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

Version number 1: Submission of protocol: CIHR (February 10, 2014); CCSRI (February 11, 2014) McGill IRB: Version number 1: Dec 16, 2014; Version number 2: June 22, 2015; Version number 3: November 25, 2016; Version number 4: June 28, 2017; Version number 5: January 9, 2016; Version number 6: November 13, 2017; Version number 7: February 11, 2019; Version number 8: November 7, 2018; Version number 9: June 10, 2019; Version number 10: September 5, 2019; Version number 11: December 16, 2019; Version number 12: May 25, 2020

Clinicaltrials.gov identifier: NCT02354144

Amendment tracking and justifications:

Approved June 22, 2015:
- *Questionnaire, recruitment materials, and consent forms amendments*

Approved November 25, 2016:
- *Inclusion criteria added to avoid participants with insufficient or too important exposure to HPV, in order to be able to identify a difference from gel use if there is one: criteria is "expect to have between 2 and 50 different partners this year".*
- *HIV-negative participants will have a rapid HIV test at baseline and 12 months (at entry and at exit) – protocol modified to remove the 6-months testing. Already correct in consent forms dated October 2015.*
- *A brief chart review will be completed at 0, 6 and 12 months for HIV-positive participants only – added in protocol (was not appearing before). Already correct in consent forms of Oct 2015.*
- *Study sites clarification; recruiting clinics can become study sites to facilitate recruitment.*

Approved June 28, 2017: Warning notice to study participants (avoid using condoms made from polyurethane) and handing out (for free) polyisoprene condoms for people with latex allergy (in-kind support from CarraShield Labs Inc.)

Approved January 9, 2016:
- *Study Modification: change to recruitment strategy*

Approved November 13, 2017 by McGill IRB, and October 30, 2017 by MUHC :
- modifying 'question 53.1' in the enrollment questionnaire

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
77: Don't remember

In light of the recent (September 2016) introduction of the Gardasil 9 vaccine (offers protection against 9 HPV types) and replacement of Gardasil (offers protection against 4 HPV types) with Gardasil 9, we added a third option (*3: Gardasil 9*) to 'question 53.1'.

- adding the following sub-question:
  53.3 When was your first HPV shot?
  [Date field: dd/mm/yyyy, and an open field]

We also made similar changes to an equivalent question in the follow-up questionnaire (question 24.1).

Approved February 11, 2019: increase compensation for participants from 175$ to 300$, if all seven study visits were to be completed, 30 January, 2019.

	Enrollment	**Follow-up**						**Total**
Compensation (CDN$)	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	All visits
Current	25	25	25	25	25	25	25	175
Amended	**50**	**40**	**40**	**40**	**40**	**40**	**50**	**300**

November 7, 2018: informed McGill IRB of relocation of the Division of Cancer Epidemiology Research Nurses' Office to the current headquarters of the Gerald Bronfman Department of Oncology, Division of Cancer Epidemiology as of September 17, 2018.

Approved June 10, 2019: collect 20 additional nurse-collected anal samples at the enrollment visit, 10 from men randomized to the treatment group and 10 from men randomized to the placebo group to assess the potential interference of HPV detection by the presence of carrageenan.

September 5, 2019: informed McGill IRB of amendment to obtain electronic consent (e-consent) from participants as we transition to Research Electronic Data Capture (REDCap) for data collection.

December 16, 2019: data are collected and managed with REDCap tools hosted as the McGill University Health Centre.

Termination May 25, 2020: trial termination based on lack of evidence of efficacy and safety concerns.

Team members who left: Dr. Agnihotram Ramanakumar (data management and randomization) and Joseph Tota (study coordinator) (2014); Samantha Shapiro: research assistant (June 2017 to August 2018); Olga Tsyruk: research assistant (August 2019-August 2020); Raphaela Rodrigues: study nurse (February 2016 to May 2020)

Current team members:

Dr. Mariam El-Zein (as of 2014): study director and data management; Allita Rodrigues: study coordinator; Cassandra Laurie: Master's student (September 2017 to August 2020) and research assistant (August 2020 to May 2021)

REVIEW OF LITERATURE SUPPORTING THE NEED FOR A TRIAL

The 2008 Medicine Nobel Prize was awarded to three individuals: Harald zur Hausen for establishing the causal link between human papillomavirus (HPV) infection and cervical carcinoma, and Luc Montagnier and Françoise Barré-Sinoussi for their discovery of human immunodeficiency virus (HIV) as the root of acquired immunodeficiency syndrome (AIDS).[1] The concurrent selection of the two discoveries reflects the grave consequences of these sexually transmitted infections (STI) and key opportunities for prevention. Recognition of HPV infection as a necessary cause of cervical cancer prompted further investigations that linked HPV to other types of cancers, like anal cancer.[2-6]

Epidemiology of anal cancer: In Canada, the anal cancer incidence rate is only 1 per 100,000 person-years.[7] But due to the higher burden among men who have sex with men (MSM), anal carcinoma has emerged as a major public health issue in this group.[8-10] Chief risk factors that place MSM at high risk include: a history of receptive anal intercourse (RAI), frequent RAI, and a high number of male sexual partners.[3,11,12] According to a recent meta-analysis based on multinational data, the annual anal cancer incidence rate among HIV-negative MSMs is 5.1 per 100,000, whereas among HIV-positive MSMs it is 45.9 per 100,000,[11] which is similar to that of cervical cancer prior to routine cervical pap screening.[12]

