in groups at high risk for anal HPV-infection and HPV-associated lesions, such as HIV-positive gbMSM.[90] A recent systematic review for screening and detection of anal cancers called for further research is this area prior to the initiation of an anal cancer screening program.[91]

Treatment of HPV infection and HPV-associated lesions

HPV infection is the most common sexually transmitted infection worldwide.[92] Among sexually active adolescents, 50-80% will be infected with HPV.[93] Currently, there is no available treatment for HPV infection; however, HPV-associated lesions can be treated. Yet, treatment of precursor lesions is problematic as there is a high recurrence rate.[94] Since 2016, there were only two RCTs assessing treatment for anal lesions.[95,96] In 2010, Fox et al published the results of an

RCT with 53 HIV-positive gbMSM anal HSIL. The men were randomized to receive either imiquimod, an immune response modifier, or placebo. While a statistically significant difference was not observed between the two groups, there was a trend of reduction in lesion grade for those participants who were randomized to imiquimod.[95] In 2013, Richel et al published another RCT among HIV-positive gbMSM with SIL. They compared treatment of SIL with imiquimod, topical fluorouracil, and electrocautery. The recurrence rate of the lesions was similar amongst all treatment groups.[96]

Since there is a lack of strong evidence for treatment of SIL, there are no consensus guidelines or recommendations for the management of these lesions.[97,98] As screening and treatment of pre-cancerous lesions has not been proven to be effective at reducing anal cancer risk, there is an ongoing RCT to address this uncertainty. The goal of the Anal Cancer/HSIL Outcomes Research (ANCHOR) study is to determine if active treatment of HSIL reduces the incidence of anal cancer (Clinical trials identifier: NCT02135419).[88] HIV-positive men and women are randomized 1:1 with one group receiving treatment (surgery, heat and/or cream), and the other group receiving active monitoring. The study began in 2014 and has an estimated end date of 2022. If successful, this study will provide evidence that screening and treatment for SIL in at-risk groups reduces incidence and mortality of anal cancer, and at an acceptable level of morbidity due to frequent treatment of recurring lesions. Similarly, in Canada, the ongoing HPV Screening and Vaccine Evaluation (HPV-SAVE) study,[99] NCT02503111 aims to evaluate screening and treatment of pre-cancers and cancers among HIV-positive men. Based on data from HIV-positive MSM in North America, an anal cancer disease mathematical model was developed to assist in decision making related to anal cancer prevention.[100] These ongoing trials, complemented by a mathematical model, will provide key information to inform future anal cancer screening programs.

A CARRAGEENAN-BASED LUBRICANT AS A NOVEL PREVENTION METHOD

Carrageenan

Carrageenan is a sulfated polysaccharide derived from red algae. It is a common gelling agent, found in a variety of household items, such as food and cosmetics, as well as personal lubricants.[101] In the late 2000s, researchers demonstrated carrageenan effectively blocks HPV

transmission in vitro[101] and in animal studies.[102,103] Additionally, Rodriguez et al evaluated a carrageenan-based lubricant in the presence of seminal plasma in cell lines and in a mouse model. The results from their study suggest carrageenan maintains its anti-HPV activity even in the presence of seminal plasma.[104]

Carrageenan's proposed mechanism of action

On the cell surface, the attachment of the virion to the cell surface is thought to be mediated by glycosaminoglycans (GAGs), such as heparan sulfate. Carrageenan, a sulfated polysaccharide, mimics heparan sulfate, thereby providing competition against the virion for attachment to the cell surface. Carrageenan may also act via a heparan sulfate proteoglycan core protein (HSPG)-independent mechanism, which is thought to involve carrageenan's interference with conformational changes of the virion or carrageenan's interference with virion binding to other cellular proteins required for the infection process.[101] Carrageenan may also promote clearance of existing HPV infection if the interaction between the virion and host cell receptors is long enough to allow natural inactivation of HPV by the mucosal local innate and adaptive immune systems.[105]

Confirmation in clinical trials

Carrageenan's HPV inhibitory properties must be confirmed in clinical trials in different study populations. Two such trials are ongoing: the Carrageenan gel Against Transmission of Cervical HPV (CATCH) study that evaluates efficacy among heterosexual women[106] and the Lubricant Investigation in Men to Inhibit Transmission of HPV Infection (LIMIT-HPV) study that evaluates efficacy among gbMSM (Clinical trials identifier: NCT02354144).

Interim results from the CATCH study

In 2018, encouraging results from the interim analysis of the CATCH RCT performed by members of our research group were published.[106] Among heterosexual women, the risk of acquiring an HPV type absent at baseline was up to 45% lower in participants who used a carrageenan-lubricant compared to participants who used a placebo-lubricant: hazard ratio 0.64 (0.45-0.89). The gel was well tolerated in the heterosexual women, thereby providing evidence

of safety and acceptability previously observed in pilot studies of both heterosexual women and men.[107–109]

Safety and acceptability of carrageenan

Carrageenan is found in cosmetics and is widely used a stabilizer.[106] Studies indicate it is well tolerated for vaginal use[110,111] and in preliminary results from an RCT in women for cervical HPV infection, there were no serious adverse events, and any adverse events were not attributed to gel use.[106] Rectal application of carrageenan was also found to be safe in mice[112] and in a small human study (n=4).[113]

SUMMARY

In summary, the burden of anal HPV infection, anal cancer precursors, and anal cancer is high in gbMSM, regardless of HIV serostatus. There is a lack of guidelines for the screening and treatment of anal cancer precursors, as there is uncertainty regarding the benefits of such programs. Additionally, vaccine uptake among men is low, vaccines do not protect against all HPV types, and current recommendations exclude gbMSM over 26 years of age. For these reasons, additional prevention strategies are required. A thorough exploration of existing literature on carrageenan and its efficacy is needed. Since carrageenan-based lubricants have the potential to protect against all HPV types. An RCT is necessary to determine carrageenan's potential for both preventing new anal HPV infections and accelerating clearance of existing anal HPV infections in sexually active gbMSM. If successful, such data would provide strong evidence that this novel prevention strategy is effective. A carrageenan-based lubricant could be utilized to improve the health of the gbMSM community.

1.2 THESIS OVERVIEW AND OBJECTIVES

The overall aim of this thesis is to investigate carrageenan's ability to prevent the acquisition of human papillomavirus (HPV) infection. This manuscript-based thesis contains three manuscripts, one for each objective.

Objectives:

1. Summarize the existing evidence of carrageenan's anti-HPV activity and the rationale for its preventive value against anal HPV infection.
2. Report the design and methods of the Lubricant Investigation in Men to Inhibit Transmission of HPV Infection (LIMIT-HPV) randomized controlled trial.
3. Evaluate the efficacy and safety of a carrageenan-based gel in the interim analysis of the LIMIT-HPV randomized controlled trial.

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CHAPTER 2. Carrageenan as a preventive agent against human papillomavirus infection: a narrative review

2.1 PREFACE

Given that my thesis research involves analysis of a trial evaluating efficacy of carrageenan, I first performed a narrative review to collect articles evaluating carrageenan's anti-HPV activity to summarize the available evidence for carrageenan as a preventive agent against HPV. This manuscript will be submitted to *Clinical Microbiology and Infection*.

2.2 TITLE PAGE

Carrageenan as a preventive agent against human papillomavirus infection: a narrative review

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2.3 ABSTRACT

Background

Carrageenan, an extract from red algae, was identified over a decade ago as a potent inhibitor of human papillomavirus (HPV) infection *in vitro*. Following this discovery, several studies further evaluated carrageenan's anti-HPV activity in cells, then in experimental animals and humans. We conducted a narrative synthesis of the evidence for carrageenan's anti-HPV activity.

Sources

PubMed was searched for relevant records, using the keywords "carrageenan" and "human papillomavirus" from inception to January 1st, 2020. Ongoing or planned studies were identified by searching Clinicaltrials.gov using the keyword "carrageenan." Full-text screening of all search results was performed to determine eligibility; studies had to be conducted in humans and report on carrageenan's anti-HPV activity.

Content

Of the 32 records identified in PubMed and 23 records in Clinicaltrials.gov, 8 records were included after full-text screening: two *ex vivo* studies, three clinical studies, and three trial protocols. Four of the studies evaluated carrageenan exclusively, one considered carrageenan combined with other anti-viral agents, and the three others were study protocols. Of the latter, one will evaluate carrageenan exclusively and two will evaluate carrageenan-combination products. Most studies (n=5) evaluated (or plan to evaluate, n=1) carrageenan's ability to prevent HPV acquisition, while one study explored its ability to promote clearance of existing infection. Of the aforementioned studies, two will evaluate clearance. Two of the study protocols identified did not list an HPV-related objective. Carrageenan's anti-HPV activity was observed consistently across study designs.

Implications

This review supports the premise that carrageenan, alone or in combination with other anti-viral agents, might be a potential prevention strategy complementary to HPV vaccination. Additional evidence is needed to confirm this hypothesis.

Keywords Carrageenan; Gel; HPV; Human papillomavirus; Microbicide

2.4 INTRODUCTION

Human papillomavirus (HPV), the most common sexually transmitted infection (STI),[1] is linked to several human cancers, including anogenital and oral cancers.[2] While the incidence of some HPV-associated cancers (cervical cancer, vaginal squamous cell carcinoma) has declined, others (oropharyngeal and anal squamous cell carcinoma) have increased in incidence in the United States between 1999 and 2015.[3] Recently, the World Health Organization called for the elimination of cervical cancer through vaccination, screening, and treatment.[4]

HPV vaccination has led to significant declines in the prevalence of HPVs 16 and 18 (in females) – the most oncogenic types – and HPVs 6 and 11, causal agents of anogenital warts and other benign diseases (in males and females).[5] However, country uptake of HPV vaccination still remains a challenge, especially in low and middle-income countries. These countries also bear the greatest burden of cervical cancer.[6] Besides, HPV vaccination is only maximally effective prior to the onset of sexual activity in women[7] and men.[8] Thus, additional prevention strategies are essential to eliminate cervical cancer and decrease the incidence of other HPV-associated diseases in the future.

Carrageenan is a sulfated polysaccharide derived from red algae that is similar in structure to heparan sulfate, an HPV cell-attachment factor.[9] Hence, it may be a promising intervention to prevent HPV acquisition and accelerate clearance of existing HPV infection. The primary mechanism of action involves carrageenan blocking the heparan sulfate proteoglycan (HSPG) to virion interaction.[9] Moreover, carrageenan is hypothesized to accelerate the natural clearance of an existing infection as its presence stimulates adaptive and innate immunity.[10]

In 2006, carrageenan was identified as a potent inhibitor of HPV infection in cells.[9] This finding was subsequently confirmed in a mouse model.[11] Prior to the initiation of studies evaluating carrageenan's anti-HPV activity in humans, the safety of carrageenan-containing gels was demonstrated in several clinical trials for vaginal[12] as well as vaginal and penile use[12–14], and also in smaller scale studies for rectal use in mice[15] and humans.[16] Carrageenan's anti-HPV activity

has been demonstrated in *in vitro* and *in vivo* studies,[9,17–26] except for a single study that showed mixed results by HPV type.[27]

Although a couple of prior reviews evaluated microbicides, their primary focus was on multipurpose prevention technologies to prevent HIV and other STIs,[28 29] without providing a comprehensive overview of all the studies that evaluated the effect of carrageenan against HPV. We conducted a narrative review to provide an updated, detailed review of carrageenan as a preventive agent against HPV. We provide an overview of the individual primary studies evaluating (or planning to evaluate) carrageenan's ability to prevent HPV acquisition and/or to accelerate clearance of existing HPV infection.

2.5 METHODS

A PubMed search was conducted from inception to April 1, 2020 using the keywords "carrageenan" and "human papillomavirus". The clinical trials registry "Clinicaltrials.gov" was searched using the keyword "carrageenan" to identify proposed, ongoing, or completed studies not yet published. Our inclusion criteria were as follows: (i) empirical studies in humans evaluating carrageenan's anti-HPV activity or (ii) study protocols planning to evaluate carrageenan against STIs. Full-text records were screened for eligibility by CL. No language or study design restriction was applied. The main components (study characteristics and effect measures) were abstracted into a standardized data extraction form.

2.6 RESULTS AND DISCUSSION

As shown in Figure 1, of 32 records identified in PubMed and 23 records in "Clinicaltrials.gov", a total of eight records were included in our review: two *ex vivo* studies[23,30] (one is a phase I randomized controlled trial, RCT), two RCTs,[31,32] one cohort study,[33] and three study protocols.[34–36] The publication date ranged from 2011 to 2020. Most publications came from North America,[23,30,32] with one from South Africa[31] and one from Italy[33]; the three protocols for ongoing studies also came from North America.[34–36] The sample size ranged from 13[23] to 348[31] for published studies, and 13[36] to 380[34] for protocols. Of the five studies that evaluated carrageenan in humans (Table 1), three (a post-hoc RCT analysis,[31] a phase IIb RCT,[32] and a cohort study[33]) assessed carrageenan's efficacy against natural HPV in terms of its prevalence, incidence, and clearance, respectively. Of the two *ex vivo* studies,[23,30] one is a phase I RCT[30] and the other is a case-series with investigator-modified exposure.[23] In two *ex vivo* studies,[23,30] cervicovaginal lavages were collected from women, but HPV activity was assessed in vitro. Table 2 presents features of four studies: three are study protocols for ongoing or recently completed studies: one phase IIb trial that will evaluate carrageenan exclusively[34] and two studies (a phase I RCT[35] and a phase I open-label study[36]) that will evaluate carrageenan-combination products. The fourth is for a phase IIb RCT that has already published an interim analysis (mentioned above), but the full analysis of the trial is ongoing.[32] The timeline of included, completed studies is shown in Figure 2, and are described in detail below.

A post-hoc RCT evaluating the effectiveness of Carraguard® against high-risk HPV

The Carraguard® trial was designed to evaluate the efficacy and safety of carrageenan, specifically λ- and κ- carrageenan, against HIV.[12] While a protective effect of carrageenan against HIV infections was not demonstrated, the Carraguard® gel was deemed safe. Thus, a sub-study assessed carrageenan's effectiveness against HPV;[31] a post-hoc analysis was performed on a subset of sexually active, HIV-negative participants (n=1723) aged ≥16 years. Participants HPV status was evaluated at the end of the two-year follow-up. Using Poisson regression analysis, the unadjusted prevalence ratio was 1.03. After controlling for significant risk factors (such as "younger age, being single, an abnormal pap smear, multiple sexual partners and promiscuous behaviour without the use of a condom"), use of the Carraguard® gel was associated with a 38% protective effect compared to a placebo gel among the most compliant

users (n=348). Women were considered compliant if researchers detected the insertion of >80% of their applicators and if, by self-report, >30% of their sex acts were covered. To detect applicator insertion, the single-use applicators returned at each visit were sprayed with a substance that turns blue in the presence of vaginal mucous.[37,38] To calculate covered sex acts, investigators divided the average number of applicators used per week by the self-reported number of sex acts per week. This study was limited by the low compliance to the intervention, and due to the unavailability of baseline HPV results, it is unknown whether the high-risk HPV prevalence in the treatment and placebo arms were comparable at baseline.

Ex vivo studies testing anti-HPV activity of carrageenan

Two *ex vivo* studies demonstrated carrageenan's potent anti-HPV activity.[23,30] In these studies, a cervicovaginal lavage sample, collected from women, was spiked with HPV pseudovirions (PsV) to measure the anti-HPV activity which allows the assessment of carrageenan's inhibitory activity in the presence of a vaginal fluid.

The study by Novetsky et al. aimed to evaluate the efficacy of the carrageenan-based gel Divine 9 using a cell-based assay.[23] No assessment of safety was reported. Sexually active women (n=13) were placed (non-randomly) into one of two groups: the precoital group was instructed to apply the gel before sex only, while the other group was instructed to apply the gel both before and after intercourse, i.e., pre- and post-coitus. The cervicovaginal lavage was collected 1, 4, or 8-12 hours after intercourse for the precoital only group, and after the postcoital gel insertion for the pre- and post-coital group. Women could contribute to more than one timepoint, e.g., after a week washout period, they could use the gel again according to group (precoital only versus pre- and post-coital), and provide a sample at either the 1, 4 or 8-12 hour mark, as requested by the investigators. Women could contribute to more than one time point, i.e 1, 4, or 8-12 hours post-coitus or last gel use, depending on the group assignment. Out of the 30 samples collected at different time points, 12 samples were from the precoital group, and 18 samples were from the pre- and post-coitus group. Based on 30 time points, carrageenan was detectable in 26 of the 30 samples. Neither the level of detectable carrageenan nor the median percent PsV16 inhibition (97.5%) were statistically significantly different between the two groups (i.e., pre-coitus only

versus pre and post coitus), suggesting either dosing strategy may be effective; however, this should be confirmed in larger clinical studies.

Friedland et al. evaluated PC-1005, a combination microbicide (containing MIV-150 against HIV, zinc acetate against HSV-2, and carrageenan), for its safety, pharmacokinetics, acceptability, adherence, and pharmacodynamics.[30] In this phase I RCT, gel safety was demonstrated based on the open label portion of the study, whereas in the randomized portion the reported adverse events were similar between arms and typically mild. In the randomized portion, women were instructed to apply the gel vaginally each day for 14 days. A cervicovaginal lavage sample was collected at baseline and at the end of the study (i.e., after the final dose on day 14). The anti-HPV activity was assessed in the cervicovaginal lavage samples using a cell-based assay with HPV16 PsV. Anti-HPV activity was detected in cervicovaginal lavage samples for all seven participants whose sample was collected four hours post-dose and for four of six participants whose sample was collected 24 hours post-dose. A higher carrageenan concentration was detected in samples collected 4 hours post-dose compared to those collected 24 hours post-dose, suggesting that carrageenan's anti-HPV activity is dose dependent.

A phase IIb RCT testing the efficacy of a carrageenan-based gel against 36 HPV types

The evidence reviewed thus far supported the initiation of an ongoing clinical trial evaluating the efficacy of a carrageenan gel. The Carrageenan gel Against Transmission of Cervical HPV (CATCH) study, performed by our team, was the first *a priori* RCT evaluating carrageenan's efficacy against natural HPV infection.[32] In this study's interim analysis, sexually active women (n=280) were randomized 1:1 to the carrageenan or placebo gel arm. Women were instructed to use their assigned gel every other day for the first month, and before, during, and after intercourse for the duration of the study (12-months follow-up period). Women who used the carrageenan gel were 36% less likely to have an incident infection than those who used the placebo gel. While more participants in the carrageenan group reported an adverse event, none were deemed related to gel use.

A cohort study evaluating anti-HPV activity of a carrageenan and *Propionibacterium* extraction

A cohort study assessed a gel containing 0.02% carrageenan and Propionibacterium extract (Carvir, Depofarm SpA, Mogliano Veneto, Treviso, Italy) for safety and acceptability, as well as anti-HPV activity.[33] Sexually active HPV-positive women (n=40) – instructed to apply the gel every day for 1 month, then on alternating days for the next 3 months – were compared to a group of HPV-positive women (n=35) who did not use a gel. A five-fold reduction in the risk of HPV persistence was found among women using the CGP gel compared to controls. Two adverse events were reported by CGP users; however, the events did not lead to withdrawal from the study. While findings favored carrageenan's anti-HPV activity, the study was limited by its small sample size, non-randomized design, and lack of adjustment for potential differences between the two groups, with the exception of age.

ONGOING RESEARCH

Results from ongoing or recently completed studies evaluating carrageenan could potentially provide more evidence for (or against) carrageenan's potential as a preventive agent against HPV.[32,34–36]

Combination products

Two of the aforementioned recently completed but not yet published studies are evaluating carrageenan-containing products. Although these study protocols, NCT03408899[36] and NCT02875119[35], lack an HPV-related objective, the carrageenan-containing products have demonstrated anti-HPV activity in earlier *in vitro*, *in vivo*, and *ex vivo* studies: MZC in a gel[19,30] and intra-vaginal ring formulation,[22] and griffithsin and carrageenan in a gel[21] and fast-dissolving insert formulation.[18,39]

In earlier research, Kizima et al. demonstrated the efficacy and safety of vaginally or rectally applied MZC/PC-1005 (MIV-150 and zinc acetate in a carrageenan gel) activity in a mouse model[19] and later, in cervicovaginal samples from women.[30] Subsequently, a phase I clinical trial (NCT03408899), MTN-037, will assess the safety and efficacy of PC-1005 gel for rectal use among men and women.[36] Recruitment was completed; however, results have not yet been published.

A gel containing griffithsin and carrageenan (against HIV type 1 and HPV, respectively) demonstrated anti-HPV activity in a mouse model.[21] A fast-dissolving insert formulation also demonstrated *in vitro*[39] and *in vivo*[18] anti-HPV activity, which supported further testing. A phase I open label clinical trial (NCT02875119) will assess the safety of a griffithsin/carrageenan gel formulation in women.[35] The study was completed, but results have not yet been published.

A carrageenan-based gel

At the time of this writing, the CATCH study,[32] has almost recruited all participants to achieve the target sample size (NB: study procedures were interrupted mid-March 2019 because of the mitigation measures against the COVID-19 pandemic). Upon completion of recruitment and follow-up, the final analysis will be conducted. A similar study, also conducted by our research group, the Lubricant Investigation in Men to Inhibit Transmission of HPV Infection (LIMIT-HPV) is designed to assess the efficacy of a carrageenan lubricant against incidence and prevalent anal HPV infections among gay, bisexual, and other men who have sex with men (gbMSM).[34] Sexually active gbMSM are randomized 1:1 to the carrageenan or placebo gel arm. GbMSM are instructed to use their assigned gel during intercourse for the duration of the study (12-months follow-up period).

2.7 CONCLUSIONS

Carrageenan's inhibitory properties have been demonstrated in several *in vitro*, *in vivo*, and clinical studies in humans. Early studies from 2006 and 2007 provided evidence of carrageenan's anti-HPV activity. The accumulation of evidence over time clearly indicate how the design of studies shifted from pre-clinical research and early clinical trials, to finally culminate in ongoing phase IIb clinical trials among women[32] and gbMSM,[34] a phase I clinical trial for PC-1005,[29] and a phase I open label clinical trial for a griffithsin/carrageenan gel (NCT02875119).[18] If efficacy and safety were to be demonstrated in the former two studies, a carrageenan gel could be recommended among sexually active individuals, including women and gbMSM to decrease the incidence, and possibly accelerate the clearance, of HPV infection, thus potentially lowering the risk of HPV-associated cancers and lesions.

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2.9 FIGURES

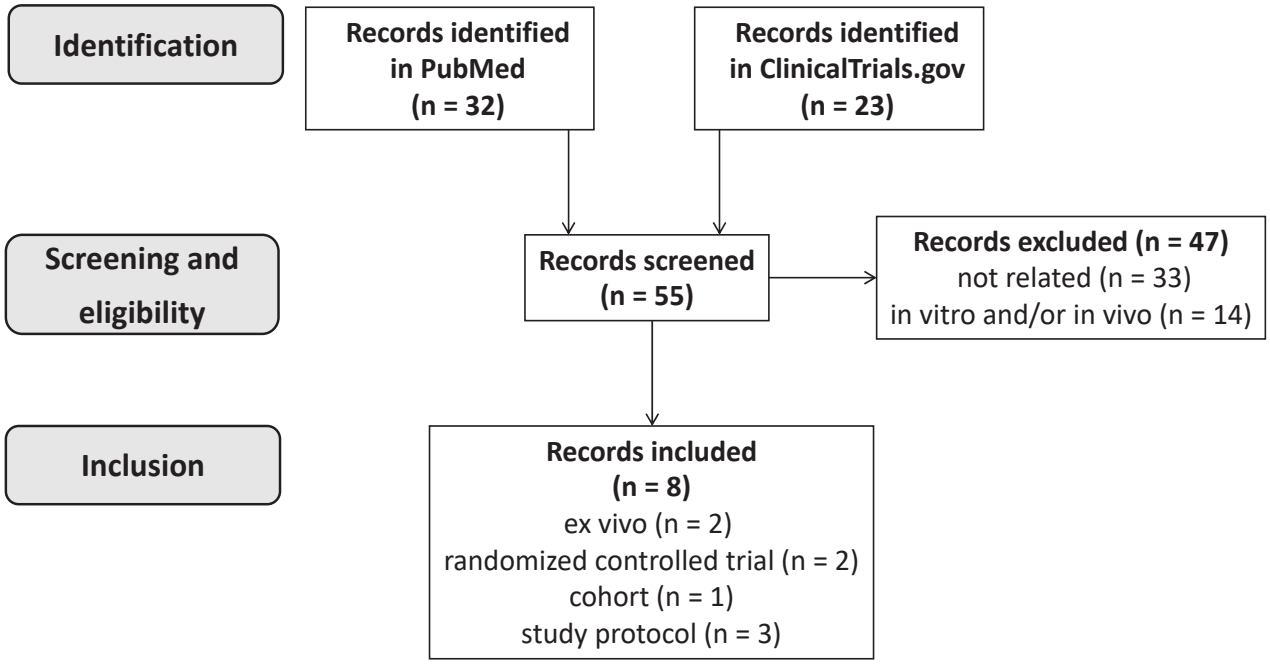

Figure 1 – Flowchart of included studies

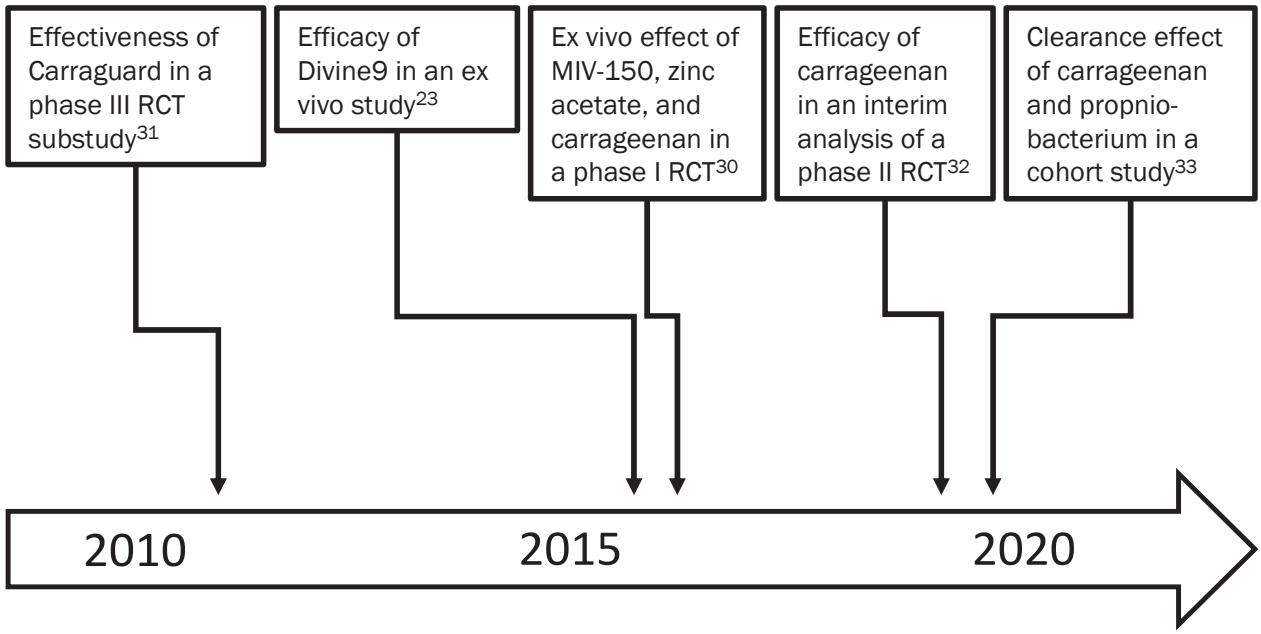

Figure 2 – Timeline of included studies

2.10 TABLES

Table 1. Summary of studies evaluating carrageenan in humans

First author, year	Study type	Sample size	Effect estimate (95% CI)	Outcome	Follow-up time	Product	HPV source	Main findings
Marais, 2011[31]	post-hoc analysis of phase III RCT	348	aOR = 0.62 (0.41-0.94)	high-risk HPV prevalence	2 years	Carraguard® (PC-515)	natural	prevalence of high-risk HPV was lower in compliant Carraguard® users than compliant placebo users
Novetsky, 2016[23]	*ex vivo*	13 (30 specimens)	97.5%	median PsV16 inhibition	NR	Divine 9	PsV16	prevents PsV infection and not negated by vaginal fluid
Friedland, 2016[30]	RCT phase I, *ex vivo*	20	anti-HPV activity in 7/7 CVLs	absence of luciferase expression	14 days	PC-1005 (MZC)	PsV 16	post-dose cervicovaginal lavage samples had anti-HPV activity
Magnan, 2019[32]	interim analysis of phase IIb RCT	277	HR = 0.64 (0.45-0.89)	incidence of HPV type not present at baseline	1 year	CG-containing gel	natural	use of a CG-based gel was associated with a reduction in the risk of HPV
Perino, 2019[33]	cohort	75	aOR = 4.9 (1.6-15.1)	clearance of HPV type positive at baseline	4 months	Carvir (CG and *Propioni-bacterium* extract)	natural	carrageenan may accelerate the clearance of existing HPV infection among HPV-positive women

Abbreviations: PsV: pseudovirion; CG: carrageenan; CI: confidence interval.

Table 2. Ongoing or recently completed studies evaluating carrageenan-containing gels

Registration number	Study name	Study type	Sample size	Product	HPV	Population	Aim
NCT02354144	LIMIT-HPV[34]	phase IIb RCT	380 (aim)	carrageenan-containing gel	natural	men	to evaluate the efficacy of a carrageenan-based lubricant against incident and prevalent anal HPV infections among gbMSM
ISRCTN96104919	CATCH[32]	phase IIb RCT	465 (aim)	carrageenan-containing gel	natural	women	to evaluate the efficacy of a carrageenan-based lubricant against incident and prevalent cervical HPV infections among women
NCT03408899	MTN-037[36]	phase I safety and PK study (open label)	13 (actual)	MZC (PC-1005)	natural	men and women	to evaluate the safety and PK of rectally administered PC-1005 in escalating doses
NCT02875119	NR[35]	phase I safety, PK and PD study (open label, followed by RCT)	15 (actual)	carrageenan and griffithsin (PC-6500)	natural	women	to evaluate the safety of vaginally administered PC-6500 for single and consecutive doses

Abbreviations: LIMIT-HPV: Lubricant Investigation in Men to Inhibit Transmission of Human Papillomavirus; RCT: randomized-controlled trial; gbMSM: gay, bisexual and other men who have sex with men; CATCH: Carrageenan gel Against Transmission of Cervical HPV; PK: pharmacokinetics; MZC: MIV-150, zinc acetate, and carrageenan; NR: not reported; PD: pharmacodynamics;

CHAPTER 3: Lubricant Investigation in Men to Inhibit Transmission of HPV Infection (LIMIT-HPV): Design and methods for a randomized controlled trial

3.1 PREFACE

In chapter 3, the narrative review provided evidence of carrageenan's anti-HPV activity across several study designs. The studies reviewed supported the initiation of additional clinical testing of carrageenan. Thus, in chapter 4, Manuscript 2 reports on the design and methods of a randomized controlled trial, the Lubricant Investigation in Men to Inhibit Transmission of HPV Infection (LIMIT-HPV) study. This trial is designed to evaluate the efficacy of a carrageenan-based lubricant for prevention of HPV infection in gbMSM in Montreal, Quebec. This manuscript was published in *BMJ Open* as of Mar 23, 2020.

3.2 TITLE PAGE

Lubricant Investigation in Men to Inhibit Transmission of HPV Infection (LIMIT-HPV): Design and methods for a randomized controlled trial

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3.3 ABSTRACT

Introduction

Gay, bisexual and other men who have sex with men (gbMSM) have an increased risk of human papillomavirus (HPV) infection and HPV-associated diseases, such as anal cancer and anogenital warts. A carrageenan-based lubricant could prevent HPV infection, thereby reducing the disease burden in this population. This paper describes the protocol for the Lubricant Investigation in Men to Inhibit Transmission of HPV Infection (LIMIT-HPV) study, an ongoing randomised controlled trial (RCT), evaluating efficacy of a carrageenan-based personal lubricant in reducing type-specific anal HPV incidence and prevalence among sexually active gbMSM, efficacy by HIV status, safety and tolerability of the gel and participant adherence to the intervention.

Methods and analysis

The study is a double-blinded, placebo-controlled RCT. Volunteer gbMSM 18 years and older are randomly assigned 1:1 to receive the treatment (a self-applied anal microbicide gel with carrageenan) or placebo (a self-applied placebo gel). At each visit, computerised questionnaires are used to collect data on sociodemographic and clinical variables, lifestyle, sexual behaviour, and the gels' safety and tolerability. At baseline and each follow-up visit (months 1, 2, 3, 6, 9, 12), nurses collect anal specimens tested for 36 HPV types (linear array assay). HIV status is determined at baseline and 12 months. The primary outcome is incidence of type-specific anal HPV infection(s) undetected at baseline. Secondary outcomes are prevalence of type-specific anal HPV infection, safety, tolerability, and adherence. We aim to recruit 380 participants to attain the study's objectives. Data will be analysed using intention-to-treat and per-protocol approaches with subgroup analyses by HIV status.

Ethics and dissemination

Ethics approval was obtained by the Research Ethics Boards of McGill University, the McGill University Health Centre, Concordia University and Centre Hospitalier de l'Université de Montréal. Trial results will be disseminated through peer-reviewed publications and conference presentations.

Trial registration number NCT02354144.

3.4 STRENGTHS AND LIMITATIONS OF THIS STUDY

- First study to explore the efficacy of carrageenan as a topical microbicide for preventing anal HPV acquisition in gbMSM
- Randomised controlled trial design comparing carrageenan lubricant gel to placebo is optimal to evaluate the efficacy of carrageenan in gbMSM with and without HIV
- Due to design limitations, dosage efficacy will not be evaluated
- The exact time of HPV acquisition will be unknown
- The proportion of incident infections that could be due to reactivation of previously acquired HPV types is unknown

3.5 INTRODUCTION

Background and rationale

Human papillomavirus (HPV) is one of the most common sexually transmitted infections (STIs) worldwide.[1] A 2012 meta-analysis found that 93% of HIV-positive gay, bisexual and other men who have sex with men (gbMSM) and 65% of HIV-negative gbMSM are currently infected with HPV.[2] Recently, an updated meta-analysis reported an HPV prevalence for HIV-positive and negative gbMSM of 81% and 47%, respectively.[3] Canadian statistics included in this meta-analysis were from a cohort study of HIV-positive gbMSM in Montreal, Quebec, which reported an HPV prevalence of 97.9%[4] and a cross-sectional study in Vancouver, British Columbia, which reported an HPV prevalence of 78.6% and 56.9% among HIV positive and negative gbMSM, respectively.[5] There is overwhelming evidence that persistent HPV infection with high oncogenic risk HPV types is the primary risk factor leading to precancerous anal lesions.[6–15]

While the incidence rate of anal cancer is 1-2 per 100000 per year,[16] the rate is 5.1 per 100000 among HIV-negative gbMSM, and 45.9 per 100000 among HIV-positive gbMSM, based on multinational data.[2] There is a lack of consensus on an anal screening strategy, and screening for high-grade lesions has not yet been shown to reduce the incidence of anal cancer.[17] The risk of other HPV-related lesions, such as genital warts, may decrease with condom use, but there is no consensus on whether condom use decreases the risk of HPV positivity.[18] Additionally, of the three current prophylactic HPV vaccines available, two are recommended for gbMSM[19] and offer protection from two (Gardasil)[20] or seven (Gardasil 9) high-risk HPV types.[21] There is thus a need for additional primary prevention measures.

Carrageenan, a gelling agent derived from red algae, is used as a stabilizer and emulsifier in food and cosmetic products.[22] Previous research demonstrated that carrageenan can block HPV transmission in vitro[23] and in animal studies.[24,25] Carrageenan interferes with virion surface proteins required for infection primarily by binding to the viral capsid thereby preventing attachment to the heparan sulfate proteoglycan receptor.[23] This interaction is long enough to allow natural inactivation of HPV by the immune system, which may increase natural HPV clearance.[26] The safety and acceptability of a carrageenan-containing gel was demonstrated for

vaginal[27] and vaginal and penile use.[28,29] Because of the high prevalence of HPV and the greater risk of anal cancer and its precursor lesions in gbMSM, compared to men in the general population, it is critical to determine whether a carrageenan-based lubricant can prevent HPV transmission among this at-risk group. Moreover, as carrageenan's primary mechanism of action against HPV may be affected by innate and adaptive immunity,[26] it is essential to verify if similar efficacy is observed in men with and without HIV. The aim of this paper is to describe the protocol for the 'Lubricant Investigation in Men to Inhibit Transmission of HPV Infection' (LIMIT-HPV) study, an ongoing, phase IIB, placebo-controlled, double-blinded randomised controlled trial (RCT) to evaluate the effect of a carrageenan-based lubricant on anal HPV infections in gbMSM.

Study objectives

The primary objective is to evaluate the efficacy of carrageenan in reducing type-specific anal HPV incidence, that is, in preventing incident infections by HPV types undetected at baseline in sexually active gbMSM, overall and by HIV status. Secondary objectives are to: (1) evaluate the efficacy of carrageenan in reducing type-specific anal HPV prevalence, that is, in accelerating clearance of existing infections in sexually active gbMSM; (2) assess the safety and tolerability of the proposed gel; and (3) assess participant adherence to the intervention.

3.6 METHODS

Study design

LIMIT-HPV is an exploratory, phase IIB, parallel group, block-randomised, placebo-controlled, RCT with 1:1 random assignment to the treatment (a self-applied anal microbicide gel with carrageenan) or placebo (a self-applied placebo gel) group. The trial was registered on clinicaltrials.gov (NCT02354144) as of February 2015. Health Canada authorised the gel for use in a clinical trial (file number 169160).

Patient and public involvement statement

Prior to study initiation, a focus group was conducted to gather recommendations from 20 volunteer gbMSM and adapt our protocol accordingly. Participants answered a self-administered questionnaire, providing their perspective on sexual behaviour; lubricant and condom usage; candidate gels; partner's support and potential impact on compliance; sample collection; willingness to enrol in the trial, as well as other concerns and suggestions. This preliminary research in itself did not inform the research question; however, the trial design was directly impacted; for example, participants were asked about the maximum frequency they would be willing to have an anal specimen collected, which directly informed the frequency of testing in the actual RCT. Additionally, the question of whether the sample should be nurse collected rather than self-collected was supported by 6/20 gbMSM, while 10/20 had no preference. Gel packaging was also adapted for their preferences. The recommended average monetary compensation to participate in the trial was $26.50 per visit.

Setting and recruitment

Participants are recruited at the participating clinical sites or via advertisements in various media (classified ads on Kijiji, Craigslist, and Les Pacs; Facebook; Fugues magazine, Quebec's gay and lesbian magazine; McGill and Concordia Classifieds; an interview on McGill/Montreal CKUT Campus Community radio station; promotional videos; 'What's New' blurbs emailed to McGill students; study announcements emailed to Université de Montréal students; and class presentations) and through printed promotional materials, including posters, business cards, posters and button pins. Study recruitment began in February 2016 and study visits are conducted at the following clinical sites: McGill University Health Centre (MUHC), Clinique

Médicale Urbaine du Quartier-Latin, Clinique OPUS, McGill Health Service Clinic, Concordia Health Services or at the Gerald Bronfman Department of Oncology at the Division of Cancer Epidemiology of McGill University.

Study population and procedures

Individuals are screened directly for eligibility at the clinical sites or prior to that over the telephone (online supplementary appendix 1). Alternatively, subjects interested in the study can first fill out an online, self-administered eligibility pre-enrollment questionnaire (online supplementary appendix 2). If eligible, they are contacted to confirm their eligibility and schedule the enrollment visit. Otherwise, they are emailed to thank them for their interest and explain their ineligibility.

- Eligibility is based on the following criteria:
- Men aged 18 or older.
- Living in Montreal and planning to remain in the city for the next 12 months,
- Having had receptive anal sex with one or more men during the previous 3 months and intend to continue being sexually active for the duration of their involvement in the study, irrespective of whether their sexual partner will change.
- Planning on having receptive anal sex with one or more men, but less than 50 different partners per year.
- Understanding French or English.
- Willing to follow study instructions and comply with follow-ups for 12 months.
- Willing to do an HIV-test (for men who were never tested seropositive for HIV).

Exclusion criteria:

- Participants must not be receiving treatment for anal or perianal condylomas or anal intraepithelial neoplasia lesions during the trial.
- Must not have a known allergy or hypersensitivity to any of the ingredients in either gels.

Study procedures according to each visit are summarised in Figure 1. Eligible men attend an enrolment visit, where the research nurse obtains written, site-specific informed consent (online supplementary appendix 3 McGill site) and instructs the participant on proper gel use. A one-month gel supply is provided, and the first specimen is collected. The nurse also provides details about HPV infection and advice about condom use and sexual health (ie, importance of condom

use to prevent HIV and other STIs). At subsequent visits, additional bottles of gel are provided, and patients are reminded to use the gel.

Randomisation and blinding

Once written informed consent is obtained and HIV status is confirmed, participants are randomised 1:1 to receive either a carrageenan-containing gel or a placebo gel. Intervention assignment occurs via a computer-assisted block randomisation with randomly variable block sizes. Each participant is assigned an individual code for the duration of the study, which is used to match him to the study arm. The trial is double-blinded: participants, care providers, investigators and outcomes assessors are unaware of treatment allocation. To ensure blinding, the two gels and their containers look and feel almost identical. Additionally, four random product codes are assigned to the treatment gel and a different set to the control gel (eight in total) to minimise the risk of unblinding. The success of blinding is evaluated at 6 and 12 months by asking subjects to guess their assignment. If the majority guess correctly, it would suggest that blinding was ineffective.

Intervention

The intervention and placebo gels used in this trial are two commercially available gels. The differentiating feature is that one gel contains carrageenan (intervention) and the other does not (placebo). Both gels are water based, latex condom compatible, clear, odourless, tasteless, and have similar viscosity. Both are packaged in a plastic bottle with a disk cap that can be operated with one finger and must be applied prior to receptive anal intercourse (RAI) during the entire study period. Participants are instructed to dispense around 15ml of the gel into the hand and apply directly to genital, anal and condom surfaces prior to and as needed during RAI. When sexual activity ceases, the water-based formulation of the gel allows it to be easily removed with lukewarm water. Participants are asked to use the assigned gel for the entire 12 months of follow-up, independently of other methods of protection against STIs (eg, condoms).

Adherence

To improve adherence, participants are provided with an unlimited gel supply until the end of the study. Up until April 2019, a monetary compensation of $25/visit was provided to each

participant. This amount was since increased to $50 for visits 1 and 7 and $40 for visits 2-6 to better reflect the market for compensation in clinical research, to improve recruitment and to help retain participants.

Concomitant care

The nurse informs unvaccinated individuals that the HPV vaccine has now been approved for men between 9 and 26 years of age and reminds them that protection is prophylactic and restricted to nine vaccine-target types. In addition to the required intervention gel, we recommend condom use for the prevention of HIV and other STIs. Condoms are easily accessible: many community organisations in Montreal such as REZO, a community-based organisation dedicated to health promotion and prevention of HIV/AIDS and other STIs, already provide condoms free of charge as a public health intervention. We also offer participants with latex allergies non-latex condoms free of charge that are compatible with the study gels. Condoms are available from the study nurse upon request.

Sample size

Data from the Montreal HIPVIRG (Human Immunodeficiency and Papilloma Virus Research Group) cohort study of gbMSM living with HIV[4] and a multinational meta-analysis representing both gbMSM subgroups[2] informed our calculation of sample size. The reported prevalence in the HIPVIRG population[4] was very similar to studies that were conducted outside of Montreal in gbMSM living with HIV [2], justifying adopting incidence data from gbMSM without HIV from settings outside of Montreal. The technique of Dupont and Plummer was used to estimate the hazard rate of acquisition.[30] Among HIV-negative gbMSM, we estimated a conservative preventive effect size of 50% based on the expert opinion of Dr. John Schiller who discovered carrageenan's inhibitory properties (personal communication).[23] We expect a lower effect size of 30% among HIV-positive gbMSM, as carrageenan's primary inhibition mechanism relies on the immune response. The power calculations were separately tailored to satisfy our primary endpoint in each gbMSM population; however, if results are homogeneous across groups, we will consider pooling results to improve the precision of our estimates. Additionally, we specified 80% power to evaluate our primary objective with a type 1 error of 0.05 and two-sided hypothesis. Assuming an incidence proportion of 30% at 12 months

among HIV negative gbMSM[2] and accounting for 10% loss to follow-up, the sample size required for an effect size of 50% was calculated to be 270. Similarly, assuming an 85% incidence of HPV infection at 12 months among HIV-positive gbMSM[2] and accounting for 10% loss to follow-up, the estimated sample size required for an effect size of 30% was calculated to be 107. Hence, recruiting 380 participants (110 HIV-positive and 270 HIV-negative) would ensure sufficient power at the end of follow-up to assess the study's objectives. With the high frequency of new sex partners among gbMSM in a similar study by our group,[4] a 1-year follow-up period would be sufficient to allow HPV exposure opportunity and evaluate compliance.

Data collection

The initial visit takes approximately 30min, while all subsequent follow-up visits (1, 2, 3, 6, 9 and 12 months) require about 20min each. Men are asked to abstain from RAI and gel use 48hours before specimen collection to minimise the risk of contamination.[31]

Computerised questionnaire

Participants complete a self-administered baseline questionnaire at enrolment, and six follow-up questionnaires (online supplementary appendices 4 and 5, respectively). These measure HPV risk factors, compliance, and monitor the gels' safety and tolerability. Between follow-up visits, participants are asked to log into a secure web module at least once a week to answer questions on daily sexual activities, condom and study gel use, and adverse events (AEs). To minimise recall bias, information can only be updated for the past 7 days (incomplete surveys expire after a week). Web-based diaries have been shown to be effective for logging sexual activities, and superior to questionnaires completed during visits for reducing recall bias.[32] This ensures high compliance and improves data quality. Responses are employed to evaluate adherence and assist in developing future studies.

Reporting AEs

To gauge the severity of AEs related to the study intervention, we refer to the Rectal Genital Grading Table for Use in Microbicide Studies[33] and Male Genital Grading Table for Use in Microbicide Studies[34]. If a stable, chronic condition is noted in the enrolment medical history questionnaire, but does not exacerbate during the trial, symptoms are recorded in the AE report but are not considered to be attributable to the gel. Subjects are advised to promptly notify the

nurse of any AE; the event is documented, and the participant is triaged and treated at the discretion of the study physicians. Nonetheless, should subjects fail to immediately report an AE, they are also asked about any recent medical visits/AEs at each follow-up visit in the questionnaire.

Anal sample collection

HPV infection status is assessed by testing anal specimens. Trained study nurses collect specimens according to the Protocol for Anal Swab Collection (online supplementary appendix 6).[4] The swab sample is immediately preserved in PreservCyt and kept at 4°C pending transfer to FC's laboratory, a WHO-accredited HPV diagnostics centre. Samples are batched and transported every 2-3 months.

HPV DNA detection and typing

The swab sample is subject to centrifugation at 13000*g* for 15min at 22°C; the supernatant is discarded, and the pellet is resuspended in 300µL of 20mmol/L Tris buffer (pH 8.3). DNA is purified using a Master-Pure Kit (Epicentre) and tested in each polymerase chain reaction (PCR) assay.[35] HPV detection and typing is done via the PGMY PCR protocol coupled with the linear array method, commercially available from Roche.[36] This test permits testing and typing for 36 different genital HPV types.[36] These types can be categorized into three alphapapillomavirus subgenera based on oncogenicity and tissue tropism: subgenus 1 includes low oncogenic risk types (HPVs 6, 11, 40, 42, 44, 54), subgenus 2 includes high oncogenic risk types (HPVs 16, 18, 26, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, 82), and subgenus 3 includes mostly commensal types (HPVs 61, 62, 71, 72, 81, 83, 84, 89).[37–40]

HIV testing

For participants who report being HIV-negative, the nurse performs a rapid HIV test at baseline and at 12 months, as is standard of care in high risk populations (online supplementary appendix 7). If positive, the participant is referred immediately to AdP at the MUHC to ensure rapid engagement with HIV care. For HIV-positive participants, a brief chart review is done at 0, 6 and 12 months to collect information on CD4 count, HIV viral load and current antiretroviral regimen.

Loss to follow-up

Discontinuing participation of a study subject occurs if the participant voluntarily withdraws from the trial, or has AEs, illness, or other medical conditions determined by a physician to be serious enough to terminate his involvement in the study. Loss to follow-up is described as failure to reach a participant for a follow-up visit 6 months post-randomisation, or the potential for a participant to jeopardise the study's integrity through protocol non-compliance.

Outcome measures

The primary outcome is presence of a newly detected anal infection of a specific HPV type(s) in an individual who was negative for that HPV type(s) at enrolment. The secondary outcome is clearance of type-specific anal HPV infections found at baseline. Analyses will be conducted for a conservative (one negative HPV result after a positive result) and liberal (two consecutive negative results after a positive result) definition of clearance. Other secondary outcomes include participant adherence and AEs reporting.

Data management

Study and data management are facilitated through the use of a secure, password-protected web-based database to record and manage study procedures. The database is used to record participant and clinic visit information, plan visits and export data. It is only accessible from specific IP addresses. A coded numeric system is used to identify subjects. All data, including but not limited to records, case report forms and laboratory results remain confidential and stored in a secure location. Research staff are the only individuals with access to these personal documents. They are available to the study sponsor or participating regulatory agencies on request. For quality control, data are downloaded from the server each month and checked for possible errors. Data management is done using SAS V.9.4 (SAS Inc., Cary, NC, USA). Any missing data will be handled by multiple imputations if appropriate.

Data analysis

Analyses will be conducted separately among gbMSM with and without HIV, and pooled if appropriate. These will use intention-to-treat (ie, including all participants who were randomized and received at least 1 month's supply of gel) and per-protocol (ie, including only 'adherent'

participants who complied with the protocol) approaches. Because of randomisation, we expect the rates of type-specific HPV infections to be comparable between study arms at enrolment.

Primary aim 1 (prevention)

Carrageenan's efficacy will be evaluated by testing the null hypothesis of no difference in time to anal type-specific HPV incident infection between treatment groups using the log rank test. Time to HPV infection will be defined as the difference in days between an incident HPV detection date and time zero at enrolment. We will use Cox proportional hazards regression to estimate the HR and 95% CI of HPV infection for treatment versus placebo. If the proportionality assumption is not met or the HR changes over time, we will fit a discrete-time hazards model.[41]

A sensitivity analysis will be conducted restricting to the most adherent participants in terms of gel usage. Adherence will be calculated as the number of times the gel was used during RAI divided by the number of RAIs reported in the same interval. A participant will be considered adherent if he reported, as recommended, gel use at least >50% of the time prior to every act of intercourse. Additional analyses will allow for time-varying adherence, defined as adherence since the last administered questionnaire.

Secondary aim 1 (clearance)

Time-to-event analysis techniques will be used to measure type-specific clearance of HPV infections present at enrolment, according to the intervention. Time to clearance and HRs of clearance will be calculated as above.

Secondary aim 2 (Safety, tolerability, and adherence)

Safety and tolerability of the interventions will be evaluated using the AE reports from both groups. For each participant, mean adherence will be calculated for the time period between two consecutive visits and for the whole follow-up period, and it will be compared between the intervention and placebo groups using a t-test. If adherence is not normally distributed, median adherence will be compared between groups using the Mann-Whitney test. As mentioned previously, adherence will also be evaluated as a binary variable and compared between groups using the χ^2 test, for each interval and overall.

Monitoring

An independent data safety monitoring board oversees the trial to ensure that it is conducted in accordance with the ethical principles of good clinical practice. The board will review the results of the interim analysis and make recommendations regarding safety concerns, and/or suspension or early termination of the study (eg, unequivocal evidence of efficacy). The same board members also oversee the Carrageenan gel Against Transmission of Cervical HPV (CATCH) RCT, which is similar in design to LIMIT-HPV, however it evaluates the efficacy of a carrageenan gel among heterosexually active women.[42]

3.7 ETHICS AND DISSEMINATION

This is the seventh study protocol version, last revised 30 January 2019. When 50% of the targeted population (380 gbMSM) are recruited, an interim analysis will be conducted. Reports of trial findings – in the form of abstracts and manuscripts to be submitted, respectively, to peer-reviewed journals and conferences – will be presented according to the CONsolidated Standards of Reporting Trials statement.[43] The co-investigators involved in the study will assist in dissemination of research findings directly to health clinics and the gbMSM community.

3.8 DISCUSSION

Presently, there is no effective way to treat anal HPV infections. With the potential for broad-spectrum anti-HPV activity, carrageenan could be a useful adjunct to HPV vaccination as a primary means of preventing HPV infections. Given the high burden of HPV infections in the gbMSM community, regular application of a carrageenan-based lubricant could be a cost-effective preventive approach, especially considering that most gbMSM regularly use lubricants for anal sex. Furthermore, treatments for condyloma and high-grade lesions are costly and often need to be repeated, as the recurrence rate is very high (particularly among people with HIV).[44] Also, vaccination is generally only maximally effective at preventing infection if administered prior to becoming sexually active.[45]

To the best of our knowledge, the LIMIT-HPV study is the first to test carrageenan against anal HPV infections. Its main strength is the blinded RCT design. Additionally, considering HIV positive and negative gbMSM would allow for the evaluation of the gel's efficacy in both groups. There are study limitations. An evaluation of dosage efficacy is not possible, as we do not collect information on the exact amount of gel used. While biannual[4,46–48] and

annual[49–53] anal HPV sampling in longitudinal studies is common, that length of follow-up will not give sufficient detail to evaluate the study's objectives. In an ideal research setting, HPV status would be ascertained daily to have a more precise measurement of the time of HPV acquisition; however, to minimise burden on the patient, the current schedule was deemed optimal. HPV incidence is consequently interval-censored, that is, infection date occurs sometime between the last negative and the first positive test, but the exact date is unknown. However, as the time interval between each visit is relatively short, the interval would represent an appropriate approximation. An additional limitation is the possibility that some 'incident' HPV infections are due to reactivation of previously acquired HPV, as opposed to acquisition from sexual activity.[54] However, because the proportion of incident infections that could be due to viral latency is expected to be balanced between groups as a result of (successful) randomisation, the effect on the risk estimate could be biased towards the null.

The LIMIT-HPV study may show a similar protective effect as was demonstrated in an interim analysis of a related study (CATCH-RCT) conducted by our team. A reduction in the risk of incident HPV infection among participants randomised to the carrageenan gel was demonstrated, and importantly, the gels appeared safe: none of the reported AE were attributed to the gels.[42] If efficacy of the carrageenan gel is demonstrated, the current trial has the potential to improve the health of individuals in the gbMSM community by providing protection against all HPV genotypes and ultimately reducing the risk of HPV-associated diseases in this at-risk group.

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Collaborators

LIMIT-HPV study team members: Affiliated with the Division of Cancer Epidemiology, McGill University, Montréal, Canada: Allita Rodrigues (study coordinator); Natalia Morykon and Raphaela Rodrigues (management of subject participation and specimen collection); Sheila Bouten and Samantha Shapiro (data management). Affiliated with Clinique OPUS: Roger Leblanc; Affiliated with Clinique Médicale Urbaine du Quartier Latin: Benoit Trottier (clinical collaborators). Affiliated with the Research Institute of the McGill University Health Centre, Montréal, Québec, Canada: Christina de Castro and Karène Proulx-Boucher (study coordination and management of subject participation); Guillaume Theriault (specimen collection). Affiliated with the Service de Microbiologie Médicale et service d'Infectiologie, Départements de Médecine et de Biologie médicale, Centre Hospitalier de l'Université de Montréal, Montréal, Québec, Canada: Julie Guénoun (HPV testing and genotyping).

Contributors

ELF, AdP, FC, and PPT conceived and designed the study. JT contributed to the grant application writing. ME-Z managed the study. CL drafted the manuscript under the supervision of ELF, AdP and ME-Z. All authors reviewed the manuscript and approved the final version.

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Competing interests

AdP's clinic participates in pharmaceutical clinical trials for HIV antiretrovirals and HCV treatments (ViiV Healthcare, Janssen, Merck and Gilead), received honoraria for consulting on HIV antiretroviral regimen for ViiV Healthcare, and received grants from CIHR and FRQ-S outside the submitted work. EF reports grants and personal fees from Merck, grants, personal fees and non-financial support from Roche and personal fees from GSK, outside the submitted work. JT is a Merck employee. FC reports grants from Réseau FRQS-SIDA during the conduct of the study and grants to his institution for HPV-related work but outside of the submitted work from Merk Sharp and Dome, Roche Diagnostics and Becton Dickinson.

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3.11 FIGURE

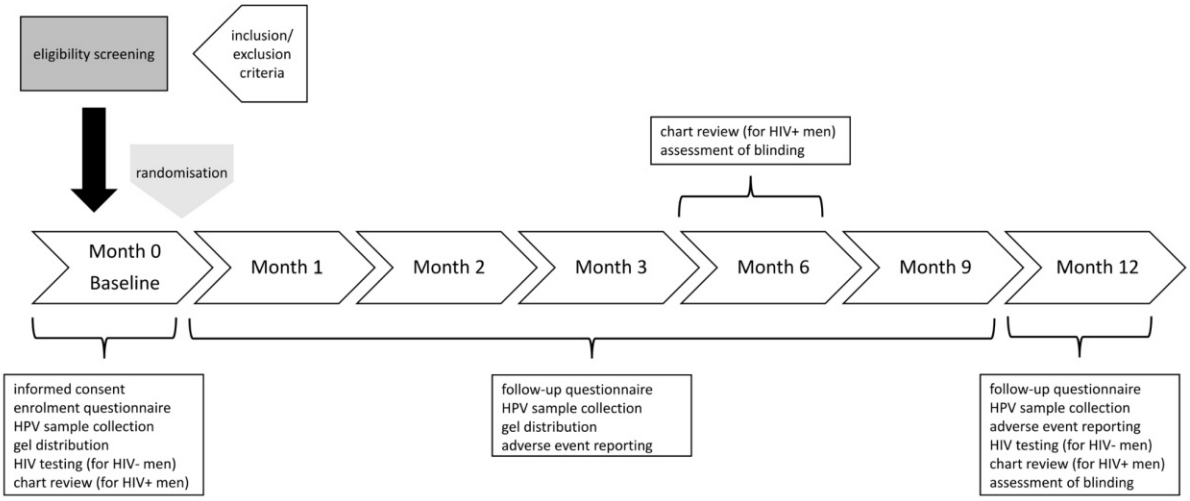

Figure 1 - Study procedures according to visit

Potential participants are screened for eligibility based on predefined inclusion and exclusion criteria. Eligible participants provide informed consent and are randomised 1:1 to receive either the carrageenan-based gel or the placebo gel. They fill out an enrolment questionnaire, provide an anal HPV sample, receive a supply of the gel, are tested for HIV if HIV-negative and a chart review is completed if HIV-positive. The participants return for each follow-up visit (months 1, 2, 3, 6, 9 and 12). At follow-up visits (months 1–9), participants fill out a follow-up questionnaire, provide an HPV sample, are provided gel and report on adverse events. At month 6 and 12, there is a blinding assessment and a chart review is completed for HIV-positive participants. At month 12, participants fill out a follow-up questionnaire, provide an HPV sample and report on adverse events, and HIV-negative men are tested again for HIV.

CHAPTER 4: Lubricant Investigation in Men to Inhibit Transmission of HPV Infection (LIMIT-HPV): interim analysis of a phase II, placebo-controlled randomized controlled trial

4.1 PREFACE

The prior chapter reported on the methods and design for the LIMIT-HPV study. In chapter 5, Manuscript 3 will report the interim analysis results for the LIMIT-HPV study, with the primary objective of evaluating the efficacy of a carrageenan-based gel against incident HPV infection. The justification for conducting an interim analysis are for the reasons following: a parallel study conducted by our team, the CATCH[1] (Carrageenan gel Against Transmission of Cervical HPV) randomized controlled trial, evaluates carrageenan's efficacy against incident HPV infection among sexually active women. Based on a recently published interim analysis, they found a 36% protective effect of the carrageenan compared to the placebo gel. We anticipated that given the protective effect observed in women and the greater compliance to gel use expected in gbMSM, there was potential for LIMIT-HPV study to show a large benefit. An interim analysis would assess whether the LIMIT-HPV study would replicate the same or greater protection seen in CATCH. If this hypothesis were confirmed, it could become unethical to sustain randomization based on the lack of clinical equipoise. Thus, an interim analysis of the LIMIT-HPV study was conducted. The LIMIT-HPV study is the first randomized controlled trial evaluating efficacy of a carrageenan-based gel against anal HPV infection. This manuscript will be submitted to the *Journal of Infectious Diseases*.

1. Magnan, S. et al. Efficacy of a Carrageenan gel Against Transmission of Cervical HPV (CATCH): interim analysis of a randomized, double-blind, placebo-controlled, phase 2B trial. Clin. Microbiol. Infect. 25, 210–216 (2019).

4.2 TITLE PAGE

Lubricant Investigation in Men to Inhibit Transmission of HPV Infection (LIMIT-HPV): efficacy of a carrageenan gel in an interim analysis of a phase II, placebo-controlled randomized controlled trial

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4.3 ABSTRACT

Background

Carrageenan, a non-toxic gelling agent derived from red algae, has demonstrated potent anti-HPV activity in *in vitro* and animal studies. We aimed to assess the efficacy of a carrageenan-based gel in reducing the risk of anal human papillomavirus (HPV) infections among gay, bisexual, and other men who have sex with men (gbMSM).

Methods

The LIMIT-HPV study is a phase IIb, placebo-controlled randomized controlled trial conducted in Montreal, Canada. gbMSM were randomly assigned (1:1) to receive a carrageenan-based or placebo gel. Questionnaire data and anal samples were collected at 0, 1, 2, 3, 6, 9, and 12 months. We assessed incident anal HPV infection(s) using Cox proportional hazards models.

Results

Participants recruited between February 2016 and December 2019 were randomly assigned to the carrageenan (n=127) or placebo (n=128) arm. Efficacy analyses included 201 participants. Median follow-up time was 7.6 months (range: 0-28.5) in the carrageenan group and 9.3 months (range: 0-40.7) in the placebo group. The hazard ratio was 1.21 (95% confidence interval: 0.86-1.70); 69.2% and 65.1% incident HPV infections were detected in the carrageenan and placebo arms, respectively. Safety analyses included 210 participants. There were more adverse events (AE) reported by participants in the carrageenan compared to placebo arm (59.8% vs. 39.8%).

Conclusions

This interim analysis did not demonstrate a protective effect of carrageenan on the risk of incident anal HPV infection among gbMSM. Carrageenan-gel use was associated with a higher proportion of AEs. The trial has been terminated.

Keywords Carrageenan; Gel; HIV; HPV; Human papillomavirus; Gay, bisexual, and other men who have sex with men; Microbicide; Randomized controlled trial

4.4 INTRODUCTION

Introduction

While anal cancer is more common in women than men[1], certain groups – namely HIV-positive gay, bisexual, and other men who have sex with men (gbMSM) – have a much greater risk of anal cancer than the general population.[2,3] Up to 90% of anal cancers are attributable to infection with human papillomavirus (HPV).[4] Prevalence of HPV infection is particularly high in HIV-positive and -negative gbMSM.[2]

Three prophylactic HPV vaccines are available for prevention of infection with certain types of HPV.[5–7] Of the 123 countries with national HPV vaccination programs (or plans for), the vast majority are however girls-only programs.[8] Canada's National Advisory Committee recommends vaccination with Gardasil and Gardasil9 for males 9-26 years old.[9] In Canada, the HPV vaccine is provided to young gbMSM at no cost, however, early analyses suggest subpar vaccine uptake.[10] On the other hand, HPV vaccination is maximally effective when administered prior to sexual activity,[11] leaving gbMSM largely unprotected, especially as they are unlikely to benefit from herd immunity following the vaccination of girls.[12]

There is a lack of formal recommendations[13] and limited access to resources[14] for anal cancer screening in Canada. It is also unknown whether anal cancer screening confers a benefit.[15] Ongoing studies aim to address this question. In the United States, the Anal Cancer/HSIL Outcomes Research phase III clinical trial is comparing treatment of high-grade squamous intraepithelial lesions to active monitoring for anal cancer prevention.[16] In Canada, the HPV Screening and Vaccine Evaluation study[17] (NCT02503111) aims to assess if treatment of anal high-grade squamous intraepithelial lesions reduces anal cancer incidence among HIV-positive gbMSM. Until these studies can provide more evidence in favor of screening and treatment, a key consideration is the exploration of alternative HPV prevention measures.

Carrageenan is a safe and non-toxic gelling agent naturally derived from three species of red algae and has a long history of human use as a stabilizer and emulsifier in food and cosmetic products.[18] There is evidence that this anionic polymer blocks HPV transmission in vitro,[19] in

vivo,[20] and in women.[21,22] Rectal application of Carraguard®, a carrageenan-containing lubricant, was demonstrated to be safe in mice[23] and in a small study in humans (n=4).[24]

Given the high burden of HPV in gbMSM and previous evidence for carrageenan's anti-HPV activity, we conducted the Lubricant Investigation in Men to Inhibit Transmission of HPV Infection (LIMIT-HPV) study, a phase IIB randomized controlled trial aimed to assess among sexually active gbMSM overall and by HIV status the 1) efficacy of a carrageenan-based lubricant in reducing type-specific anal HPV incidence and prevalence; 2) safety and tolerability of the gel; and 3) participants' adherence to the intervention.

4.5 METHODS

We followed the CONSORT (Consolidated Standards of Reporting Trials) 2010 Checklist[25] and CONSORT for harms[26] to report the findings of this study.

Trial design

The LIMIT-HPV study is an exploratory, phase IIB, parallel group, block-randomized, placebo-controlled RCT; full details of its design and study procedures were recently published.[27] Briefly, gbMSM were followed for 12 months; data were collected at enrollment and each follow-up visit (months 1, 2, 3, 6, 9 and 12).

Settings and locations where the data were collected

Study recruitment began in February 2016 in Montreal, Canada. HIV-positive participants were enrolled at the McGill University Health Centre (MUHC) and two other clinics that offer support and medical care for people living with HIV. HIV-negative participants were enrolled at two student health services clinics (McGill and Concordia universities) between April 2016 and September 2018, after that they were seen at the research clinic of McGill's Division of Cancer Epidemiology.

Participants

Men aged 18 and older were considered eligible if they lived in Montreal and planned to remain in the city for one year; reported receptive anal intercourse (RAI) with at least one man in the previous 3 months and expected to continue being sexually active over the follow-up, with the same or a different sexual partner; planned to have RAI with 2 to 50 different partners per year; understood French or English; were willing to follow study instructions; and were willing to do an HIV-test (for men who were never tested seropositive for HIV).

Randomisation and blinding

Written informed consent was obtained by the research nurse. Eligible gbMSM were randomized 1:1 (via a computer-assisted randomization with randomly variable block size) to receive a carrageenan-based (treatment) or placebo (control) anal gel. To ensure blinding, the containers were identical, and both gels were water-based, latex-condom compatible, clear, odourless,

tasteless, and of similar viscosity. Additionally, four random product codes were assigned to the treatment gel and a different set to the control gel (eight in total). Participants, lab technician(s) performing DNA genotyping, and study nurses were blinded to intervention assignment.

Intervention

The study gels are commercially available, self-applied anal gels. During the study period (12 months of follow-up), participants were instructed to apply around 15mL of the gel prior (and as needed) to each RAI, irrespective of the use of other protection methods against STIs (e.g., condoms).

Outcomes and procedures

The primary outcome was the incidence of a newly detected anal infection of a specific HPV type(s) that was absent at baseline. Testing of anal samples was performed using the Roche Linear Array PCR assay for detecting and genotyping of 36 HPV types.[28] These HPV types can be subdivided based on oncogenic risk and tissue trophism. Subgenera 1 consists of low-risk HPVs 6, 11, 40, 42, 44, and 54; subgenus 2 consists of high-risk HPVs 16, 18, 26, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, and 82; and subgenus 3 consists of HPVs 61, 62, 71, 72, 81, 83, 84, and 82.[29–31] Overall, β-globin was negative (indicating the DNA content of samples was inadequate) for 6.2% (74/1187) of anal samples, and genotyping results for 3.6% (43/1187) samples were not available at the time of manuscript preparation. These visits were thus censored in efficacy analyses. Secondary outcomes included the evaluation of participant's adherence to the intervention, AE reporting, and tolerability of the intervention.

At each visit, a study nurse collected an anal sample and instructed participants to complete a self-administered, computerized questionnaire to collect sociodemographic, sexual behaviour, and information on the gel's safety and tolerability. A weekly, online calendar completed by participants captured sexual behaviour, gel use, and AEs.

Statistical methods

Sample size

Assuming a cumulative incidence of 30% and 85% at 12 months among HIV-negative and HIV-positive gbMSM, respectively,[2] 270 HIV-negative and 110 HIV-positive MSM would have to be recruited to assess the study's objectives. This was based on 80% power, an alpha of 0.05, and assuming a 30% and 50% effect size for HIV-positive and HIV-negative gbMSM, respectively.

Data analysis

Baseline characteristics were compared between study arms using the Student's t-test or Wilcoxon rank-sum test for continuous data and χ^2 or Fisher's exact test (if small numbers) for categorical data. In the primary analysis, we estimated hazards ratios (HR) and 95% confidence intervals (CI) for the incidence of any HPV (and in each subgenera) type using univariate Cox proportional hazards regression models with treatment group as the main effect (there was no statistical evidence that the proportional hazards assumption was violated). The primary analysis was performed on complete cases i.e., including participants with at least 2 valid HPV samples (n=201). Sensitivity analyses comprised using the following single imputation methods for missing outcome data[32,33] (i.e., missing HPV results): 1) worst-best analyses assumed that all participants with missing HPV data in the carrageenan arm had incident infections and all those in the placebo arm were HPV-negative, and 2) best-worst analyses assumed that all participants with missing HPV data in the carrageenan arm were HPV-negative and all those in the placebo arm had an incident HPV-infection.

To account for multiple HPV infections per participant, we used a type-specific Cox proportional hazards model to estimate the HR of all incident HPV infections acquired over the follow-up, whereby the unit of analysis was each individual HPV type (each participant could contribute up to 36 observations, each corresponding to an HPV type).

Post-hoc subgroup analyses were performed to assess differences in intervention effects for main baseline characteristics of participants, in addition to cumulative compliance, other gel use, and bleeding following RAI. Compliance was defined as the number of RAI divided by the number of times the gel was used in the seven days prior to the study visit. Safety analyses included all participants with at least 1 follow-up visit, i.e., 210 participants. Data analysis was done using Stata software (version 16.0; StataCorp).

Ethics

The study was approved by the Research Ethics Boards of McGill University, the McGill University Health Centre (MUHC), Concordia University, and Centre Hospitalier de l'Université de Montréal (CHUM). The study protocol was registered on ClinicalTrials.gov (identifier NCT02354144). The gel was authorized for use by Health Canada (file number 169160).

4.6 RESULTS

Recruitment for the LIMIT-HPV study began February 2016. **Figure 1** shows the flow of participants during the trial. Of 403 individuals assessed for eligibility, 255 eligible gbMSM were willing to enroll. They were subsequently randomized to the carrageenan (n=127) and placebo (n=128) arms. Overall, 41.2% (105/255) withdrew early: 40.2% (51/127) in the carrageenan arm and 42.2% (54/128) in the placebo arm. Compared to active participants, those who withdrew were younger (median age of 28.0 years versus 38.5 years) and had a different educational distribution, fewer lifetime sexual partners, less HIV-positivity, and lower HPV prevalence (55.2% versus 64.7%) (**Table S1**). Of the 255 randomized participants, 210, 190, 164, 145, 124, and 99 completed visits 2, 3, 4, 5, 6, and 7, respectively. Of the 210 participants who completed at least 2 visits, 9 were excluded due to unavailable HPV results, resulting in a sample size of 201 participants in the efficacy analyses. Safety analyses included 210 participants, excluding participants with only 1 visit (n=45).

The baseline and follow-up characteristics of participants were balanced between groups (**Table 1**). Overall, median age at study entry was 33.6 years (range: 18.22-71.7). Most gbMSM were French Canadian (38.4%) and highly educated; half completed university. The median age at first intercourse was 17 years. The majority (63.1%) reported having had a new sexual partner in the last month. HPV prevalence at baseline was high; 60.8% tested positive for any HPV type. A total of 26 participants (10.2%) had missing HPV genotyping data (19 were invalid and seven were not yet tested by the laboratory). HPV prevalence data at baseline and over follow-up are shown in **Tables S4-7**. Most participants (77.7%) were not vaccinated against HPV. Median follow-up time was 8.02 months, and median number of visits was 5.

As shown in **Table 2**, 69.4% of participants in the carrageenan and 65.1% of placebo groups acquired at least one HPV infection, corresponding to a HR of 1.21 (95% CI: 0.86-1.70). The non-significant increased risk was consistently observed when stratifying by HIV status and subgenera (with the exception of HIV-positive participants for the infections belonging to subgenus 1). The actuarial mean time to incident infection (which accounts for censoring) was typically shorter in the carrageenan compared to the placebo arm. This is consistent when restricting only to participants who acquired an incident infection (arithmetic mean). Exceptions

to these observations were: in the HIV-positive stratum, the actuarial mean was longer in the carrageenan arm for subgenus 1, and the arithmetic mean was the same in each group overall but longer in the carrageenan arm for each subgenera. Our sensitivity analyses (**Table S2**) demonstrated that under the worst-best scenario, a 53% increased risk of incident HPV infection (HR=1.53, 95% CI 1.09-2.16) was observed. Conversely, under the best-worst scenario, the HR was 0.96 (95% CI: 0.68-1.34), which is consistent with a null effect for gel use.

The cumulative incidence of HPV infection by intervention group is shown in **Figure 2.** Whereas **Figure 2A** considers the first HPV infection detected (**Figure S1** by HIV status), **Figure 2B** considers all new HPV infections acquired over follow-up (**Table S2** by HIV status). When considering all new HPV infections acquired over follow-up, a total of 189 and 200 incident infections were detected in the carrageenan and placebo arm, respectively, corresponding to a HR of 1.02 (95% CI: 0.74-1.40).

Sub-group analyses showed (**Figure 3**) variable results. The following sub-groups had point estimates below the null: participants over the median age, ethnicity other than caucasian, a lower number of lifetime sexual partners (between one and 27), having no sex partners in the last month, being HPV-negative at baseline, being vaccinated, and never using another lubricant gel during the study. These analyses were for exploratory purposes only to assess heterogeneity of the estimates.

Data on AEs (**Table 3**) were collected and tabulated from the calendar, follow-up surveys, and clinic visits. There were significantly more AEs reported in the carrageenan compared to placebo arm. Notably, a significantly higher proportion of gonorrhea cases was reported in the carrageenan (22.6%) compared to placebo (9.3%) arm, and one case of HIV seroconversion in the carrageenan arm.

4.7 DISCUSSION

This study demonstrated that use of a carrageenan-based gel did not confer protection against acquisition of anal HPV infections compared to the use of a placebo gel among gbMSM. Consistent results were observed for low and high-risk HPV types, and by HIV status. Significantly more AE were reported in the treatment compared to placebo arm, questioning the safety of the study gel. In April 2020, these results were presented to the data safety monitoring board who recommended early termination of the trial due to safety concerns and the lack of a protective effect of carrageenan.

The findings of this study contradict the wealth of literature published on carrageenan's anti-HPV activity, including in vitro,[19,34] in vivo,[20] ex vivo,[35,36] and clinical studies.[21,22] The first clinical study was a sub-study of an RCT evaluating carrageenan against HIV in women. While unsuccessful against HIV, in a subset of women, use of carrageenan was associated with a 38% reduction in prevalent high-risk HPV infection; however, this data was only collected at study end, so it is cross-sectional.[21] The other clinical study, Carrageenan gel Against Transmission of Cervical HPV (CATCH), conducted by our team was specifically design to evaluate carrageenan's efficacy in women. An interim analysis of the CATCH study demonstrated a 36% reduction in the risk of acquiring an incident HPV infection.[22] A greater protective effect among gbMSM was expected, as compliance was presumed to be higher in this population who are more likely to require lubricants for intercourse. The gbMSM were in fact more compliant than heterosexual women: a cumulative compliance of greater than 75% was observed for 49.7% of participants in CATCH and 71.3% of participants in LIMIT-HPV (data not shown); however, a protective effect of carrageenan among gbMSM was not observed. As mentioned previously, the CATCH and LIMIT-HPV studies reported hazard ratios of 0.64 (95% CI: 0.45-0.89) and 1.21 (95% CI: 0.86-1.70), respectively. This lack of protective effect could be related to a combination of factors, including the rheological properties of the gel, differences in anatomical sites, and AEs.

While the rheological properties of the study gel were tested in an in vitro study and deemed hypo-osomolal,[37] later testing of the gel revealed after trial termination that the gel is hyper-osmolal (personal communication). Previous research demonstrated some hyper-osmolal

lubricants can cause epithelial injury in the rectum,[38] and consistent lubricant use was identified as an independent predictor of prevalent STI infection.[39] This could potentially explain the increase in AE (e.g., more reports of discomfort in the carrageenan arm), as well as the (non-significant) increased risk of incident HPV infection in the carrageenan arm. If the lubricant gel is damaging the epithelium, pathogens could potentially gain access more easily, leading potentially to more HPV infection, and other STIs (e.g. significantly more gonorrhea cases were reported in the carrageenan arm). This adverse effect could be more problematic for anal intercourse than vaginal intercourse, due to self-lubricating differences between these two sites, and to the delicate and absorbent nature of the rectal epithelium compared to the vaginal epithelium.[23] A study in macaque found while a hyper-osmolar lubricant caused was cytotoxic, it did not increase the risk of simian/human immunodeficiency virus.[40] This observation does not preclude the possibility that a hyper-osmolar gel may increase susceptibility to HPV. Additionally, if the study gel has a low viscosity (as reported by Rodriguez et al.), the gel may easily wash away, leading to lower carrageenan concentrations and possibly less protection.[37] Less lubrication could lead to greater irritation and damage, thereby increasing the number of adverse events/reactions experienced by participants, and potentially increasing the risk of HPV infection. In both the CATCH and LIMIT-HPV studies significantly more AEs were reported in the carrageenan arm compared to the placebo arm (44.0% vs. 30.9% in CATCH[22] and 59.8%% vs. 39.8% in LIMIT-HPV); however, no withdrawals resulted from the AE in the CATCH study, while there were some AE-related withdrawals in the LIMIT-HPV study. Presumably, this could be related to severity of the AE.

Several study limitations need to be acknowledged. First, there were 26 invalid anal samples at baseline, for which the next visit with available HPV data was considered as baseline. For these participants, a proportion of HPV types present and detected at the subsequent visit (i.e., the second visit) could represent incident infections (i.e., they were not present at baseline), but would not be classified as such; however, the proportion of participants with invalid HPV results at baseline was similar between the two groups (7.9% in the carrageenan and 12.5% in placebo arm). This potential for left censoring could bias the estimate towards the null. The proportion of invalid anal samples in the study (6.2%) is similar to a multi-national study among HIV-negative MSM that reported 9.1% of anal samples were β-globin negative.[41] While the invalid HPV rate

was not reported in a cohort study of men living with HIV, repeated anal sampling (up to 3 times) over a 6 month period was done if an insufficient sample was obtained.[42] This would be a useful strategy to mitigate invalid anal samples. As a result of invalid HPV genotyping samples, a complete case analysis was performed. Complete case analyses are common in RCTs with missing data.[43] In the present study, the overall missing/invalid rate was 9.9%, which is slightly higher than the recommended 5% to use complete case analysis.[32] However, the complete case analysis was supplemented with singly imputed best-worst and worst-best scenarios.[32] Taken together, based on the observed and imputed data, a protective effect of carrageenan was not demonstrated and is unlikely to be demonstrated if the missing data was available. Second, prior research suggests some incident HPV infections could be reactivations of previously acquired infection.[44] Further, if RAI occurred close to the study visit, a detected HPV type could have been recently deposited from intercourse, as was demonstrated for vaginal sex.[45] Reactivations or depositions of HPV would be expected to be balanced between study arms, however, these 'incident' infections could bias the estimate towards the null. Third, an attrition rate of 10% was expected, but 41.2% of participants withdrew early. Although this may not have had a great impact on the effect estimate, as 28.6% (31/105) of participants who withdrew early acquired an incident HPV infection. Fourth, compliance to the intervention was also an issue for 80% of all visits, participants reported 100% compliance to gel use during each RAI; however, 52.9% of participants ever reported using another gel during the interval, indicating at least some of the time, they were non-compliant to the intervention.

The LIMIT-HPV RCT is the first study to evaluate efficacy of a carrageenan gel among gbMSM. The study has several strengths, including its design; a randomized, double-blinded, and placebo-controlled trial. The intervals between anal HPV sampling were relatively short, which captures more granular information on HPV incidence and clearance. Further, testing with the Linear Array Assay allowed for detection of 36 HPV types,[28] which expands our knowledge of HPV prevalence in gbMSM in Montreal. To conclude, the LIMIT-HPV study demonstrated a null effect of a carrageenan-based lubricant in a gbMSM population, and more AEs were reported in the carrageenan arm. Alternate HPV prevention strategies are needed for this at-risk population.

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Trial registration

This trial is registered with clinicaltrials.gov (NCT02354144). The full trial protocol is available here: Laurie 2020 BMJ Open.[27]

Contributors

ELF, AdP, FC, and PPT conceived and designed the study. MZ managed the study. CL drafted the manuscript under the supervision of ELF, AdP and MZ. All authors interpreted the data, critically reviewed the manuscript, and approved the final version.

Declaration of interests

AdP received honoraria for consulting on HIV antiretroviral regimen for ViiV Healthcare, and received grants from CIHR and FRQ-S, outside the submitted work.

ELF reports grants and personal fees from Merck, grants, personal fees and non-financial support from Roche, and personal fees from GSK, outside the submitted work.

FC reports grants from Réseau FRQS-SIDA during the conduct of the study and grants to his institution for HPV-related work from Merck Sharp and Dome, Roche Diagnostics and Becton Dickinson, outside of the submitted work.

MZ, PPT, and CL have nothing relevant to this article to declare.

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4.10 FIGURES

Figure 1. Trial profile: design and subject allocation

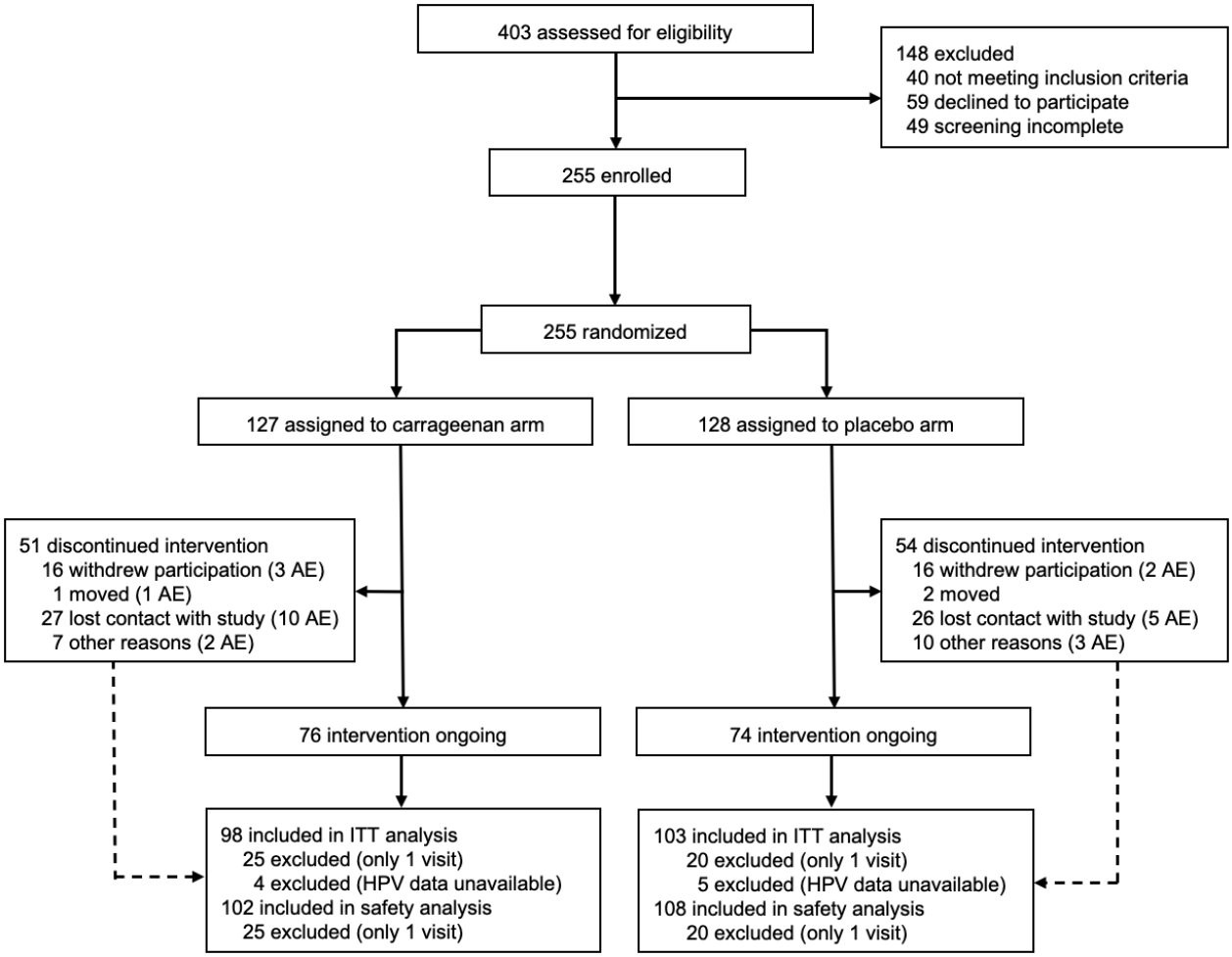
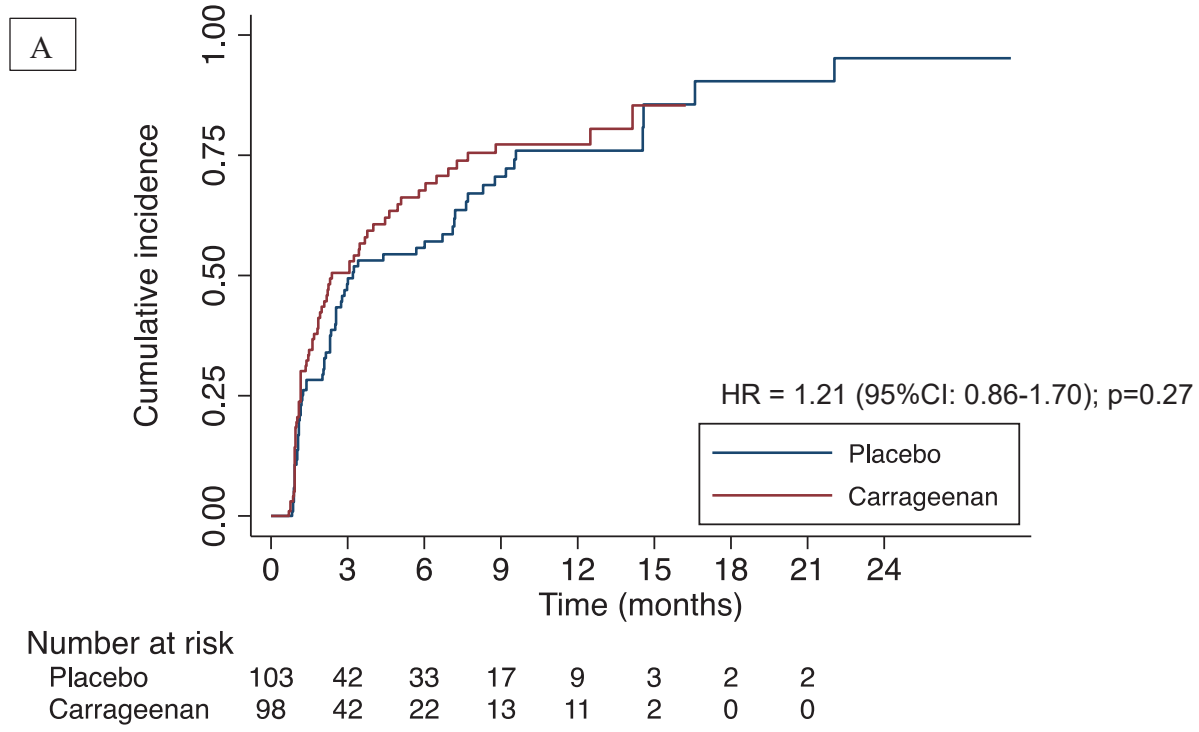


Figure 1 legend

The flow diagram presents the number of participants at enrollment, intervention allocation, follow-up, and data-analysis. A total of 201 participants were included in the intention to treat analyses (ITT) and 210 participants were included in the safety analyses.

Figure 2. Cumulative incidence of HPV infections by arm



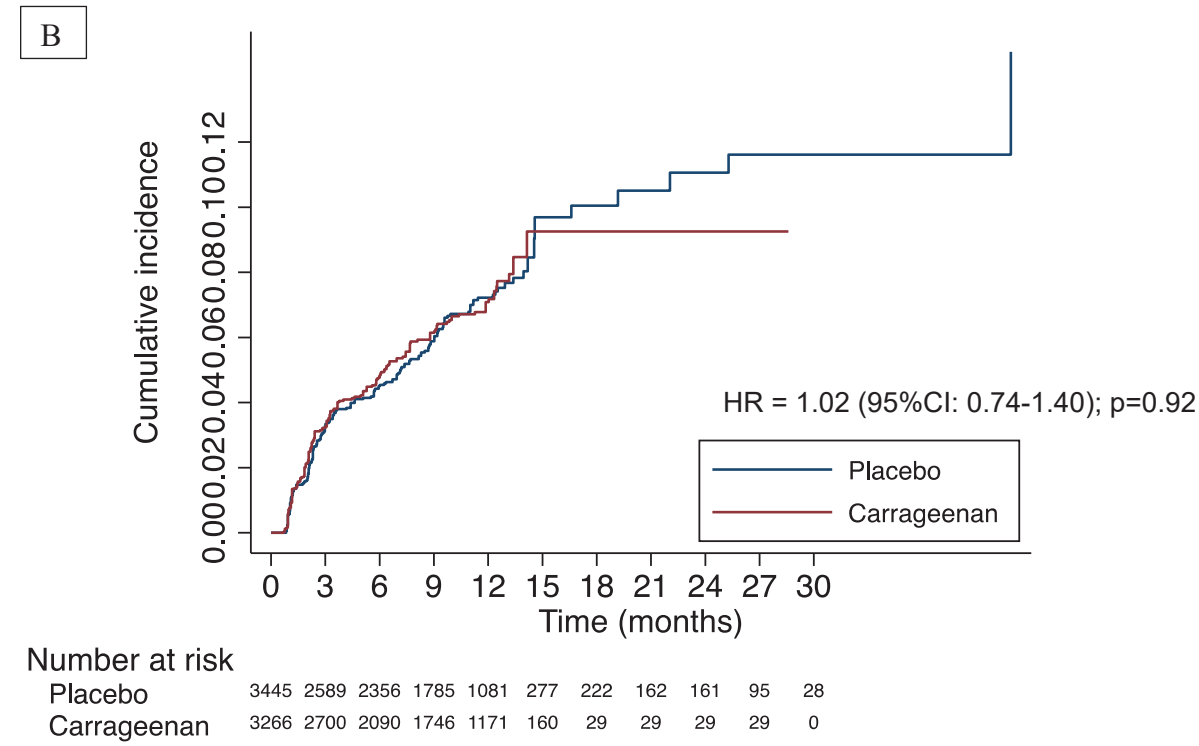
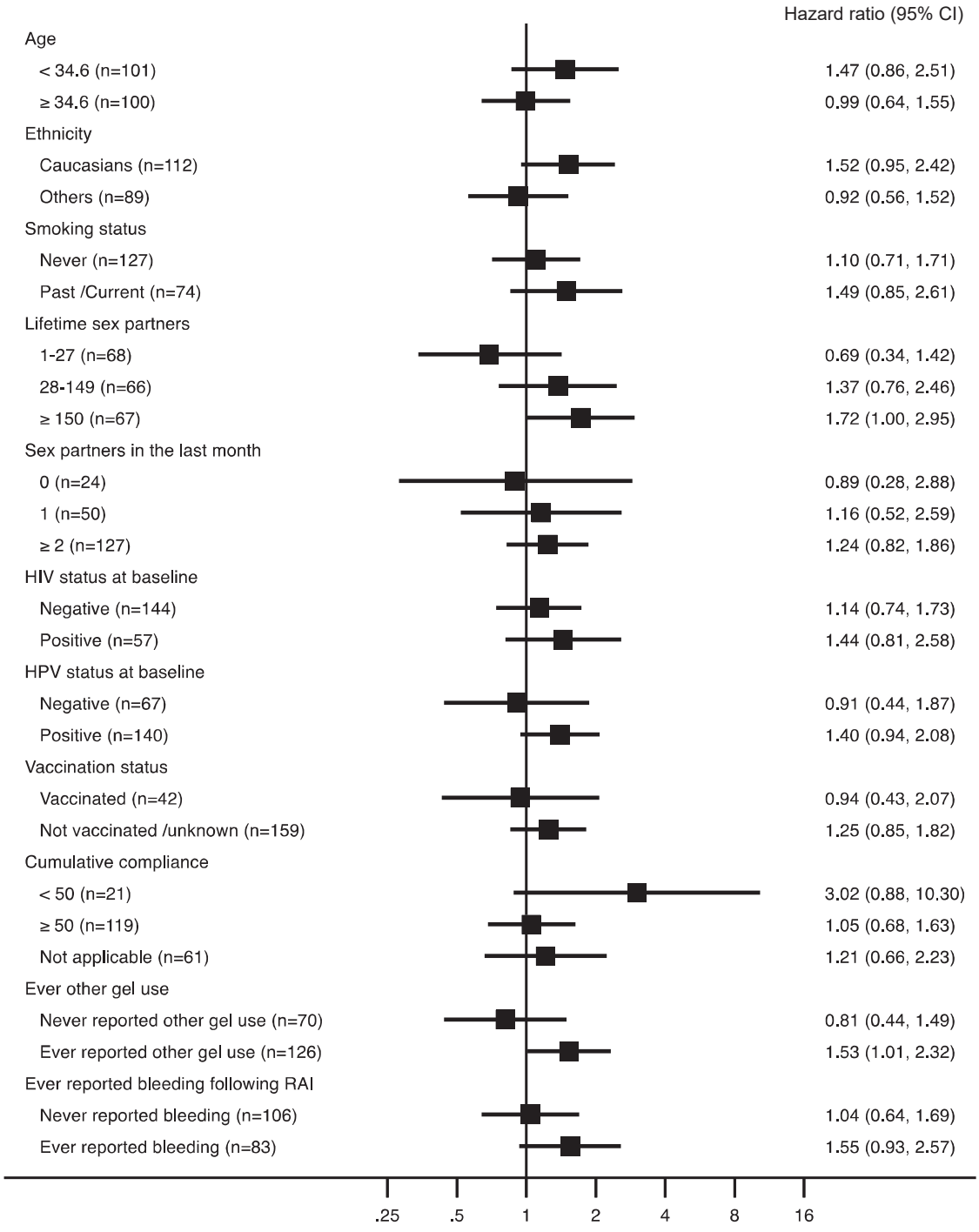


Figure 2 legend

Fig2A plots the first HPV infection detected. Fig2B plots all new HPV infections, using a type-specific analysis; the number at risk corresponds to the number of infections a participant could have acquired at subsequent visits. Each participant could have acquired any of the 36 HPV types, excluding any HPV types that were positive at baseline for that participant.

Figure 3. Sub-group analyses



4.11 TABLES

Table 1. Baseline and follow-up characteristics of participants, overall and by intervention

	Overall (n=255)	Treatment (n=127)	Placebo (n=128)	p value
Age – years				
Mean (SD)	36.9 (14.3)	36.7 (14.2)	37.0 (14.4)	0.82
Median	33.6	33.6	33.7	
Range	18.2 – 71.7	18.3 – 71.7	18.2 – 70.3	
Ethnicity - n (%)				0.26
French Canadian	98 (38.4)	48 (37.8)	50 (39.1)	
English Canadian	37 (14.5)	17 (13.4)	20 (15.6)	
Black Canadian	5 (2.0)	0 (0)	5 (3.9)	
Aboriginal	1 (0.4)	1 (0.8)	0 (0)	
American	6 (2.4)	1 (0.8)	5 (3.9)	
Latin American	29 (11.4)	14 (11.0)	15 (11.7)	
Haitian	4 (1.6)	2 (1.6)	2 (1.6)	
European	32 (12.6)	20 (15.8)	12 (9.4)	
African	2 (0.8)	1 (0.8)	1 (0.8)	
South Asian	8 (3.1)	4 (3.2)	4 (3.1)	
East Asian	13 (5.1)	6 (4.7)	7 (5.5)	
Middle Eastern	13 (5.1)	7 (5.5)	6 (4.7)	
Other	7 (2.8)	6 (4.7)	1 (0.8)	
Education - n (%)				0.24
Elementary	2 (0.8)	0 (0)	2 (1.6)	
Secondary	55 (21.6)	23 (18.1)	32 (25.0)	
College	66 (25.9)	33 (26.0)	33 (25.8)	
University	132 (51.8)	71 (55.9)	61 (47.7)	
Smoking status - n (%)				0.06
Never	160 (62.8)	86 (67.7)	74 (57.8)	
Former	53 (20.8)	27 (21.3)	26 (20.3)	
Current	42 (16.5)	14 (11.0)	28 (21.9)	
Age at first intercourse				
Mean (SD)	17.3 (5.5)	17.2 (5.7)	17.4 (5.4)	0.23
Median	17.0	16.0	17.0	
Range	4.0 – 42.0	5.0 - 42.0	5.0 – 42.0	
Lifetime sex partners – quintiles, n (%)				0.82
1-19	48 (18.8)	23 (18.1)	25 (19.5)	
20-40	54 (21.2)	25 (19.7)	29 (22.7)	
45-95	45 (17.7)	25 (19.7)	20 (15.6)	
100-460	54 (21.2)	25 (19.7)	29 (22.7)	
> 500	54 (21.2)	29 (22.8)	25 (19.5)	
New sexual partner in the last month – n (%)				0.13
Yes	161 (63.1)	86 (67.7)	75 (58.6)	
No	94 (36.9)	41 (32.3)	53 (41.4)	
HIV status, n (%)				0.81

Yes	68 (26.7)	33 (26.0)	35 (27.3)	
No	187 (73.3)	94 (74.0)	93 (72.7)	
HPV DNA status, n (%)				
Any HPV	155 (60.8)	84 (66.1)	71 (55.5)	
Negative	74 (29.0)	33 (26.0)	41 (32.0)	0.19
Missing PCR results	26 (10.2)	10 (7.9)	16 (12.5)	
Subgenus 1	81 (31.8)	44 (34.7)	37 (28.9)	0.47
Subgenus 2	128 (50.2)	69 (54.3)	59 (46.1)	0.34
Subgenus 3	91 (35.7)	49 (38.6)	42 (32.8)	0.50
Vaccination status, n (%)				0.91
Yes	57 (22.4)	28 (22.1)	29 (22.7)	
No	198 (77.7)	99 (78.0)	99 (77.3)	
Follow-up time – months				0.24
Mean	7.8 ± 6.5	7.3 ± 5.7	8.4 ± 7.2	
Median	8.02	7.6	9.3	
Range	0 – 40.7	0 – 28.5	0 – 40.7	
Number of visits/man				0.65
Mean	4.7 ± 2.4	4.6 ± 2.4	4.7 ± 2.3	
Median	5	5	5.5	
Range	1 - 7	1 - 7	1 - 7	

Table 2. Incidence of HPV infection, overall and by arm and HIV status

		Carrageenan				Placebo				Effect estimate	
		Incident cases/ number at risk (%)	Actuarial mean [a] (95% CI)	Arithmetic mean [a] (95% CI)	Median [a] (95% CI)	Incident cases / number at risk (%)	Actuarial mean [a] (95% CI)	Arithmetic mean [a] (95% CI)	Median [a] (95% CI)	Hazard ratio (95% CI)	p value
Overall (N=201)	Overall	68/98 (69.4%)	5.5[b] (4.3-6.8)	2.7 (2.1-3.4)	2.4 (1.9-4.0)	67/103 (65.1%)	7.3[b] (5.4-9.3)	3.8 (2.8-4.9)	3.2 (2.5-7.2)	1.21 (0.86-1.70)	0.27
	Subgenus 1	21/98 (21.4%)	21.2[b] (18.3-24.1)	4.1 (2.4-5.8)	NR	22/103 (21.4%)	25.6[b] (18.2-33.0)	4.7 (2.7-6.7)	NR	1.03 (0.57-1.88)	0.92
	Subgenus 2	55/98 (56.1%)	8.0[b] (6.5-9.5)	3.5 (2.6-4.4)	6.0 (3.5-9.2)	54/103 (52.4%)	10.1[b] (7.4-12.8)	4.3 (3.0-5.5)	7.7 (3.4-11.2)	1.12 (0.77-1.63)	0.56
	Subgenus 3	35/98 (35.7%)	10.5[b] (9.0-11.9)	3.5 (2.3-4.8)	13.4 (8.8-)[c]	33/103 (32.0%)	17.8[b] (14.6-21.0)	3.9 (2.0-5.7)	25.3 (19.2-)[c]	1.22 (0.75-1.98)	0.42
HIV-negative (N=144)	Overall	44/72 (61.1%)	6.5[b] (4.9-8.1)	2.8 (2.0-3.7)	3.5 (2.1-6.5)	44/72 (61.1%)	8.0[b] (5.6-10.4)	4.5 (3.1-5.9)	6.7 (2.5-7.7)	1.14 (0.74-1.73)	0.56
	Subgenus 1	15/72 (20.8%)	20.8[b] (17.2-24.4)	4.3 (1.9-6.6)	NR	12/72 (16.7%)	27.0[b] (18.7-35.2)	5.8 (2.5-9.0)	NR	1.37 (0.64-2.94)	0.42
	Subgenus 2	33/72 (45.8%)	9.3[b] (7.4-11.2)	3.4 (2.3-4.5)	7.3 (3.8-)[c]	35/72 (48.6%)	10.9[b] (7.7-14.1)	4.9 (3.3-6.6)	8.8 (5.7-13.4)	1.00 (0.62-1.62)	1.00
	Subgenus 3	19/72 (26.4%)	11.8[b] (10.2-13.4)	3.6 (1.8-5.4)	NR	19/72 (26.4%)	19.4[b] (15.7-24.0)	5.0 (1.8-8.2)	25.2 (19.2-)[c]	1.17 (0.61-2.25)	0.64
HIV-positive (N=57)	Overall	24/26 (92.3%)	3.3[b] (1.8-4.8)	2.5 (1.3-3.6)	1.4 (1.1-2.2)	23/31 (74.2%)	4.9 (2.9-6.9)	2.5 (1.2-3.9)	2.3 (1.2-3.2)	1.44 (0.81-2.6)	0.22
	Subgenus 1	6/26 (23.1%)	11.6[b] (9.9-13.4)	3.6 (0.7-6.6)	NR	10/31 (32.3%)	11.0[b] (8.8-13.2)	3.3 (0.9-5.8)	NR	0.64 (0.23-1.77)	0.38
	Subgenus 2	22/26 (84.6%)	5.1[b] (3.2-7.0)	3.6 (1.9-5.2)	2.2 (1.2-5.8)	19/31 (61.3%)	6.8 (4.5-9.0)	3.1 (1.3-4.8)	2.7 (1.4-)[c]	1.40 (0.75-2.27)	0.29
	Subgenus 3	16/26 (61.5%)	7.3[b] (5.1-9.4)	3.5 (1.6-5.3)	5.6 (2.0-)[c]	14/31 (45.2%)	8.4[b] (6.1-10.8)	2.3 (1.2-3.4)	NR	1.30 (0.63-2.67)	0.47

[a]Time in months. The actuarial mean accounts for censoring, whereas the arithmetic mean excludes participants who did not acquire a new HPV type.
[b] Mean was underestimated since the largest observed analysis time is censored.
[c] Upper confidence limit was undetermined since the survival function does not fall below 0.5.
[d]Subgenus 1 consists of HPVs 6, 11, 40, 42, 44, and 54.
[e]Subgenus 2 consists of HPVs 16, 18, 26, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, and 82.
[f]Subgenus 3 consists of HPVs 61, 62, 71, 72, 81, 83, 84, and 82.
NR: Not reached

Table 3. Adverse events by arm, overall and by data collection tool

Adverse events	Carrageenan (n=102)	Placebo (n=108)	p value
Any *(including bleeding)*	74 (72.6)	68 (63.0)	0.14[a]
Any *(excluding bleeding)*	61 (59.8)	43 (39.8)	0.004[a]
Adverse event reported in calendar[b]	11 (14.7)	2 (2.6)	0.009
Unusual pain during anal sex	4 (5.3)	0 (0)	0.057
Rectal bleeding in between anal sex	6 (8.0)	0 (0)	0.013
Unusual abdominal pain	2 (2.7)	0 (0)	0.24
Unusually painful defecation	0 (0)	0 (0)	-
Flatulence, constipation, urgency and/or fecal incontinence or diarrhea	0 (0)	1 (1.3)	1.00
Rectal abscess/ulcer/fistulae	0 (0)	0 (0)	-
Anal discharge	2 (2.7)	0 (0)	0.24
Hemorrhoids	0 (0)	0 (0)	-
Itching, burning, edema or pain in the anorectal area	4 (5.3)	0 (0)	0.057
Anal fissures	0 (0)	0 (0)	-
Other	3 (2.9)	1 (0.9)	0.36
Adverse event/reaction reported in survey[c] *(including bleeding)*	60 (58.8)	47 (43.5)	0.04[a]
Use of the gel caused discomfort/adverse reactions to you	18 (18.2)	8 (7.8)	0.035
Use of the gel caused discomfort/adverse reactions to your partner(s)	13 (13.1)	2 (1.9)	0.003
Reported bleeding following RAI	47 (49.5)	43 (42.6)	0.39[a]
Adverse event/reaction reported in survey[d] *(excluding bleeding)*	25 (25.2)	9 (8.7)	0.002[a]
Use of the gel caused discomfort/adverse reactions to participant	18 (18.2)	8 (7.8)	0.035
Use of the gel caused discomfort/adverse reactions to partner	13 (13.1)	2 (1.9)	0.003
Conditions reported by nurse at clinic visit	25 (24.5)	23 (21.3)	0.58[a]
Warts	6 (5.9)	7 (6.5)	1.00
Erythema	6 (5.9)	9 (8.3)	0.60
Abrasions	4 (3.9)	2 (1.9)	0.44
Inflammation	2 (2.0)	0 (0)	0.24
Fissures	3 (2.9)	2 (1.9)	0.68
Abscesses	1 (1.0)	1 (0.9)	1.00
Hemorrhoids	11 (10.8)	7 (6.5)	0.33
Infections/conditions reported by participant	34 (35.1)	23 (24.7)	0.012[a]
Venereal warts or condylomas[e]	2 (2.1)	3 (3.3)	0.68
Chlamydia	14 (13.9)	12 (11.1)	0.68
Lymphogranuloma vereneum	0 (0)	1 (0.9)	1.0
Anal or genital herpes	5 (5.0)	5 (4.7)	1.0
Syphilis	7 (6.9)	7 (6.5)	1.0
Gonorrhea	23 (22.8)	10 (9.4)	0.008[a]
Ulcers or genital sores	0 (0)	1 (0.9)	1.0
Hepatitis B	0 (0)	1 (0.9)	1.0
Hepatitis C	0 (0)	2 (1.9)	0.5
HIV	1 (1.1)	0 (0)	1.0
Anal precancer	0 (0)	1 (0.9)	1.0
Cancer	0 (0)	1 (0.9)	1.0
Other	0 (0)	1 (0.9)	1.0
Death	0 (0)	1 (0.9)	1.0

[a]p-value calculated using χ^2. Fisher's exact test was used otherwise.
[b]Non-response for 27 carrageenan and 31 placebo
[c]Non-response for 3 carrageenan and 3 placebo
[d]Non-response for 3 carrageenan and 5 placebo
[e]Non-response for 7 carrageenan and 16 placebo

CHAPTER 5: DISCUSSION

5.1 SUMMARY OF RESEARCH FINDINGS

This thesis explored the evidence for carrageenan as a preventive agent against HPV. Manuscript 1 summarized the existing evidence of carrageenan's anti-HPV activity. In Manuscript 2, the protocol for a randomized controlled trial (RCT), whose primary aim is to determine the efficacy of a carrageenan-based gel among gbMSM, was described in detail.[1] In the final manuscript (Manuscript 3), data collected from this RCT were used to evaluate the efficacy and safety of a carrageenan-based gel among gbMSM. Findings from Manuscript 3 suggested the lack of a protective effect of the carrageenan-gel, and significantly more adverse events (AEs) were reported in the treatment arm. Thus, the trial was stopped based on recommendations by the Data Safety and Monitoring Committee. Importantly, this trial highlights a known safety concern of hyper-osmolal gels[2] and a potential safety concern of carrageenan-based gels for rectal use. Prior research has found carrageenan-based gels to be safe for vaginal,[3] vaginal and penile,[4,5] and anal use.[6] However, hyper-osmolal gels are damaging to the rectal epithelium.[2] If there is a protective effect of carrageenan, AEs related to hyper-osmolality could mask the protection; however, it cannot be ruled out that the AEs are attributable to carrageenan itself.

5.2 STRENGTHS AND LIMITATIONS

To the best our knowledge, the LIMIT-HPV study is the first RCT to explore efficacy of a carrageenan-based gel against incident anal HPV infections. An inherent strength of the trial was its randomized, placebo controlled, double-blinded, and prospective study design. In addition, use of the Linear Array Assay allowed for detection of 36 different HPV types,[7] including 6 low-risk, 22 high-risk, and 8 mostly commensal HPV types,[8–11] hence enabling the evaluation of carrageenan's efficacy by subgenera. Ascertaining the status of multiple HPV types also allowed for modelling to take into account the incidence of each new HPV type acquired by participants using a type-specific Cox regression model.

A limitation of the LIMIT-HPV study is that the findings are based on interim data analyses, not the full sample data (study did not reach the target sample size). Based on the targeted sample size, the study is underpowered to detect a significant difference between the carrageenan and

placebo arms, if one exists. Duplicating the dataset to mimic the full sample size lead to the same non-significant increase in risk of HPV infection, although the lower bound of the confidence interval was closer to 1. The (non-significant) increase in the risk of incident HPV infections was detected prior to study end, which allowed for prevention of future harm that could result from study participation, especially given the finding that more AEs were reported by participants in the carrageenan arm.

5.3 FUTURE RESEARCH

The findings suggest the formulation of gel used in this study should not be recommended to gbMSM for prevention of HPV infection, given the lack of a beneficial effect and more AEs in the carrageenan arm. This observation may extend to women, who should be advised to avoid using this gel formulation for anal intercourse. The final analyses of the Carrageenan gel Against Transmission of Cervical HPV (CATCH) RCT,[12] evaluating efficacy of a carrageenan gel among sexually active women, is of key interest in the evaluation of carrageenan. Additionally, a recently completed but not yet published study is evaluating a carrageenan-combination product for rectal use in women and men, however based on very few participants (n=13).[13] This study could provide evidence for or against the hypothesis that carrageenan may cause rectal damage, as the study gel is formulated with Carraguard®, which is a nearly iso-osmolal gel, and iso-osmolal gels are not known to cause rectal damage.[14] Further analyses of the LIMIT-HPV trial are needed to disentangle the effect of hyper-osmolality and carrageenan. The secondary objective of the LIMIT-HPV study is to evaluate carrageenan's efficacy in reducing type-specific anal HPV prevalence, i.e., accelerating the clearance of existing infections in sexually active gbMSM. This analysis will be conducted in the near future. The data collected from the placebo arm of the LIMIT-HPV study could also be used for additional analyses, such as identifying determinants of compliance to gels, and identifying risk factors of anal HPV incidence, clearance, and persistence to improve the understanding of anal HPV infection in gbMSM.

5.4 CONCLUSIONS

This thesis evaluated carrageenan as a preventive agent against HPV. While the narrative review supported carrageenan's anti-HPV activity in pre-clinical studies and clinical studies in women, the interim analysis of the LIMIT-HPV study did not demonstrate a protective effect of carrageenan when comparing the carrageenan to placebo group. Further follow-up data from a similar study (CATCH) in heterosexual women will provide more evidence for or against the hypothesis that carrageenan prevents the acquisition of HPV infection. Ultimately, this thesis served to explore and evaluate an alternative avenue for prevention of HPV infection and adds to the body of work in this important area of research.

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6. APPENDIX

6.1 Manuscript 1 Appendix

Appendix 1 - Telephone screening questionnaire

LIMIT HPV Telephone Screening Questionnaire

Thank you for your interest in our study which aims to evaluate the efficacy of a lubricant gel used during receptive anal intercourse to protect against HPV infection in men having sex with men.

I will now ask you some questions to determine whether or not you are eligible. This will take about 10 minutes. Some of these questions may be of a personal nature. I would like to assure you that all your answers will be kept strictly confidential. Is that OK with you?

[Button] *Verbal consent obtained* [/Button – when pressed record date, show generated LIMIT ID at the top of the page]

1. Are you a man aged over 18 years old?

   [For programming:

   Yes [→ELIGIBLE]

   No [→ NOT ELIGIBLE]

2. For how long do you plan to stay in Montreal?

   Less than a year [→ NOT ELIGIBLE]

   A year or more [→ ELIGIBLE]

3. Are you fluent in either English or French?

   Yes [→ELIGIBLE]*No* [→ NOT ELIGIBLE]

4. How long ago did you last have receptive anal intercourse with a male partner?

   Less than 3 months ago [→ ELIGIBLE]

   3 or more months ago [→ NOT ELIGIBLE]

5. Do you think you will have receptive anal intercourse with a male partner within the next 3 months?

   Yes [→ ELIGIBLE]

   No [→ NOT ELIGIBLE]

   Don't know [→ ELIGIBLE]

6. Based on the past few years, do you expect to have less than 2 or more than 50 DIFFERENT partners in the next year?

Yes [→ NOT ELIGIBLE]

No [→ ELIGIBLE]

7. Eligible men must not be receiving treatment for anal or perianal condylomas or anal intraepithelial neoplasia (AIN) during the course of this study. Are you ok with this criterion?

   Yes [→ ELIGIBLE]

   No [→ NOT ELIGIBLE]

8. Are you currently participating in another study of intervention or treatment of Human Papillomavirus (HPV) or HPV-related disease (condylomas, AIN, anogenital cancer)?

   Yes [→ NOT ELIGIBLE]

   No [→ ELIGIBLE]

9. Do you have any allergies or hypersensitivities to personal lubricants?

   Yes [→ NOT ELIGIBLE]

   No [→ ELIGIBLE]

   Don't know [→ ELIGIBLE]

10. The lubricants that we will use in this study may contain: Propylene Glycol, Glycerin, Carrageenan, Aloe barbadensis leaf juice, Cellulose Gum, Citric Acid, Diazolidinyl urea, Saccharin, Tetrasodium EDTA. Do you have any known allergies to any of these substances?

    Yes [→ NOT ELIGIBLE]

    No [→ ELIGIBLE]

[If participant status=INELIGIBLE at this point, DISPLAY SCRIPT 1 and END]

"Thank you for answering my questions. Unfortunately, you are not eligible for our study. We appreciate your interest in our study."

[IF ELIGIBLE, DISPLAY FOLLOWING QUESTIONS]

11. Thank you, I just have a few more questions.

In this study, participants will be given a lubricant gel to use during all receptive anal intercourses during one year, and will be asked to visit the clinic seven times over that year (at 0, 1, 2, 3, 6, 9 and 12 months). At each visit, a short online survey will be completed and a nurse will collect an anal swab. Participants will also keep track of their sexual activities and lubricant use through an online calendar. Do you think you will be able to follow these procedures?

Yes [→ ELIGIBLE]

No [→ NOT ELIGIBLE]

[IF 11=No, participant status=INELIGIBLE, DISPLAY SCRIPT 2 and END]

"Thank you for answering my questions. Unfortunately study participation requires the fulfillment of specific study procedures. Nonetheless, we greatly appreciate your interest in our study."

12. So far, it looks like you are eligible for this study. Both HIV-positive and HIV-negative men are enrolled in this study. We need to know your HIV-status to see if the effect of the gel differs according to the HIV status, and to plan recruitment at the different study sites. If you never tested positive for HIV previously, we will do an HIV test by pricking your finger to obtain a drop of blood. Please choose the best answer:

    I am HIV positive [→ ELIGIBLE]

    I never tested positive for HIV before, and I am ok with doing an HIV-test for this study. [→ ELIGIBLE]

    I never tested positive for HIV before, but I am NOT ok with doing an HIV-test for this study. [→ NOT ELIGIBLE]

[IF participant status= INELIGIBLE, DISPLAY SCRIPT 2 and END]

Great, thank you for your time. So far you are eligible for the study. We can now plan your enrolment visit.

At this point, record the name, contact information and study ID number to transmit to nurse.

The Research Coordinator could also note in a separate excel sheet on where/how did the caller hear about the study (i.e., poster, Facebook ad, email, friends, etc.).

Appendix 2 - Pre-eligibility questionnaire

LIMIT-HPV Study - Eligibility, Pre-screening questionnaire

(Content template for production of google form)

McGill University has several innovative research projects on Human Papillomavirus (HPV). HPV is the most common sexually transmitted disease (STD) in the world and touches more than 75% of Canadians in their lifetime.

This study investigates whether a lubricant that contains carrageenan is effective in clearing and preventing HPV infection (LIMIT study). Overall, the objective of these studies is to improve the health of ALL sexually active individuals. Results from these studies will be useful to the prevention efforts deployed in Canada as well as elsewhere in the world.

The purpose of the questionnaire below is to assess your eligibility to LIMIT. Thank you for your interest in our studies.

Best regards,

Division of Cancer Epidemiology, McGill University.

* Required

1. How did you hear about us? *
    1. *Posters*
    2. *Classified ads (Kijiji, AnnonceDonc, LesPacs, Craiglist, etc.)*
    3. *Facebook*
    4. *Word of mouth (class presentation, friends, familly, collegue)*
    5. *Emails (from your department, research assistant, etc.)*
2. What is your gender? *
    1. *Man*
    2. *Woman*
    3. *Other:*
3. What is your age? *
    1. *17 years old and under*
    2. *Between 18 and 45 years old*
    3. *46 years old and over*

4. When was your last sexual intercourse? Here, you must specify with as much accuracy as possible the time elapsed since your last relationship (in week, month or year). *

5. Are you sexually active? *That is, at least one sexual partner in the last 3 months. *

1. *Yes*
2. *No*

6. Are you planning to stay in Montreal and its surrounding areas for the next year? *

1. *Yes*
2. *No*
3. *Not sure*

Additional Information

Just a few questions left! Based on your answer, it may be possible that you're eligible for one of our studies. Please leave us your contact information, we will reach you shortly. In all cases, we will inform you of the outcome of this questionnaire, whether you are eligible or not.

*Name (and/or nickname) **

*Phone number **

*E-mail address **

Appendix 3 - Informed consent form

INFORMATION AND CONSENT FORM

Research Project: Lubricant Investigation in Men to Inhibit Transmission of HPV Infection (LIMIT-HPV)

Principal investigators: Dr. Eduardo Franco and Dr. Alexandra de Pokomandy

Institution: Division of Cancer Epidemiology and Family Medicine Department, McGill University

Funding Source: The Canadian Institutes of Health Research (CIHR) and Canadian Cancer Society Research Institute (CCSRI)

You are invited to take part in a clinical trial on human papillomavirus (HPV) infection prevention. Clinical trials include only people who choose to take part. Should you decide to participate in this study, you will be given a copy of this consent form. It provides you with a detailed description of the study, describing all the procedures that will be followed. If you have any questions concerning what is explained here, do not hesitate to ask us. Please take all the time you need to read this form.

INTRODUCTION

HPV is the most common sexually transmitted infection, and most sexually active men will be infected with HPV over their lifetime. Usually these infections go unnoticed or only cause anal or genital warts (condylomas). Although benign, genital warts are difficult to treat and may lead to social embarrassment. Even if most HPV infections are temporary and will be cleared naturally, certain types cause more persistent infections that can progress to cancer.

Researchers identified that *carrageenan*, an inexpensive gelling agent that is already commonly used in food and cosmetics, is able to interfere with HPV infection. This study will examine if a personal sex lubricant containing carrageenan, directly applied to the skin and used during sexual activities, can decrease HPV infection. Such an inexpensive intervention would help reduce the burden of genital warts, and HPV-associated cancers in a cost-effective way. The gel being studied is already commercialized and sold as a personal lubricant.

PURPOSE OF THE STUDY

This study will investigate whether or not a lubricant gel that contains carrageenan is effective in preventing anal HPV infection in men who have sex with men. We will recruit 380 adult men participants in Montreal, including 110 HIV-seropositive men.

STUDY PROCEDURE

Duration and number of visits

Your participation in this study will be for 12 months and will include 7 visits.

Table of study visits and procedures

	Visit 1 (Enrollment)	**Visit 2** (1 mo)	**Visit 3** (2 mo)	**Visit 4** (3 mo)	**Visit 5** (6 mo)	**Visit 6** (9 mo)	**Visit 7** (12 mo)
Questionnaire	x	x	x	x	x	x	x
Anal HPV test	x	x	x	x	x	x	x
HIV test*	x						x
Estimated duration	60 min	30 min	30 min	30 min	30 min	30 min	30 min

* For participants not known to be seropositive for HIV.

Randomization

If you are eligible and consent to enroll in this study, you will be "randomized" into one of the study groups described below. Randomization means that you are put into a group by chance. A computer program will place you in one of the study groups. Neither you nor the study staff can choose the group you will be in. You will have an equal chance of being placed in either group.

If you are in Group 1 you will receive a personal lubricant that contains carrageenan.

If you are in Group 2 you will receive a personal lubricant that does not contain carrageenan.

Neither you nor the study staff will know if you are getting the carrageenan gel or the comparison gel. The reason for this is that if men know which gel they are using, it may affect what they think and say about it. If the study staff is aware of participants' group assignment, it may affect how they interpret what they see and hear in the exams and interviews. Only after the study is over will the researchers be able to find out the intervention that each participant was assigned, and whether or not the study results show a difference between the 2 groups. If you want to know which gel you were using, you will be able to find out when the whole study is finished.

Clinic Visits

You will be asked to visit the research nurse 7 times for the collection of anal specimens for HPV testing over the 12 months of your participation. At your first visit, a research nurse will provide you with instructions on how and when to apply the study gel. The nurse will collect your first anal HPV specimen in a private room at the clinic, by inserting and rotating a cotton-tip swab in your anus. This procedure is not painful, and only takes few seconds. In addition, you will be asked to complete an electronic survey. This first survey will ask questions about your background, medical and sexual history, condom and lubricant usage, smoking habits and alcohol consumption. The research nurse or coordinator will be available at all times should you need help. Before leaving, you will be provided with a one-month supply of gel. This first visit will last about one hour.

You will visit the clinic at 1 month, 2 months, 3 months, 6 months, 9 months and 12 months after your first visit. You will be asked to abstain from receptive anal sex and gel use for at least 48 hours prior to each visit. At the clinic, the nurse will collect an anal specimen for HPV testing in a private room, and you will be asked to complete a follow-up survey about your recent sexual activities, use of study gel, and medical history. Each survey will take about 15 minutes to complete. At your one- and two-month visit, the research nurse will provide you with a one-month supply of study gel. At every visit thereafter (except for your final visit), the research nurse will provide you with 3 months' supply of gel, i.e., enough to last you until the next visit. Each visit will last about 30 minutes.

You will be asked to continue using the assigned intervention for the complete follow-up period (12 months), along with condom use for prevention of other sexually transmitted infections.

Online calendars

You will be given an access code with which you can log on to a secure internet website to complete confidential electronic calendars. You will be asked to track your sexual activities and study gel use on a weekly basis using the online calendar. The calendars will take approximately 5 minutes per week to update. Help will be available through email and telephone should you need assistance.

Laboratory testing of anal specimens

The anal specimens collected for HPV testing will be sent to the laboratory and will be tested for 36 strains of HPV, including the most common types of HPV that can cause anal cancer. HPV testing is only done for research purposes and it is not used in standard clinical care of men. Therefore, we are not planning to reveal individual test results unless specifically requested. In such a case, we can send them to your doctor at the end of your study participation. It is important to know that an HPV infection can last for a very long time. Thus, a positive test for an HPV infection does not mean that it was recently acquired.

We also ask you for permission to store the samples for future studies on HPV infection using more sophisticated techniques not yet available.

HIV status

- **If you have never been diagnosed HIV-seropositive:** For participants who never tested seropositive for HIV, the nurse will conduct a rapid HIV test with a drop of your blood obtained through a finger prick. We will test you at enrolment and at study exit. This will serve to verify the HIV status of participants. Please note that if your rapid test gives a HIV-seropositive result, we will need to confirm this finding with a second test using a regular blood sample and more accurate laboratory equipment. We will then ask you to abstain from sexual activities or to use protection when engaging in sex until this result can be confirmed. The nurse will immediately refer you to a physician for follow-up. If you are found to be HIV-positive at study entry (not at study exit), then the following paragraph will apply to you as well.
- **If you are living with HIV:** For participants living with HIV, a review of your medical chart (at your usual HIV clinic) will be done to collect data about your HIV medical history and HIV lab results (CD4 counts, HIV viral load). We will therefore ask you to provide us with the contact information of your HIV physician.

End of study participation

Your participation in the study will be stopped early if you consider it to be in your best interest or for personal reasons, or if a physician considers it to be in your best interest because of safety reasons or your well-being.

BENEFITS

You should not expect any direct health benefits from participating in this study. While researchers hope that the intervention under study will be useful in protecting against infection with HPV, **there is no proof of this yet**. The information from this study will help researchers learn more about carrageenan as a potential treatment and preventative against anal HPV infection and anal cancer.

RISKS

Using either carrageenan or the comparison gel may cause itching, burning or pain, but these symptoms are unlikely (<5% chance). If you experience any side effects, discontinue use of the gel and contact the study nurse. Since the study gel (with or without carrageenan) does not prevent other sexually transmitted infections, you will be asked to continue using condoms for the duration of the trial.

The collection of an anal specimen for HPV testing is a safe procedure. There is a possibility of slight discomfort during the insertion of the cotton-tip swab to collect the specimen.

For the HIV-negative participants, there may be small amount of pain during the finger prick for the HIV tests. There may be psychological distress associated with testing positive for HIV. In the event that you test positive, you will receive counseling and referral for standard care.

If you experience any adverse events during your involvement in the study, you will be referred to the McGill University Student Health Services Clinic located at 3600 McTavish Street West or the MUHC Chronic Viral Illnesses Service clinic located on the 2nd floor of the MUHC Glen site 1001 Décarie.

CONFIDENTIALITY

The results from the laboratory testing of your specimens and the responses you give in the surveys will be treated with strict confidentiality. All the information that you provide online will be stored in a secure server. Only researchers who are part of the study will have access to the data. No names or other information that could identify yourself as a participant will be released. All the data from this study will be analyzed as groups without linkage of names to any data.

The actual specimens will not be made available to investigators that are not involved with this study, nor will they be sold for commercial use. They will only be used for the purposes outlined

in this consent form. They will be securely stored at University of Montreal (laboratory of co-investigator, Dr. Francois Coutlée) for as long as they are needed for the verification of laboratory results, testing with additional methods, and for research audit purposes. Your name will not be linked to any specimen.

Health Canada and the McGill University Faculty of Medicine Institutional Review Board may review the study data and files to ensure sound management of this study. For this reason, the records derived from the trial will be kept for 25 years. These records will be destroyed after 25 years.

YOUR RIGHTS

Your participation in this study is completely voluntary. You are free to withdraw from the study at any time. Your decision to withdraw will have no effect on your current or future health care. As a participant, you will be informed of any new information that may affect your willingness to participate in the trial.

By accepting to participate in this research project, you are not waiving any of your legal rights nor discharging the researchers, the sponsor or the institution, of their civil and professional responsibility.

COST

There are no costs to you, direct or indirect.

COMPENSATION

You will receive between $40 and $50 per completed study visit in compensation for costs (transport, parking, food) and/or loss of income incurred from your participation in this study. The maximum total compensation for this study (7 study visits) is $300.

ADDITIONAL INFORMATION

If at any time during your study participation you have questions about HPV or this study, you may contact one of our research staff Mrs. Natalia Morykon, at 514-398-3710 or Mrs. Karène Proulx-Boucher at 514-934-1934 extension 32146. You may also contact Dr. Mariam El-Zein, Associate Director for Research at the Division of Cancer Epidemiology, at 514-398-1489 or mariam.elzein@mcgill.ca.

If you have questions regarding your rights as a research participant, please contact Ms. Ilde Lepore, Senior Ethics Administrator of the Institutional Review Board, Faculty of Medicine at 514-398-8302 or ilde.lepore@mcgill.ca.

ETHICS APPROVAL

Health Canada has authorized the use of carrageenan for this investigational study. The McGill Institutional Review Board has reviewed this study for ethical acceptability.

Research Project: Lubricant Investigation in Men to Inhibit Transmission of HPV Infection (LIMIT-HPV)

I. Participant's consent

I have read this consent form. I have been informed of the purpose of this study. I am aware of the study procedures and the risks and benefits of my participation. I have been informed that my participation in this study is voluntary, and that I can withdraw from this study at any time without giving a reason. I consent to take part in this study. I do not give up any of my legal rights by signing this consent form.

A dated and signed copy of the present information and consent form was given to me.

_____

Name of participant

_____ _____

Signature of participant Date

II. Signature of the person who obtained consent

I have explained the terms of the present information and consent form to the research participant and I answered all his questions.

_____

Name of person obtaining consent

_____ _____

Signature of person obtaining consent Date

Appendix 4 - Enrolment questionnaire

LIMIT-HPV Study - Enrolment Questionnaire

(Content template for production of computerized instrument)

IMPORTANT INFORMATION

Questions and instructions appear in regular text. Responses appear in italicized text. Notes and skip patterns for programming appear in [square brackets]. Questions that must be answered are marked [*REQUIRED]. All other questions are optional

For multiple choice questions, the number or letter that appears before each response option indicates the coding or numbering for the response, the number/letter is for programming purposes only and is not to appear in the participant questionnaire

Codes: 99 – skipped by the skip pattern or not applicable; 88 – left blank by the participant; 77 – don't remember/don't know

Introduction

Thank you for being part of the study! Your participation helps us answer questions about the potential effectiveness of a Carrageenan-containing gel in reducing Human Papillomavirus (HPV) transmission.

This 30-minute survey will ask questions about you, your health and sexual history, and recent sexual behaviours. We understand that some of these questions may be sensitive and of a personal nature. We assure you that all your answers will be kept completely confidential. There is no right or wrong answer to any question. Some questions ask you to think back over your adult years, or over the past several months, to recall specific information. Please take your time to consider each question carefully. We would greatly appreciate your efforts to answer all questions as best as you can. It is crucial for a research study to have complete and accurate information and we need your help in making this study successful.

Most questions require that you simply click on the response that applies to you. Other questions ask you to enter a specific answer, such as a number, a date, or a short text. Depending on your answer for some questions, you may be skipped past some questions. This is to save you time so that you won't have to answer questions that do not apply to you.

Ready to start? Press continue!

General information
This part of the questionnaire concerns general information about you and where you live.

1. What is your date of birth?

   [Date field: dd/mm/yyyy - *REQUIRED]

2. In what country were you born?

   [Selection field]

[IF 2=Canada] 2.1 In which province were you born?

   [Selection field]

3. The Montreal area is made up of many ethnic groups. We would like to know in which group you would place yourself. Please indicate the most appropriate category.

   1. *French Canadian*
   2. *English Canadian*
   3. *Black Canadian*
   4. *Aboriginal*
   5. *American*
   6. *Latin American*
   7. *Haitian*
   8. *European*
   9. *African*
   10. *South Asian*
   11. *East Asian*
   12. *Middle Eastern*
   13. *Other, please specify:* [text field 3.1]

4. What is the highest degree of education that you have completed?

   1. *Less than elementary*
   2. *Elementary*
   3. *Secondary (High school)*
   4. *College or CEGEP*
   5. *University*

4.1 What is your current work or life situation?

   1: Working full time (30 hours/week or more)

2: Working part time (<30 hours/week)

3: On parental leave

4: On temporary sick leave

5: Looking for work

6: No longer able to work

7: No longer wish to work

8: Other, please specify: [text field 4.1.1]

5. How long have you lived in Montreal?

   [#field] [options field 5.1- choose unit - 1: months; 2: years] *months OR years*

Smoking History

The following questions are about your tobacco smoking habits. Please try to be as specific as possible in your answers.

6. Have you ever smoked cigarettes regularly – that is, one cigarette or more each day for a year or more?

   1: Yes

   0: No

[IF 6=No, SKIP to 10]

7. At what age did you start to smoke regularly?

   Age in years: [# field]

8. Do you still smoke regularly?

   1: Yes

   0: No

[IF 8=No] 8.1 At what age did you last stop smoking regularly?

Age in years: [# field]

9. During your smoking years, how many cigarettes, on average, did you smoke per day?

   Cigarettes per day: [# field]

Alcohol and Drug Consumption

The next few questions are about your alcohol consumption during the **past year**. A drink refers to 1 can/bottle (375 mL) of beer, 1 glass of wine, 1 can or bottle of wine cooler, 1 cocktail, or 1 shot of liquor.

10. During the **past year**, on average, how many days per week or days per month did you have at least one drink of any alcoholic beverage?

[#field] [options field 10.1- choose unit: per week, per month] *per week OR per month*

0: Did not drink

[IF 10=Did not drink, SKIP to 13]

11. On the days when you drank in the **past year**, about how many drinks did you drink on average?

Average number of drinks per drinking day: [# field]

12. Considering all types of alcoholic beverages, how many times in an average month over the **past year** did you have 5 or more drinks on an occasion?

Times per month: [# field]

13. Have you **ever** injected yourself with substances or drugs?

1: Yes

0: No

[If 13 =Yes, answer 13.1 and 13.2]

13.1 When was the **FIRST** time you ever used injection drugs (approximately)?

[date field mm/yyyy]

77: Don't remember

13.2 When was the **LAST** time you used injection drugs (approximately)?

[date field mm/yyyy]

77: Don't remember

Lifetime Sexual History

The next questions are about your sexual history. We realize that this is a personal subject, but it is very important to the study of Human Papillomavirus (HPV). Please take the time to recall this information as accurately as possible. Some questions in this section refer to your sexual experience over your lifetime, whereas others refer only to recent experience. Please remember that all the information you give will be kept entirely confidential.

Throughout this survey, we will refer to various specific sexual acts. These terms are explained below so that everyone attaches the same meanings to them. Note that female genitals were kept in the definitions to account for sexual activities participants may have or have had with women too. Please be sure to read these definitions. If you need any further help or explanation, please ask the Research Nurse.

Oral sex:	A person's mouth on a sex partner's genital area (penis, vulva or

	vagina, but NOT the anus which we will refer to as rimming).
Rimming:	A person's tongue around the anus rim or in the anal canal (for this questionnaire, it includes any type of contact between a tongue and an anus).
Anal sex:	A man's penis in a sex partner's anus or rectum.
Receptive anal sex:	Being penetrated by the penis of your sex partner(s) during anal sex (being bottom).
Mutual masturbation:	Hand stimulation of a person's anal or genital area by his/her partner, NOT involving penetration of the penis in the mouth, vagina or anus.
Fisting:	Penetration of the hand (fist) in a partner's anus or rectum.
Sexual activity:	Mutual masturbation, oral sex, vaginal sex, or anal sex.
Sex partner(s):	People who have engaged in sexual activities together – whether once, or just a few times, or as regular partners, or as married partners.

14. Please think about all the people with whom you have engaged in sexual activity. In total, with how many people have you engaged in any sexual activity in your **lifetime**? [# field 14]
    How many were male (i.e. possessing male genitals)? [# field 14.1]
    How many were female (i.e. possessing female genitals)? [# field 14.2]
15. How old were you when you had your **first** sexual activity with a person of same sex? [Selection field]
16. Since you first started having sexual activities with men, with how many different men per year, on average, would you say you had sexual activities with?
    1. *None*
    2. *One per year*
    3. *2 – 5 per year*
    4. *6 – 9 per year*
    5. *10 – 14 per year*
    6. *15 – 24 per year*
    7. *25 – 49 per year*
    8. *50 – 100 per year*
    9. *More than 100 per year*

17. In the **last year only**, how many different male sex partners have you had?

    1. *None*
    2. *One per year*
    3. *2 – 5 per year*
    4. *6 – 9 per year*
    5. *10 – 14 per year*
    6. *15 – 24 per year*
    7. *25 – 49 per year*
    8. *50 – 100 per year*
    9. *More than 100 per year*

18. In the last **month**, have you had one or more **new** male sex partner(s)?

    1: Yes

    0: *No*

19. Do you currently have a stable male sex partner (i.e. someone with whom you have sexual activities with on a regular basis, but not necessarily an exclusive partner)?

    1: Yes

    0: *No*

[IF 19=No, SKIP to 22]

20. Do you only have anal sex with your stable male sex partner?

    1: Yes

    0: *No*

21. Does your stable male sex partner have sex with other men?

    1: Yes, or I think so

    0: *No, or I don't think so*

    77: Don't know

22. Did you ever receive fisting in your anus (i.e. penetration of your sex partner's fist in your rectum)?

    1: Yes, or I think so

    0: *No, or I don't think so*

    77: Don't know

[IF 22=Yes] 22.1 How many times in your lifetime, did you receive fisting? [# field 14]

For the next questions, we only refer to the times you engaged in receptive anal sex.

23. Have you ever had receptive anal sex, i.e. the penis of your sex partner penetrates your anus?

1: Yes

0: *No*

[IF 23=No, SKIP to 27]

24. In the **last year only**, how many men have you had receptive anal sex with?

1. *None*
2. *One per year*
3. *2 – 5 per year*
4. *6 – 9 per year*
5. *10 – 14 per year*
6. *15 – 24 per year*
7. *25 – 49 per year*
8. *50 – 100 per year*
9. *More than 100 per year*

25. In the **last year only**, how often did your sex partner(s) wear a condom (rubber) when you had receptive anal sex?

0: Never (0%)

1: Rarely (1-24%)

2: Occasionally (25-49%)

3: Often (50-74%)

4: Almost always (75-99%)

5: Always (100%)

26. Have you ever experienced bleeding from your anus following receptive anal sex?

1: Yes

0: *No*

Sexual Activities in the Past Month

The next questions are about sexual activities during the past month, that is, **between dd/mm/yyyy** [CALCULATE TODAY's DATE-30] **and today**.

27. During that period, did you engage in sexual activity with one or more partner(s)?

1: Yes

0: No

[IF 27=No, SKIP to 47]

28. How many sex partners did you have in the **past month**? [# field 28]

    How many were male (i.e. possessing male genitals)? [# field 28.1]

    How many were female (i.e. possessing female genitals)? [# field 28.2]

29. Considering all your sex partners in the **past month**, how many times in total did you engage in sexual activities? By sexual activity, we mean any of mutual masturbation, oral, vaginal, anal sex, rimming or fisting.

    [#field] [options field 29.1- choose unit – 1: per week; 2: in total] *per week OR in total*

30. In the **past month**, how many times in total did you engage in the following specific sexual activities?

    30.1 receiving oral anal (rimming), i.e. any contact between the tongue of your sex partner and your anus?

    [#field] [options field 30.1- choose unit – 1: per week; 2: in total] *per week OR in total*

    30.2 receiving fingers of your sex partner in your anus?

    [#field] [options field 30.2- choose unit – 1: per week; 2: in total] *per week OR in total*

    30.3 receiving an object (dildo/vibrator or other) in your anus or rectum (by your partner or yourself)?

    [#field] [options field 30.3- choose unit – 1: per week; 2: in total] *per week OR in total*

    30.4 receiving fisting (i.e. the fist of your partner in your anus or rectum?

    [#field] [options field 30.4- choose unit – 1: per week; 2: in total] *per week OR in total*

For the next questions, we only refer to the times you engaged in <u>receptive</u> anal sex.

31. With how many male partners did you engage in <u>receptive</u> anal sex in the **past month**?

    [#field]

32. How many times did you have <u>receptive</u> anal sex in the **past month**?

    [#field] [options field 32- choose unit – 1: per week; 2: in total] *per week OR in total*

33. When was the last time you had <u>receptive</u> anal sex?

    [date field dd/mm/yyyy]

    77: Don't remember

34. How often did you use condoms during <u>receptive</u> anal sex in the **past month**?

    0: Never (0%)

1: Rarely (1-24%)

2: Occasionally (25-49%)

3: Often (50-74%)

4: Almost always (75-99%)

5: Always (100%)

[IF 34=Never, SKIP to 38]

When you used condoms for <u>receptive</u> anal sex (i.e. you were bottom) in the **past month**…

35. Did the condom **ever** break or slip off?

1: Yes

0: No

77: Don't remember

36. Did your partner **always** put the condom on before starting to penetrate you?

1: Yes

0: No

77: Don't remember

37. Did your partner **ever** take the condom off then continued to penetrate you without the condom?

1: Yes

0: No

77: Don't remember

Personal lubricants are liquids used during sexual activities to reduce friction between body parts or between body parts and other objects. We do not include saliva as a lubricant here.

38. How often did you use a lubricant when you were receiving <u>receptive anal sex</u> in the **past month**?

0: Never (0%)

1: Rarely (1-24%)

2: Occasionally (25-49%)

3: Often (50-74%)

4: Almost always (75-99%)

5: Always (100%)

39. How often did you use a lubricant during other receptive anal sexual activities in the **past month** (i.e. while you were receiving object or fisting in your anus or rectum)?

0: Never (0%)

1: Rarely (1-24%)

2: Occasionally (25-49%)

3: Often (50-74%)

4: Almost always (75-99%)

5: Always (100%)

[IF 38 and 39=Never/SKIPPED, SKIP to 43]

When you used lubricants in the **past month**…

40. Where did you or your partner apply the lubricant? (Mark all that apply)

1. *Around own anus*
2. *Inside own rectum*
3. *On partner's penis*
4. *Outside of the condom*
5. *Inside of the condom*
6. *Around partner's anus*
7. *Inside partner's rectum*
8. *On a sex toy that was placed on your genitals or inside your anus*
9. *Elsewhere (please specify):* [text fields up to 3: 40.1-40.3]

41. How many teaspoons (approximate average) were used per sexual activity in the **past month**?

1. *Greater than or equal to 1, but less than 2*
2. *Greater than or equal to 2, but less than 3*
3. *Greater than or equal to 3, but less than 4*
4. *Greater than or equal to 4, but less than 5*
5. *Greater than 5*

42. What specific brand(s) of gel lubricant(s) did you use in the **past month**? (Mark all that apply)

a: Astroglide *i: Pink*

b: Bioglide
c: ID
d: JO
e: K-Y
f: Liquid Silk
g: Maximus
h: OMY
j: PJUR
k: Slippery Stuff
l: Sylk
m: Uberlube
n: WET
o: *Other (please specify):* [text fields up to 3: 19.1-19.3]

Sexual Activities in the Past Week

The next questions are about sexual activities during the past 7 days, that is, **between dd/mm/yyyy** [CALCULATE TODAY's DATE-7] **and today**.

43. How many times did you have <u>receptive</u> anal sex with a man in the past 7 days?
[Drop down selection menu: numbers 0-20]

44. How many times did you use condoms during <u>receptive</u> anal sex in the **past 7 days**?
[Drop down selection menu: numbers 0-20]

45. How many times did you use personal lubricants during <u>receptive</u> anal sex in the **past 7 days**?
[Drop down selection menu: numbers 0-20]

46. How many times in total did you engage in the following specific sexual activities in the **past 7 days**?

46.1 receiving oral-anal (rimming), i.e. any contact between the tongue of your sex partner and your anus?
[Drop down selection menu: numbers 0-20]

46.2 receiving fingers of your sex partner in your anus?
[Drop down selection menu: numbers 0-20]

46.3 receiving an object (dildo/vibrator or other) in your anus or rectum (by your partner or yourself)?
[Drop down selection menu: numbers 0-20]

46.4 receiving fisting (i.e. the fist of your partner in your anus or rectum?
[Drop down selection menu: numbers 0-20]

Medical History

The next questions ask about medical conditions or health problems you may have currently or had in the past.

47. Has a doctor **ever** told you that you were HIV-positive?

1: Yes

0: No

48. Has a doctor **ever** diagnosed you with any chronic health conditions (other than HIV)?

1: Yes

0: No

[If 48=Yes] 48.1 What chronic health conditions have you been diagnosed with (excluding HIV)? [textbox 48.1]

49. Do you currently take any medications prescribed by a doctor [this includes medication you may take against HIV if the case]?

1: Yes

0: No

[If 49=Yes] 49.1 Please list all the medications prescribed by a doctor that you currently take [textbox 48.1]

50. Do you have, or have you had, any allergies?

1: Yes

0: No

[If 50=Yes] 50.1 What are/were you allergic to? [textbox 50.1]

51. Have you **ever** had surgery?

1: Yes

0: No

[If 51=Yes] 51.1 Which surgeries did you have? [textbox 51.1]

52. Have you **ever** been hospitalized?

1: Yes

0: No

[If 52=Yes] 52.1 What were the reasons for your hospitalization(s)? [textbox 52.1]

53. Have you **ever** been vaccinated against HPV (i.e. with Gardasil or Cervarix)?

1: Yes

0: No

[IF 53=Yes, answer 53.1 and 53.2]

53.1 Which HPV vaccine did you receive?

1: Gardasil

2: Cervarix

3: Gardasil 9

77: Don't know or don't remember

53.2 How many vaccine doses did you receive?

[Drop down selection menu: numbers 1-3 or simple choice between 1, 2 or 3]

77: Don't know or don't remember

53.3 When was your first HPV shot?

[Date field: dd/mm/yyyy, and an open field]

54. Did a doctor **ever** tell you that you had one of the following conditions or sexually transmitted infections (STIs)?

Condition	*1:**Yes***	If yes, check if it was within the **last 6 months** [only available if yes, *0/1*]	*0: **No***	*77: **Don't know***
a) Venereal warts or condylomas				
b) Chlamydia				
c) Lymphogranuloma Venereum (LGV)				
d) Anal or genital herpes				
e) Syphilis				
f) Gonorrhea				
g) Ulcers or genital sores				
h) Hepatitis B				
i) Hepatitis C				
j) Anal high grade dysplasia OR anal intraepithelial neoplasia grade 2 or 3 (AIN 2 or 3) OR anal precancer				

k) Anal cancer				

55. In the **last five years only**, have you ever experienced pain in the anus caused by hemorrhoids?

    0. Never

    1. Rarely

    2. Sometimes

    3. Frequently

56. In the **last five years only**, have you ever had a discharge, other than blood, from your anus?

    0. Never

    1. Rarely

    2. Sometimes

    3. Frequently

57. Have you ever had sex with a partner whom you know had condyloma or genital warts?

    1: Yes

    0: No

Thank you very much for your participation!

All the information you have provided will be kept strictly confidential.

Appendix 5 - Follow-up questionnaire

LIMIT-HPV Study – Follow-up Questionnaire

(Content template for production of computerized instrument)

IMPORTANT INFORMATION

Questions and instructions appear in regular text. Responses appear in *italicized text*. Notes and skip patterns for programming appear in [square brackets]. Questions that must be answered are marked [*REQUIRED]. All other questions are optional.

For multiple choice questions, the number or letter that appears before each response option indicates the coding or numbering for the response, the number/letter is for programming purposes only and is not to appear in the participant questionnaire

Codes: 99 – skipped by the skip pattern or not applicable; 88 – left blank by the participant; 77 – don't remember/don't know

Introduction

Thank you for returning to complete your follow-up questionnaire! We appreciate your continued participation.

This 20 minute survey will be asking you to update your personal and medical information, as well as your recent sexual behaviour. Please take your time to consider each question carefully. A good guess is always better than no information at all. You can leave blank any questions that you feel uncomfortable answering or do not know the answer to. We would greatly appreciate your efforts to answer all questions as best as you can.

We will also ask you about your experience with the study lubricant. Remember that you are not being evaluated on your use of the study lubricant, so please answer all questions as honestly as possible. The accuracy of this information is valuable to us.

Ready? Press continue to begin!

Sexual Behaviour Update

The next questions are about sexual behaviour you may have engaged in since your last survey on **dd/mm/yyyy** [LAST QUESTIONNAIRE DATE].

We realize this is a personal subject, but it is very important to the study of Human papillomavirus (HPV). Please take the time to recall this information as accurately as possible. Please remember that all the information you give will be kept entirely confidential.

Throughout this survey, we will refer to various specific sexual acts. These terms are explained below so that everyone attaches the same meanings to them. Note that female genitals were kept in the definitions to account for sexual activities participants may have or have had with women too. Please be sure to read these definitions. If you need any further help or explanation, please ask the Research Nurse.

Oral sex:	A person's mouth on a sex partner's anal or genital area (penis, vulva or vagina, but NOT the anus which we will refer to as rimming).
Rimming:	A person's tongue around the anus rim or in the anal canal (for this questionnaire, it includes any type of contact between a tongue and an anus).
Anal sex:	A man's penis in a sex partner's anus or rectum.
Receptive anal sex:	Being penetrated by the penis of your sex partner(s) during anal sex (being bottom).
Mutual masturbation:	Hand stimulation of a person's anal or genital area by his/her partner, NOT involving penetration of the penis in the mouth, vagina or anus.
Fisting	Penetration of the hand (fist) in a partner's anus or rectum.
Sexual activity:	Mutual masturbation, oral sex, vaginal sex, or anal sex.
Sex partner(s):	People who have engaged in sexual activities together – whether once, or just a few times, or as regular partners, or as married partners.

58. Since your **last** survey, did you engage in sexual activity with one or more partner(s)?

1: Yes

0: No

[IF 1=No, SKIP to 24]

59. How many sex partners did you have since your **last** survey? [# field 2]

    How many were male (i.e. possessing male genitals)? [# field 2.1]

    How many were female (i.e. possessing female genitals)? [# field 2.2]

60. Since your **last** visit, how many different male sex partners have you had?

    1. *None*
    2. *One*
    3. *2 – 5*
    4. *6 – 9*
    5. *10 – 14*
    6. *15 – 25*
    7. *More than 25*

61. Since your **last** visit, did you have at least one **new** male sex partner?

    1: Yes

    0: *No*

62. Since your **last** visit, have you had receptive anal sex (i.e. you were bottom)?

    1: Yes

    0: *No*

[IF 5=No, SKIP to 11]

63. Since your last visit, during receptive anal sex did your partner wear a condom (rubber)?

    0: Never (0%)

    1: Rarely (1-24%)

    2: Occasionally (25-49%)

    3: Often (50-74%)

    4: Almost always (75-99%)

    5: Always (100%)

[IF 6=Never, SKIP to 10]

When you used condoms for receptive anal sex **since your last survey** …

64. Did the condom **ever** break or slip off?

    1: Yes

    0: No

    77: Don't remember

65. Did your partner **always** put the condom on before starting to penetrate you?

1: Yes

0: No

77: Don't remember

66. Did your partner **ever** take the condom off then continue to penetrate you without the condom?

1: Yes

0: No

77: Don't remember

67. Since your **last** visit**,** have you ever experienced bleeding from your anus following receptive anal sex?

1: Yes

0: *No*

68. Since your **last** visit, how many times in total did you engage in the following specific sexual activities?

11.1 receiving oral anal (rimming), i.e. any contact between the tongue of your sex partner and your anus? [#field] [options field 11.1- choose unit – 1: per week; 2: in total] *per week* OR *in total*

11.2 receiving fingers of your sex partner in your anus?
[#field] [options field 11.2- choose unit – 1: per week; 2: in total] *per week* OR *in total*

11.3 receiving an object (dildo/vibrator or other) in your anus or rectum (by your partner or yourself)? [#field] [options field 11.3- choose unit – 1: per week; 2: in total] *per week* OR *in total*

11.4 receiving fisting (i.e. the fist of your partner in your anus or rectum?
[#field] [options field 11.4- choose unit – 1: per week; 2: in total] *per week* OR *in total*

The Study Gel

69. Since your **last** visit, have you used the study gel during sexual activities?

1: Yes

2: No

[IF 12=No, SKIP to 19]

70. Since your **last** visit, where did you or your partner apply the study gel during sexual activities OTHER than receptive anal sex? (Mark all that apply)
    1. *Around own anus*
    2. *Inside own rectum*
    3. *On partner's penis*
    4. *Outside of the condom*
    5. *Inside of the condom*
    6. *Around partner's anus*
    7. *Inside partner's rectum*
    8. *On a sex toy that was placed on your genitals or inside your anus*
    9. *Elsewhere (please specify):* [text fields up to 3: 13.1-13.3]
71. Since your **last** visit, have you used the study gel during receptive anal sex?
    1: Yes
    0: No

[IF 14=No, SKIP to 18]

72. When was the last time you used the study gel during receptive anal sex?
    [date field dd/mm/yyyy]
    77: Don't remember
73. During receptive anal sex, how did you or your partner apply the study gel? (Mark all that apply)
    1. *Around own anus*
    2. *Inside own rectum*
    3. *On partner's penis*
    4. *Outside of the condom*
    5. *Inside of the condom*
    6. *Around partner's anus*
    7. *Inside partner's rectum*
    8. *On a sex toy that was placed on your genitals or inside your anus*
    9. *Elsewhere (please specify):* [text fields up to 3: 16.1-16.3]

74. How many teaspoons (approximate average) were used per round of receptive anal sex since your **last** survey?

0: Greater than or equal to 1, but less than 2

1: Greater than or equal to 2, but less than 3

2: Greater than or equal to 3, but less than 4

3: Greater than or equal to 4, but less than 5

4: Greater than 5

75. Was there anything that made it difficult for you to use (or not to use) the study gel during receptive anal sex? (Mark all that apply)

a: Application of the study gel is too difficult

b: The packaging is too inconvenient

c: You did not have the study gel on you at the time of intercourse

d: You forgot to use the study gel

e: You did not want to use lubricants

f: You preferred other brands to the study gel

g: You think that the quality of the study gel is poor (e.g., odour, feel, etc.)

h: Use of the gel caused discomfort/adverse reactions to you (please inform the nurse)

i: Partner(s) does/did not want to use lubricants

j: Partner(s) is/are allergic to ingredients of the study gel

k: Partner(s) preferred other brands to the study gel

l: Partner(s) think(s) that the quality of the study gel is poor (e.g., odour, feel, etc.)

m: Use of the gel caused discomfort/adverse reactions to your partner(s) (please inform the nurse)

n: Other: [text fields, up to 3: 18.1-18.3]

o: Nothing, it was easy to use

76. Since your **last** survey, did you use any lubricants other than the study gel?

1: Yes

0: No

[IF 19=Yes] 19.1 What other brand(s) of gel lubricant(s) did you use **since your last survey**?

a: Astroglide *i: Pink*

b: Bioglide *j: PJUR*

c: ID	*k: Slippery Stuff*
d: JO	*l: Sylk*
e: K-Y	*m: Uberlube*
f: Liquid Silk	*n: WET*
g: Maximus	o: *Other (please specify):* [text fields up to 3: 19.1-19.3]
h: OMY	

Sexual Activities in the Past Week

The next questions are about sexual activities during the past 7 days, that is, **between dd/mm/yyyy** [CALCULATE TODAY's DATE-7] **and today**.

77. How many times did you have receptive anal sex in the past 7 days?
[Drop down selection menu: numbers 0-20]

78. How many times did you use condoms during receptive anal sex in the **past 7 days**?
[Drop down selection menu: numbers 0-20]

79. How many times did you use the study gel during receptive anal sex in the **past 7 days**?
[Drop down selection menu: numbers 0-20]

80. In the **past 7 days**, how many times in total did you engage in the following specific sexual activities?

23.1 receiving oral-anal (rimming), i.e. any contact between the tongue of your sex partner and your anus? [Drop down selection menu: numbers 0-20]

23.2 receiving fingers of your sex partner in your anus? [Drop down selection menu: numbers 0-20]

23.3 receiving an object (dildo/vibrator or other) in your anus or rectum (by your partner or yourself)? [Drop down selection menu: numbers 0-20]

23.4 receiving fisting (i.e. the fist of your partner in your anus or rectum?
[Drop down selection menu: numbers 0-20]

Medical Update

The next questions refer to your medical history **since your last survey on dd/mm/yyyy** [LAST QUESTIONNAIRE DATE].

81. Have you received any vaccine shot against HPV (i.e. with Gardasil or Cervarix)?

1: Yes

0: No

[IF 24=Yes] 24.1 Which HPV vaccine did you receive?

1: *Gardasil*

2: Cervarix

3: Gardasil 9

77: Don't know or don't remember

24.2 How many vaccine doses did you receive?

[Drop down selection menu: numbers 1-3 or simple choice between 1, 2 or 3]

77: Don't know or don't remember

24.3 When was your first HPV shot? [Date field: dd/mm/yyyy, and an open field]

82. Since your **last** survey, did a doctor tell you that you had one of the following conditions/sexually transmitted infections (STIs)?

Condition	*1: **Yes***	*0: **No***	*77: **Don't Know***
a) Venereal warts, condylomas, or papilloma virus infection			
b) Chlamydia			
c) Lymphogranuloma Venereum (LGV)			
d) Genital Herpes			
e) Syphilis			
f) Gonorrhoea			
g) Ulcers of genital sores			
h) Hepatitis B			
i) Hepatitis C			
j) Anal high grade dysplasia OR anal intraepithelial neoplasia grade 2 or 3 (AIN 2 or 3) OR anal precancer			
k) Anal Cancer			

83. Since your **last** visit, have you experienced pain in the anus caused by hemorrhoids?

4. *Never*
5. *Rarely*
6. *Sometimes*
7. *Frequently*

84. Since your **last** visit, have you had a discharge, other than blood, from your anus?

4. *Never*
5. *Rarely*
6. *Sometimes*
7. *Frequently*

85. Since your **last** visit, have you had sex with a partner whom you know had condyloma or genital warts?

1: Yes

0: No

[29 will only be visible if patient answered No for question 46 in the Enrolment Questionnaire]

86. Since your **last** visit, has a doctor told you that you were HIV-positive?

1: Yes

0: No

87. Did you see a doctor for any medical problems since your **last** survey?

1: Yes

0: No

[IF 30=Yes] 30.1 What condition did you see a doctor for?

[textbox 30.1]

88. Were you diagnosed with any medical conditions since your **last** survey**?**

1: Yes

0: No

[IF 31=Yes] 31.1 Which medical conditions were you diagnosed with?

[textbox 31.1]

89. Have you been hospitalized **since your last survey**?

1: Yes

0: No

[IF 32=Yes] 32.1 What were the reasons for your hospitalization?

[textbox 32.1]

90. Since your **last** visit, have you injected yourself with substances or drugs?

1: Yes

0: No

91. Since your **last** visit, have you begun smoking regularly?

1: Yes

0: No

[35 will only be visible if (today's date>enrolment date + 150 AND today's date<enrolment date+210) OR (today's date>enrolment date + 330)]

92. To the best of your knowledge, which study product do you think you've been assigned?

    1. *The gel that contains carrageenan*
    2. *The gel that does not contain carrageenan*

    77. Don't know

93. To the best of your knowledge, do you think that your sex partner(s) was(were) involved in the current study?

    1: Yes

    0: No

Thank you very much for completing your follow-up survey!

All the information you have provided will be kept strictly confidential.

Appendix 6 - Protocol for anal swab collection

Protocol for Anal Swab Collection

At each clinic visit, nurses will collect an anal swab specimen from participants for HPV testing. These will occur at months 0, 1, 2, 3, 6, 9, and 12, resulting in seven specimens in total per male. Specimens will be collected using a Dacron™ swab.

Specimen collection materials

1. One Dacron™ applicator
2. A cone tube to hold the swab during collection
3. A Styrofoam holder to hold the vial upright during collection
4. One vial with PreservCyt
5. Gloves

Provision of instructions to participants

Men will be asked to abstain from receptive anal intercourse and anal gel use a minimum of 48 hours before specimen collection. This will minimize the risk of contamination with residual epithelial cells, urethral secretions, and/or semen.

Written instructions provided to the study nurses

1. Put on gloves.
2. Remove the Dacron™ swab from the wrapping, being very careful not to touch anything with it and place it in saline solution (to soften the cotton).
3. Ask the participant to remove their clothes from the waist down.
4. The individual will be asked to assume a comfortable position on their side (supine position) on the examination table and hold one cheek of their buttocks to the side.
5. Hold the swab three to five cm (about 1.5-2 inches) from the tip and insert it into their anus until the tip of your fingers touches the outside of their anus (at 5 cm you should feel a bit of resistance).
6. If there is too much resistance before the swab is deep enough: take away swab, then pull down skin or lift up skin and change angle of entry. If the swab has become contaminated, get a new swab.
7. Release your hold on the swab and grasp it halfway down the shaft.

8. Rotate the swab in a large circular motion, pressing gently against the sides of the anal canal.
9. Withdraw the swab gently in a twirling motion, being very careful not to touch any surface.
10. Place the swab directly into the Universal Collection Medium (UCM)-containing collection vial. Rub the swab against the inside side of the vial.

Storage and transport

The research nurse will remove the swab from the tube, agitate the swab in the vial with PreservCyt, and then press it against the sides of the vial to express the solution. The swab is then disposed of; it is NOT stored in the vial. The vial is labeled with the participant's identifier and date. All samples will be stored in a refrigerator at 4ºC pending transfer to Dr. Coutlée's laboratory. Samples will be batched and transported to the lab. At the lab, they will be stored at 4ºC until being processed.

Appendix 7 - HIV testing

LIMIT-HPV INSTI HIV-1 Antibody Test Procedure

**To be used by the research nurses for HIV-negative participants.

The nurse will conduct a rapid HIV test at the enrolment/baseline and at the exit visit using the INSTI HIV-1 Antibody Test Kit. This test involves using a lancet to obtain a drop of the participants blood through a finger prick.

This will be used to monitor the patient's HIV infection status throughout the clinical trial.

Be sure to read the INSTI HIV-1 Antibody Test Kit package insert before performing test.

Check test kit expiration date.

Test collection materials:

- Personal Protective Equiptment - Disposable gloves and protective eyewear
- Alcohol swab
- INSTI HIV-1 Antibody Test Kit – includes: membrane unit, sample diluent, colour developer, and clarifying solution
- Single-use Lancet
- Single-use Pipette
- Cotton Guaze

Procedure:

1. Gather materials including: alcohol swab, lancet, pipette, one sealed test pouch containing INSTI membrane unit, and one vial each of the sample diluent, colour developer and clarifying solution.
2. Wash and dry hands.
3. Put on pair of disposable gloves and protective eyewear.
4. Select a finger to perform the test. Avoid using a finger that is calloused or injured in any way. Choose a bare finger since a ring can constrict circulation.
5. Massage the finger to allow the blood to move to the surface (fingertip will become pink). The hand must be positioned at waist level or lower.
6. Clean the test area with an alcohol swab. Allow area to dry thoroughly before perfoming test.
7. As soon as the finger is dry, twist off the green protective cap from the lancet and pull it straight out. (See figure A on package insert)

8. Press the finger firmly at the point just below where the lancet will be applied.
9. Use your other hand to hold the lancet by the body and press the lancet body firmly against the finger to activate the device and to make a small puncture on the side of the test finger. (See figure B on package insert)
10. Discard the lancet in a sharps container.
11. Apply slight pressure to the distal (far end) of the finger to produce a large drop of blood.
12. Hold the pipette horizontally and touch the tip of the pipette to the blood sample. The blood will automatically flow to the fill line and then stop. Never squeeze the tube while filling. (See figure C on package insert)
13. If you do not get enough blood to reach the fill line, gently apply intermittent pressure near the puncture site. If blood amount is inadequate, perform a second puncture using a new lancet.
14. Use guaze to have the participant apply gentle pressure to the puncture site to stop the bleeding.
15. Transfer the blood in the pipette to the Sample Diluent vial by aligning the tip of the pipette with the vial. Squeeze the pipette bulb to dispense the blood. Note: If the blood will not expel, hold the pipette vertically and slide a finger over (without pressing) the vent hole. Then squeeze the bulb. (See figure E on package insert)
16. Recap the Sample Diluent vial and mix the contents with inversion.
17. Dispose of pipette in biohazard container.
18. Tear open the pouch and carefully remove the Membrane Unit without touching the center well. The tab of the Membrane Unit can be labelled with the participants name or study ID number.
19. Place the unit on a level surface.

NOTE: At this point it is important that the following steps be performed immediately and in sequence

20. Remix the Sample Diluent/blood mixture and pour the entire contents in the center of the Membrane Unit well. NOTE: this needs to be done **within 5 minutes** of adding the blood to the Sample Diluent vial contents. The sample should be absorbed through the membrane within 30 seconds (times may vary).
21. Take the Colour Developer and slowly invert to mix the solution thoroughly.

22. Open the Colour Developer and add the entire contents to the center of the Membrane Unit well. This coloured solution should absorb through in about 20 seconds.
23. Open the Clarifying Solution and add entire contents to the center of the Membrane Unit well. This will lighten the background colour and help with reading the results.
24. Immediately read the results while the membrane is still wet. Do not allow more than 5 minutes to pass after adding the Clarifying Solution before reading results.
25. Discard all specimens and materials used for the test in a biohazard waste container.
26. Thoroughly wash hands.

Reading Results:

Please refer to the INSTI HIV-1 Antibody Test Kit package insert for diagrams and how to interpret results.

A BLUE dot in the control spot indicates that the procedure was performed correctly and will appear on all valid tests.

Possible results include:

1. **Non Reactive (Negative)** result: only one blue dot appeas on the memberane at the Control Spot. No dot should be visible in the Test Spot (below the Control Spot).
2. **Reactive (Preliminary Positive)** result: two blue dots appear on the membrane at both the Control and Test spots. This means that the specimen contained HIV-1 antibodies. One dot may be darker than the other.
3. **Invalid Results:** (test performed incorrectly or there is a problem with the sample or device). Invalid test results need to be repeated using all new test collection materials.
    a. No dot appears on the membrane
    b. The test dot appears without the control dot
    c. There is a uniform tint across the membrane
    d. Only blue specks appear on the membrane
4. **Intermediate Results:** a faint background ring appears at the Test Spot along with the blue control dot.

If the INSTI HIV-1 Antibody test result is REACTIVE or INDETERMINATE:

Notify the participant of the test result and explain that this is a preliminary result. Another blood test will be performed and confirmed by a laboratory once he is seen by a physician.

The participant is to be referred **immediately** to Dr de Pokomandy (at MUHC Chronic Viral Illnesse Service) for follow-up.

It is important that we ensure that Dr. de Pokomandy responds and a follow-up appointment is made. (MUHC Chronic Viral Illnesses Service, tel: (514) 934-1934 Ext. 32146 - Karène Proulx-Boucher, research coordinator at the Glen site).

Explain to the participant that it is advisable to abstain from sexual activities or to use protection when engaging in sexual activities until the result can be confirmed.

6.2 Manuscript 3 Appendix

Supplementary Table 1. Baseline characteristics of participants who withdrew versus participants who completed or were on study

	Completed or on study (n=150)	Withdrew (n=105)	p value
Group			
Carrageenan	76 (50.7)	51 (48.6)	0.74
Placebo	74 (49.3)	54 (51.4)	
Age – years			
Mean (SD)	39.5	33.1	
Median	38.5	28.0	0.82
Range	18.2-71.7	18.3-70.3	
Ethnicity - n (%)			0.26
French Canadian	62 (41.3)	36 (34.3)	
English Canadian	21 (14.0)	16 (15.2)	
Black Canadian	4 (2.7)	1 (1.0)	
Aboriginal	1 (0.7)	0 (0.0)	
American	3 (2.0)	3 (2.9)	
Latin American	19 (12.7)	10 (9.5)	
Haitian	2 (1.3)	2 (1.9)	
European	14 (9.3)	18 (17.1)	
African	1 (0.7)	1 (1.0)	
South Asian	3 (2.0)	5 (4.8)	
East Asian	7 (4.7)	6 (5.7)	
Middle Eastern	8 (5.3)	5 (4.8)	
Other	5 (3.3)	2 (1.9)	
Education - n (%)			0.24
Elementary	2 (1.3)	0 (0.0)	
Secondary	24 (16.0)	31 (29.5)	
College	36 (24.0)	30 (28.6)	
University	88 (58.7)	44 (41.9)	
Smoking status - n (%)			0.06
Never	91 (61.3)	68 (64.8)	
Former	35 (23.3)	18 (17.1)	
Current	23 (15.3)	19 (18.1)	
Age at first intercourse			
Mean (SD)	17.7	16.8	0.23
Median	17.0	16.0	
Range	4.0-42.0	5.0-33.0	
Lifetime sex partners – quintiles, n (%)			0.82
1-19	23 (15.3)	25 (23.8)	
20-40	22 (14.7)	32 (30.5)	
45-95	29 (19.3)	16 (15.2)	
100-460	38 (25.3)	16 (15.2)	
> 500	38 (25.3)	16 (15.2)	

New sexual partner in the last month – n (%)			0.13
Yes	51 (34.0)	43 (41.0)	
No	99 (66.0)	62 (59.1)	
HIV status, n (%)			0.81
Positive	52 (34.7)	16 (15.2)	
Negative	98 (65.3)	89 (84.8)	
HPV DNA status, n (%)			
Any HPV	96 (64.0)	53 (50.5)	0.35
Negative	40 (26.7)	40 (38.1)	
Missing PCR results	14 (9.3)	12 (11.4)	
Subgenus 1	50 (33.3)	31 (29.5)	0.47
Subgenus 2	82 (54.7)	41 (39.1)	0.57
Subgenus 3	46 (30.7)	19 (18.1)	0.95
Vaccination status, n (%)			0.91
Yes	38 (25.3)	19 (18.1)	
No	112 (74.7)	86 (81.9)	

Supplementary Table 2. Summary of incident HPV infections under best-worst, worst-best, and duplication of dataset

	Number of infections / number at risk		**Hazard Ratio (95% CI)**
	Carrageenan	**Placebo**	
Best-worst scenario	65/102	71/108	0.96 (0.68-1.34)
Worst-best scenario	74/102	61/108	1.53 (1.09-2.16)
Duplicated dataset	136/196	134/206	1.21 (0.95-1.54)

Supplementary Table 3. Summary of type-specific incident HPV infections by arm, overall

	Carrageenan	**Placebo**	**Effect estimate**	
	Number of incident infections / number at risk (%)	**Number of incident infections / number at risk (%)**	**Hazard Ratio (95% CI)**	**p-value**
Overall	189/3266 (5.8%)	200/3445 (5.8%)	1.02 (0.74-1.40)	0.92
HIV-positive	75/824 (9.1%)	96/994 (9.7%)	0.85 (0.54-1.34)	0.49
HIV-negative	114/2442 (4.7%)	104/2451 (4.2%)	1.18 (0.79-1.78)	0.42

This model takes into account all incident HPV infections acquired over follow-up.

Supplementary Table 4. HPV prevalence [n, (%)] by visit, overall

HPV type/groups	Visit 1 (n=255)	Visit 2 (n=210)	Visit 3 (n=190)	Visit 4 (n=164)	Visit 5 (n=145)	Visit 6 (n=124)	Visit 7 (n=99)
HPV6	30 (11.8)	24 (11.4)	25 (13.2)	19 (11.6)	19 (13.1)	16 (12.9)	13 (13.3)
HPV11	12 (4.7)	10 (4.8)	8 (4.2)	7 (4.3)	9 (6.2)	8 (6.5)	3 (3.0)
HPV16	27 (10.6)	21 (10.0)	23 (12.1	24 (14.6)	16 (11.0)	15 (12.1)	9 (9.1)
HPV18	13 (5.1)	9 (4.3)	9 (4.7)	7 (4.3)	9 (6.2)	11 (8.9)	2 (2.0)
HPV26	5 (2.0)	6 (2.9)	3 (1.6)	4 (2.4)	3 (2.1)	1 (0.8)	0 (0)
HPV31	17 (6.7)	12 (5.7)	14 (7.4)	14 (8.5)	8 (5.5)	10 (8.1)	5 (5.1)
HPV33	9 (3.5)	9 (4.3)	9 (4.7)	11 (6.7)	9 (6.2)	6 (4.8)	3 (3.0)
HPV34	2 (0.8)	2 (1.0)	2 (1.1)	2 (1.2)	0 (0)	1 (0.8)	2 (2.0)
HPV35	15 (5.5)	10 (4.8)	9 (4.7)	9 (5.5)	10 (6.9)	11 (8.9)	5 (5.1)
HPV39	25 (9.8)	22 (10.5)	23 (12.1)	20 (12.2)	15 (10.3)	10 (8.1)	14 (14.1)
HPV40	8 (3.5)	8 (3.8)	6 (3.2)	5 (3.1)	4 (2.8)	3 (2.4)	1 (1.0)
HPV42	26 (10.2)	19 (9.1)	22 (11.6)	20 (12.2)	14 (9.7)	13 (10.5)	7 (7.1)
HPV44	18 (7.1)	17 (8.1)	17 (9.0)	14 (8.5)	12 (8.3)	15 (12.1)	10 (10.1)
HPV45	19 (7.5)	10 (4.8)	14 (7.4)	12 (7.3)	13 (9.0)	11 (8.9)	9 (9.1)
HPV51	24 (9.4)	19 (9.1)	22 (11.6)	20 (12.2)	18 (12.4)	20 (16.1)	11 (11.1)
HPV52	18 (7.1)	16 (7.6)	10 (5.3)	21 (12.8)	16 (11.0)	17 (13.7)	11 (11.1)
HPV53	31 (12.2)	30 (14.3)	24 (12.6)	26 (15.9)	28 (19.3)	20 (16.1)	14 (14.1)
HPV54	18 (7.1)	14 (6.7)	13 (6.8)	11 (6.7)	7 (4.8)	14 (11.3)	5 (5.1)
HPV56	13 (5.1)	5 (2.4)	10 (5.3)	13 (7.9)	9 (6.2)	7 (5.7)	4 (4.0)
HPV58	17 (6.7)	14 (6.7)	17 (9.0)	11 (6.7)	13 (9.0)	12 (9.7)	9 (9.1)
HPV59	17 (6.7)	16 (7.6)	13 (6.8)	16 (9.8)	11 (7.6)	10 (8.1)	5 (5.1)
HPV61	29 (11.4)	20 (9.5)	22 (11.6)	21 (12.8)	20 (13.8)	14 (11.3)	12 (12.1)
HPV62	30 (11.8)	20 (9.5)	25 (13.2)	21 (12.8)	18 (12.4)	20 (16.1)	14 (14.1)
HPV66	20 (7.8)	20 (9.5)	13 (6.8)	20 (12.2)	17 (11.7)	11 (8.9)	6 (6.1)
HPV67	8 (3.1)	3 (1.4)	2 (1.1)	5 (3.1)	1 (0.7)	0 (0)	1 (1.0)
HPV68	12 (4.7)	10 (4.8)	8 (4.2)	12 (7.3)	9 (6.2)	10 (8.1)	6 (6.1)
HPV69	7 (2.8)	2 (1.0)	4 (2.1)	5 (3.1)	4 (2.8)	1 (0.8)	2 (2.0)
HPV70	20 (7.8)	20 (9.5)	18 (9.5)	20 (12.2)	14 (9.7)	11 (8.9)	9 (9.1)
HPV71	3 (1.2)	2 (1.0)	1 (0.5)	0 (0)	2 (1.4)	0 (0)	0 (0)
HPV72	12 (4.7)	13 (6.2)	9 (4.7)	6 (3.7)	7 (4.8)	4 (3.2)	6 (6.1)
HPV73	17 (6.7)	15 (7.1)	13 (6.8)	14 (8.5)	11 (7.6)	8 (6.5)	9 (9.1)
HPV81	15 (5.9)	13 (6.2)	17 (9.0)	13 (7.9)	10 (6.9)	11 (8.9)	7 (7.1)
HPV82	10 (3.9)	10 (4.8)	6 (3.2)	8 (4.9)	9 (6.2)	7 (5.7)	1 (1.0)
HPV83	10 (3.9)	12 (5.7)	13 (6.8)	12 (7.3)	10 (6.9)	8 (6.5)	6 (6.1)
HPV84	25 (9.8)	26 (12.4)	24 (12.6)	21 (12.8)	19 (13.1)	15 (12.1)	15 (15.2)
HPV89	25 (9.8)	22 (10.5)	23 (12.1)	20 (12.2)	15 (10.3)	10 (8.1)	14 (14.1)
Negative	74 (29.0)	54 (25.7)	47 (24.7)	39 (23.8)	33 (22.8)	27 (21.2)	20 (20.2)
Any HPV	**155 (60.8)**	**138 (65.7)**	**124 (65.3)**	**112 (68.3)**	**98 (67.6)**	**85 (68.6)**	**64 (64.7)**
Subgenus 1[a]	81 (31.8)	65 (31.0)	64 (33.7)	54 (32.9)	46 (31.7)	45 (36.3)	28 (28.3)
Subgenus 2[b]	123 (48.2)	102 (48.6)	89 (46.8)	87 (53.1)	74 (51.0)	72 (58.1)	49 (49.5)
Subgenus 3[c]	65 (25.5)	59 (28.1)	56 (29.5)	50 (30.5)	46 (31.7)	40 (32.3)	33 (33.3)
Missing[d]	26 (10.2)	18 (8.6)	19 (10.0)	13 (7.9)	14 (9.7)	12 (9.7)	15 (15.2)

[a] Subgenus 1 group consists of HPVs 6, 11, 40, 42, 44, and 54.
[b] Subgenus 2 group consists of HPVs 16, 18, 26, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, and 82.
[c] Subgenus 3 group consists of HPVs 61, 62, 71, 72, 81, 83, 84, and 89.
[d] Missing results correspond to anal samples not analyzed by laboratory or tested but invalid.

Supplementary Table 5. HPV prevalence [n, (%)] by visit for carrageenan arm

HPV type/groups	Visit 1 (n=127)	Visit 2 (n=102)	Visit 3 (n=93)	Visit 4 (n=82)	Visit 5 (n=71)	Visit 6 (n=60)	Visit 7 (n=48)
HPV6	14 (11.0)	9 (8.8)	11 (11.8)	9 (11.0)	7 (9.9)	8 (13.3)	6 (12.5)
HPV11	5 (3.9)	6 (5.9)	4 (4.3)	3 (3.7)	3 (4.2)	3 (5.0)	2 (4.2)
HPV16	12 (9.5)	10 (9.8)	11 (11.8)	11 (13.4)	8 (11.3)	8 (13.3)	4 (8.3)
HPV18	8 (6.3)	6 (5.9)	6 (6.5)	6 (7.3)	7 (9.9)	5 (8.3)	2 (4.2)
HPV26	2 (1.6)	3 (2.9)	1 (1.08)	1 (1.2)	0 (0.0)	1 (1.7)	0 (0.0)
HPV31	10 (7.9)	8 (7.8)	9 (9.7)	8 (9.8)	5 (7.0)	6 (10.0)	2 (4.2)
HPV33	7 (5.5)	6 (5.9)	6 (6.5)	8 (9.8)	7 (9.9)	4 (6.7)	3 (6.3)
HPV34	2 (1.6)	2 (2.0)	1 (1.1)	1 (1.2)	0 (0.0)	1 (1.7)	2 (4.2)
HPV35	7 (5.5)	6 (5.9)	5 (5.4)	7 (8.5)	4 (5.6)	4 (6.7)	2 (4.2)
HPV39	12 (9.5)	9 (8.8)	8 (8.6)	8 (9.8)	7 (9.9)	8 (13.3)	4 (8.3)
HPV40	4 (3.2)	3 (2.9)	3 (3.2)	3 (3.7)	2 (2.8)	0 (0.0)	0 (0.0)
HPV42	20 (15.8)	14 (13.7)	14 (15.1)	12 (14.6)	12 (16.9)	9 (15.0)	6 (12.5)
HPV44	12 (9.5)	11 (10.8)	10 (10.8)	9 (11.0)	7 (9.9)	9 (15.0)	7 (14.6)
HPV45	10 (7.9)	5 (4.9)	7 (7.5)	4 (4.9)	5 (7.0)	5 (6.7)	4 (8.3)
HPV51	11 (8.7)	10 (9.8)	10 (10.8)	11 (13.4)	10 (14.1)	8 (13.3)	5 (10.4)
HPV52	10 (7.9)	6 (5.9)	4 (4.3)	12 (14.6)	9 (12.7)	10 (16.7)	7 (14.6)
HPV53	17 (13.4)	16 (15.7)	13 (14.0)	16 (19.5)	17 (23.9)	11 (18.3)	10 (20.8)
HPV54	5 (3.9)	5 (4.9)	5 (5.4)	3 (3.7)	2 (2.8)	5 (8.3)	4 (8.3)
HPV56	4 (3.2)	1 (1.0)	2 (2.2)	5 (6.1)	3 (4.2)	4 (6.7)	1 (2.1)
HPV58	11 (8.7)	8 (7.8)	7 (7.5)	5 (6.1)	6 (8.5)	3 (5.0)	4 (8.3)
HPV59	9 (7.1)	9 (8.8)	8 (8.6)	12 (14.6)	8 (11.3)	5 (8.3)	3 (6.3)
HPV61	15 (11.8)	10 (9.8)	10 (10.8)	10 (12.2)	12 (16.9)	8 (13.3)	7 (14.6)
HPV62	17 (13.4)	9 (8.8)	12 (12.9)	9 (11.0)	10 (14.1)	8 (13.3)	8 (16.7)
HPV66	10 (7.9)	9 (8.8)	7 (7.5)	12 (14.6)	12 (16.9)	5 (8.3)	1 (2.1)
HPV67	6 (4.7)	3 (2.9)	2 (2.2)	3 (3.7)	1 (1.4)	0 (0.0)	1 (2.1)
HPV68	5 (3.9)	4 (3.9)	3 (3.2)	4 (4.9)	5 (7.0)	5 (8.3)	3 (6.3)
HPV69	3 (2.4)	0 (0.0)	2 (2.2)	0 (0.0)	2 (2.8)	0 (0.0)	1 (2.1)
HPV70	8 (6.3)	8 (7.8)	6 (6.5)	7 (8.5)	4 (5.6)	4 (6.7)	4 (8.3)
HPV71	2 (1.6)	1 (1.0)	1 (1.1)	0 (0.0)	2 (2.8)	0 (0.0)	0 (0.0)
HPV72	4 (3.2)	5 (4.9)	2 (2.2)	3 (3.7)	3 (4.2	2 (3.3)	4 (8.3)
HPV73	11 (8.7)	9 (8.8)	8 (8.6)	10 (12.2)	9 (12.7)	5 (8.3)	5 (10.4)
HPV81	5 (3.9)	2 (2.0)	6 (6.5)	3 (3.7)	5 (7.0)	6 (10.0)	3 (6.3)
HPV82	4 (3.2)	5 (4.9)	3 (3.2)	5 (6.1)	5 (7.0)	3 (5.0)	1 (2.1)
HPV83	6 (4.7)	9 (8.8)	9 (9.7)	9 (11.0)	5 (7.0)	3 (5.0)	3 (6.3)
HPV84	11 (8.7)	13 (12.8)	10 (10.8)	8 (9.8)	8 (11.3)	7 (11.7)	6 (12.5)
HPV89	17 (13.4)	16 (15.7)	15 (16.1)	14 (17.1)	12 (16.9)	7 (11.7)	9 (18.8)
Negative	33 (26.0)	29 (28.4)	23 (24.7)	17 (20.7)	14 (19.7)	14 (23.3)	10 (20.8)
Any HPV	**84 (66.1)**	**67 (65.7)**	**62 (66.7)**	**60 (73.2)**	**50 (70.4)**	**39 (65.0)**	**34 (70.8)**
Subgenus 1[a]	44 (34.7)	34 (33.3)	31 (33.3)	27 (32.9)	23 (32.4)	22 (33.7)	17 (35.4)
Subgenus 2[b]	65 (51.2)	48 (47.1)	45 (48.4)	47 (57.3)	41 (57.8)	34 (56.7)	27 (56.3)
Subgenus 3[c]	33 (26.0)	29 (28.4)	28 (30.1)	23 (28.1)	23 (32.4)	18 (30.0)	18 (37.5)
Missing[d]	10 (7.9)	6 (5.9)	8 (8.6)	5 (6.1)	7 (9.9)	7 (11.7)	4 (8.3)

[a] Subgenus 1 group consists of HPVs 6, 11, 40, 42, 44, and 54.
[b] Subgenus 2 group consists of HPVs 16, 18, 26, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, and 82.
[c] Subgenus 3 group consists of HPVs 61, 62, 71, 72, 81, 83, 84, and 89.
[d] Missing results correspond to anal samples not analyzed by laboratory or tested but invalid.

Supplementary Table 6. HPV prevalence [n, (%)] by visit for placebo arm

HPV type/groups	Visit 1 (n=128)	Visit 2 (n=108)	Visit 3 (n=97)	Visit 4 (n=82)	Visit 5 (n=74)	Visit 6 (n=64)	Visit 7 (n=51)
HPV6	16 (12.5)	15 (13.9)	14 (14.4)	10 (12.2)	12 (16.2)	8 (12.5)	7 (13.7)
HPV11	7 (5.5)	4 (3.7)	4 (4.1)	4 (4.9)	6 (8.1)	5 (7.8)	1 (2.0)
HPV16	15 (11.7)	11 (10.2)	12 (12.4)	13 (15.9)	8 (10.8)	7 (10.9)	5 (9.8)
HPV18	5 (3.9)	3 (2.8)	3 (3.1)	1 (1.2)	2 (2.7)	6 (9.4)	0 (0.0)
HPV26	3 (2.3)	3 (2.8)	2 (2.1)	3 (3.7)	3 (4.1)	0 (0.0)	0 (0.0)
HPV31	7 (5.5)	4 (3.7)	5 (5.2)	6 (7.3)	3 (4.1)	4 (6.3)	3 95.9)
HPV33	2 (1.6)	3 (2.8)	3 (3.1)	3 (3.7)	2 (2.7)	2 (3.1)	0 (0.0)
HPV34	0 (0.0)	0 (0.0)	1 (1.0)	1 (1.2)	0 (0.0)	0 (0.0)	0 (0.0)
HPV35	7 (5.5)	4 (3.7)	4 (4.1)	2 (2.4)	6 (8.1)	7 (10.9)	3 (5.9)
HPV39	7 (5.5)	10 (9.3)	8 (8.3)	7 (8.5)	10 (13.5)	8 (12.5)	4 (7.8)
HPV40	5 (3.9)	5 (4.6)	3 (3.1)	2 (2.4)	2 (2.7)	3 (4.7)	1 (2.0)
HPV42	6 (4.7)	5 (4.6)	8 (8.3)	8 (9.8)	2 (2.7)	4 (6.3)	1 (2.0)
HPV44	6 (4.7)	6 (5.6)	7 (7.2)	5 (6.1)	5 (6.8)	6 (9.4)	3 (5.9)
HPV45	9 (7.0)	5 (4.6)	7 (7.2)	8 (9.8)	8 (10.8)	7 (10.9)	5 (9.8)
HPV51	13 (10.2)	9 (8.3)	12 (12.4)	9 (11.00)	8 (10.8)	12 (18.8)	6 (11.8)
HPV52	8 (6.3)	10 (9.3)	6 (6.2)	9 (11.0)	7 (9.5)	7 (10.9)	4 (7.8)
HPV53	14 (10.9)	14 (13.0)	11 (11.3)	10 (12.2)	11 (14.9)	9 (14.1)	4 (7.8)
HPV54	13 (10.2)	9 (8.3)	8 (8.3)	8 (9.8)	5 (6.8)	9 (14.1)	1 (2.0)
HPV56	9 (7.0)	4 (3.7)	8 (8.3)	8 (9.8)	6 (8.1)	3 (4.7)	3 (5.9)
HPV58	6 (4.7)	6 (5.6)	10 (10.3)	6 (7.3)	7 (9.5)	9 (14.1)	5 (9.8)
HPV59	8 (6.3)	7 (6.5)	5 (5.2)	4 (4.9)	3 (4.1)	5 (7.8)	2 (3.9)
HPV61	14 (10.9)	10 (9.3)	12 (12.4)	11 (13.4)	8 (10.8)	6 (9.4)	5 (9.8)
HPV62	13 (10.2)	11 (10.2)	13 (13.4)	12 (14.6)	8 (10.8)	12 (18.8)	6 (11.8)
HPV66	10 (7.8)	11 (10.2)	6 (6.2)	8 (9.8)	5 (6.8)	6 (9.4)	5 (9.8)
HPV67	2 (1.6)	0 (0.0)	0 (0.0)	2 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)
HPV68	7 (5.5)	6 (6.6)	5 (5.2)	8 (9.8)	4 (5.4)	5 (7.8)	3 (5.9)
HPV69	4 (3.1)	2 (1.9)	2 (2.1)	5 (6.1)	2 (2.7)	1 (1.6)	1 (2.0)
HPV70	12 (9.4)	12 (11.1)	12 (12.4)	13 (15.9)	10 (13.5)	7 (10.9)	5 (9.8)
HPV71	1 (0.8)	1 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
HPV72	8 (6.3)	8 (7.4)	7 (7.2)	3 (3.7)	4 (5.4)	2 (3.1)	2 (3.9)
HPV73	6 (4.7)	6 (5.6)	5 (5.2)	4 (4.9)	2 (2.7)	3 (4.7)	4 (7.8)
HPV81	10 (7.8)	11 (10.2)	11 (11.3)	10 (12.2)	5 (6.8)	5 (7.8)	4 (7.8)
HPV82	6 (4.7)	5 (4.6)	3 (3.1)	3 (3.7)	4 (5.4)	4 (6.3)	0 (0.0)
HPV83	4 (3.1)	3 (2.8)	4 (4.1)	3 (3.7)	5 (6.8)	5 (7.8)	3 (5.9)
HPV84	14 (10.9)	13 (12.0)	14 (14.4)	13 (15.9)	11 (14.9)	8 (12.5)	9 (17.7)
HPV89	8 (6.3)	6 (6.6)	8 (8.3)	6 (7.3)	3 (4.1)	3 (4.7)	5 (9.8)
Negative	41 (32.0)	25 (23.2)	24 (24.7)	22 (26.8)	19 (25.7)	13 (20.3)	10 (19.6)
Any HPV	**71 (55.5)**	**71 (65.7)**	**62 (63.9)**	**52 (63.4)**	**48 (64.9)**	**46 (71.9)**	**30 (58.8)**
Subgenus 1[a]	37 (28.9)	31 (28.7)	33 (34.0)	27 (32.9)	23 (31.1)	23 (35.9)	11 (21.6)
Subgenus 2[b]	58 (45.3)	54 (50.0)	44 (45.4)	40 (48.8)	33 (44.6)	38 (59.4)	22 (43.1)
Subgenus 3[c]	32 (25.0)	30 (27.8)	28 (28.9)	27 (32.9)	23 (31.1)	22 (34.4)	15 (29.4)
Missing[d]	16 (12.5)	12 (11.1)	11 (11.3)	8 (9.8)	7 (9.5)	5 (7.8)	11 (21.6)

[a] Subgenus 1 group consists of HPVs 6, 11, 40, 42, 44, and 54.
[b] Subgenus 2 group consists of HPVs 16, 18, 26, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, and 82.
[c] Subgenus 3 group consists of HPVs 61, 62, 71, 72, 81, 83, 84, and 89.
[d] Missing results correspond to anal samples not analyzed by laboratory or tested but invalid.

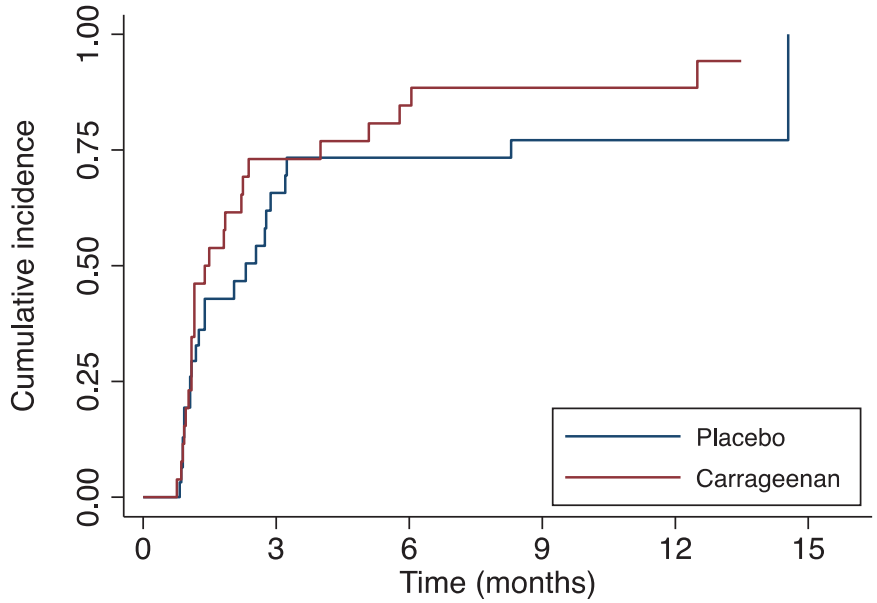
Supplementary Table 7. HPV prevalence [n, (%)] by study arm and HIV status

	HIV-positive			HIV-negative		
HPV type/groups	**Carrageenan (n=33)**	**Placebo (n= 35)**	**Overall (n= 68)**	**Carrageenan (n=94)**	**Placebo (n=93)**	**Overall (n=187)**
HPV6	2 (6.1)	10 (28.6)	12 (17.7)	12 (12.8)	6 (6.5)	18 (9.6)
HPV11	3 (9.1)	5 (14.3)	8 (11.8)	2 (2.1)	2 (2.2)	4 (2.1)
HPV16	4 (12.1)	5 (14.3)	9 (13.2)	8 (8.5)	10 (10.8)	18 (9.6)
HPV18	4 (12.1)	5 (14.3)	9 (13.2)	4 (4.3)	0 (0)	4 (2.1)
HPV26	1 (3.0)	2 (5.7)	3 (4.4)	1 (1.1)	1 (1.1)	2 (1.1)
HPV31	2 (6.1)	4 (11.4)	6 (8.8)	8 (8.5)	3 (3.2)	11 (5.9)
HPV33	3 (9.1)	0 (0.0)	3 (4.4)	4 (4.3)	2 (2.2)	6 (3.2)
HPV34	1 (3.0)	0 (0.0)	1 (1.5)	1 (1.1)	0 (0.0)	1 (0.5)
HPV35	3 (9.1)	3 (8.6)	6 (8.8)	4 (4.3)	4 (4.3)	8 (4.3)
HPV39	6 (18.2)	4 (11.4)	10 (14.7)	6 (6.4)	3 (3.2)	9 (4.8)
HPV40	1 (3.0)	1 (2.9)	2 (2.9)	3 (3.2)	4 (4.3)	7 (3.7)
HPV42	8 (24.2)	1 (2.9)	9 (13.2)	12 (12.8)	5 (5.4)	17 (9.1)
HPV44	7 (21.2)	4 (11.4)	11 (16.2)	5 (5.3)	2 (2.2)	7 (3.7)
HPV45	4 (12.1)	2 (5.7)	6 (8.8)	6 (6.4)	7 (7.5)	13 (7.0)
HPV51	4 (12.1)	6 (17.4)	10 (14.7)	7 (7.5)	7 (7.5)	14 (7.5)
HPV52	5 (15.2)	4 (11.4)	9 (13.2)	5 (5.3)	4 (4.3)	9 (4.8)
HPV53	9 (27.3)	7 (20.0)	16 (23.5)	8 (8.5)	7 (7.5)	15 (8.0)
HPV54	3 (9.1)	6 (17.1)	9 (13.2)	2 (2.1)	7 (7.5)	9 (4.8)
HPV56	1 (3.0)	4 (11.4)	5 (7.4)	3 (3.2)	5 (5.4)	8 (4.3)
HPV58	7 (21.1)	2 (5.7)	9 (13.2)	4 (4.3)	4 (4.3)	8 (4.3)
HPV59	3 (9.1)	1 (2.9)	4 (5.9)	6 (6.4)	7 (7.5)	13 (7.0)
HPV61	6 (18.2)	8 (22.9)	14 (20.6)	9 (9.6)	6 (6.5)	15 (8.0)
HPV62	8 (24.2)	6 (17.1)	14 (20.6)	9 (5.6)	7 (7.5)	16 (8.6)
HPV66	4 (12.1)	5 (14.3)	9 (13.2)	6 (6.4)	5 (5.4)	11 (5.9)
HPV67	1 (3.0)	0 (0.0)	1 (1.5)	5 (5.3)	2 (2.2)	7 (3.7)
HPV68	2 (6.1)	3 (8.6)	5 (7.4)	3 (3.2)	4 (4.3)	7 (3.7)
HPV69	0 (0.0)	2 (5.7)	2 (2.9)	3 (3.2)	2 (2.2)	5 (2.7)
HPV70	3 (9.1)	5 (14.3)	8 (11.8)	5 (5.3)	7 (7.5)	12 (6.4)
HPV71	1 (3.0)	1 (2.9)	2 (2.9)	1 (1.1)	0 (0.0)	1 (0.5)
HPV72	3 (9.1)	5 (14.3)	8 (11.8)	1 (1.1)	3 (3.2)	4 (2.1)
HPV73	3 (9.1)	1 (2.9)	4 (5.9)	8 (8.5)	5 (5.4)	13 (7.0)
HPV81	4 (12.1)	4 (11.40	8 (11.8)	1 (1.1)	6 (6.5)	7 (3.7)
HPV82	2 (6.1)	2 (5.7)	4 (5.9)	2 (2.1)	4 (4.3)	6 (3.2)
HPV83	3 (9.1)	2 (5.7)	5 (7.4)	3 (3.2)	2 (2.2)	5 (2.7)
HPV84	6 (18.2)	4 (11.4)	10 (14.7)	5 (5.3)	10 (10.8)	15 (8.0)
HPV89	8 (24.2)	2 (5.7)	10 (14.7)	9 (9.6)	6 (6.5)	15 (8.0)
HPV negative	4 (12.1)	5 (14.3)	9 (13.2)	29 (30.9)	36 (38.7)	65 (34.8)
Any HPV	**26 (78.8)**	**27 (77.1)**	**53 (77.9)**	**58 (61.7)**	**44 (47.3)**	**102 (54.6)**
Subgenus 1[a]	14 (42.4)	15 (42.9)	29 (42.7)	30 (31.9)	22 (23.7)	52 (27.8)
Subgenus 2[b]	24 (72.7)	23 (65.7)	47 (69.1)	41 (43.6)	35 (37.6)	76 (40.6)
Subgenus 3[c]	16 (48.5)	12 (34.3)	28 (41.2)	17 (18.1)	20 (21.5)	37 (19.8)
Missing[d]	3 (9.1)	3 (8.6)	6 (8.8)	7 (7.5)	13 (14.0)	20 (10.7)

[a] Subgenus 1 group consists of HPVs 6, 11, 40, 42, 44, and 54.
[b] Subgenus 2 group consists of HPVs 16, 18, 26, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, and 82.
[c] Subgenus 3 group consists of HPVs 61, 62, 71, 72, 81, 83, 84, and 89.
[d] Missing results correspond to anal samples not analyzed by laboratory or tested but invalid.

Supplementary Figure 1. Cumulative incidence of HPV infection according to treatment group and HIV status

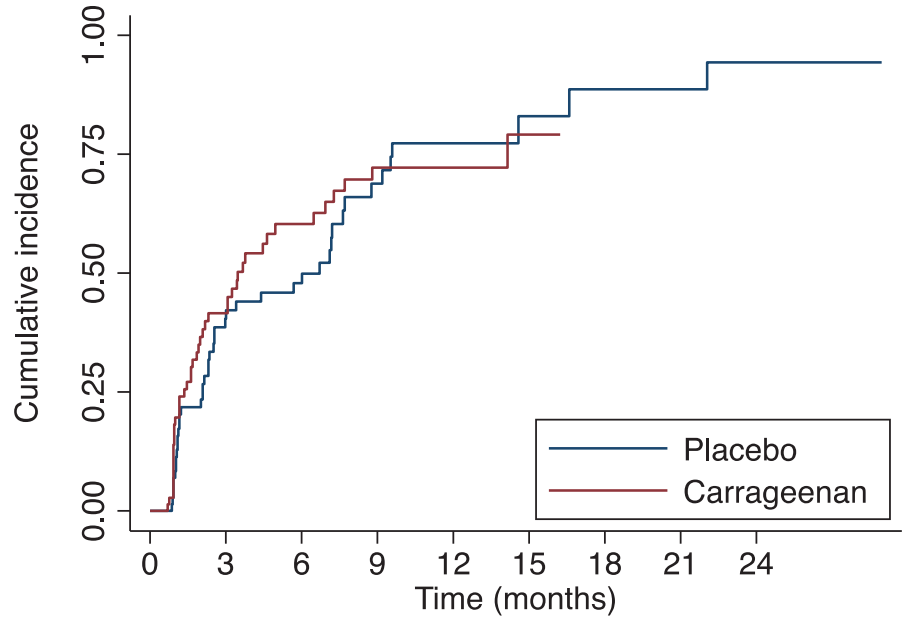
a) participants living with HIV



Number at risk

Placebo	31	9	7	6	3	0
Carrageenan	26	7	4	2	2	0

b) participants not living with HIV



Number at risk

Placebo	72	33	26	11	6	3	2	2
Carrageenan	72	35	18	11	9	2	0	0