The link between HPV and anal carcinoma: Of the over 40 different mucosotropic HPV genotypes (types for short) known to infect the anogenital tract, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66 are classified as high oncogenic risk HPV (HR-HPV) types. Much evidence supports that persistent infection by these types is the primary risk factor in the development of pre-cancerous anal lesions.[13-23] Types 16 and 18 have been documented in 72% of invasive anal cancers, and 69% of high-grade anal squamous intraepithelial lesions (HSIL).[24] Also, investigations in MSMs have found HPV infection to be present among 98% of high-grade anal intraepithelial neoplasia (AIN) lesions.[25] Co-infection by different HPV types was identified as a risk factor in the progression to HSIL.[26-30] Other genital types (i.e. 6, 11, 42-44) are of no oncogenic risk,[31-34] although they may cause subclinical and clinically visible benign lesions known as condylomata that are a major cosmetic concern among MSMs, causing physical discomfort and interference with sexual activity. Despite the causal link between HR-HPV infection and AIN, anal screening is not currently recommended.[35]

HIV and HPV infections: The introduction of HAART therapy in 1996 has had a paradoxical effect on the incidence of anal cancer in the MSM community.[32,33,36,37] Whereas patients would formerly die of AIDS-related ailments, men undergoing HAART therapy now live longer; thus, diseases with long evolution (e.g., anal cancer) will progress. There has been more than a 3-fold increase in the annual incidence of anal cancer among HIV+ MSMs in regions where HAART medications are now easily accessible (78 vs. 22 per 100,000).[11,38-41] A cohort study found that after 4 years 38% of MSMs living with HIV developed high-grade AIN, compared with 14% of MSMs without HIV.[37] The partial restoration of CD4 cell counts during HAART therapy has not led to a reduction in the prevalence of HPV-induced ASIL.[41,42] Our group's cohort study of MSMs living with HIV found that at baseline, 98% were infected with HPV (any type) in their anal canal.[39] These results are in line with other studies conducted in geographically unrelated populations, indicating that HPV infections are ubiquitous in MSMs with HIV.[26,43] Low CD4 counts and increased persistence of HPV infections have elevated the risk of ASIL in the HIV+ population to 46-84 times that of the general male population.[11,23,26,27,33,43-45] Prevalence of anal HR-HPV infections is also high among MSMs without HIV (range: 61-81%).[22,46]

Male condom: Condoms act as a protective barrier between the insertive and receptive male during sex and are impermeable to known sexually transmitted pathogens.[46] In practice, however, condoms have not been effective in greatly reducing the risk of HPV transmission in MSMs or among females.[47-49] Thus, a complementary barrier, such as a microbicide, could prove beneficial in reducing HPV transmission.[14]

Prophylactic HPV vaccine: Gardasil® (Merck) and Cervarix® (GlaxoSmithKline) target HPV types 6/11/16/18 and 16/18, respectively, and are nearly 100% effective in preventing cervical HPV infections and cervical lesions (attributable to these target types) in previously uninfected females.[50-55] Although Gardasil has now been approved for use among males aged 9-26 years,[35] this vaccine is exclusively prophylactic (i.e., it is ineffective against established HPV infections),[56] and it is only freely available to school-aged girls via provincial vaccination. Therefore, most young MSMs remain at risk for infection by these vaccine-targeted types. Encouraging results have been reported from a randomized controlled trial (RCT) of Gardasil in young men: the vaccine was 100% effective in protecting against new anal HPV 16 and 18 infections and had an overall efficacy of 90% against all 4 vaccine types.[57] It was also 90% effective in preventing condylomas.[57] Importantly, efficacy was lower in MSMs than among heterosexual males, emphasizing the need for additional HPV prevention approaches for this high-risk population. Of note, 1) vaccine-induced immunity may wane beyond 10 years, 2) only offers protection against few HR-HPV types, and 3) there is the potential for type replacement, i.e., a gradual change in HPV type distribution within vaccinated populations due to vacated ecologic niches occupied by HPV 16/18.[58]

Treating HPV infections: Although consequent lesions can be treated, it is not currently possible to treat established HPV infections, regardless of infection site or HIV status. HPV infection persistence (despite treatment of lesions) explains why these lesions recur in people with HIV. As a result, MSMs with HIV that are diagnosed with AIN 2/3 require lifelong follow-up and monitoring. Treatments for condylomas and AIN are costly and need to be repeated.[28]

Evidence for the Efficacy, Safety, and Acceptability of Carrageenan against HPV Infection

HPV inhibitory compounds (deliverable as topical microbicides) may block the spread of HPV.[59-62] Buck et al. identified *carrageenan* (a non-toxic gelling agent safe in animals and humans) as a potent HPV inhibitor.[59] It blocks genital transmission of HPV in mice and in monkeys and evaluation of its efficacy in humans is now warranted.[61,62] HPV vaccination only protects against infection by select HPV types; however, we propose to clinically investigate a compound with proven inhibitory properties both in vitro and in vivo against *all* HPV types. The MSM population is ideal to evaluate the efficacy of a carrageenan-based lubricant against HPV. MSMs generally are found to have a high HPV transmission rate compared to heterosexual female or male populations, which favours study power.[39] A recent meta-analysis estimated prevalence of HPV infection in MSMs with and without HIV to be 93% and 65%.[11] Our Montreal HIV+ MSM study found the incidence of HPV (any type) to approach 90% in one year, with 66% of the population reporting more than 10 sexual partners over the same period.[39] Furthermore, as anal intercourse frequently requires lubrication, the MSM cohort is expected to have higher compliance with lubricant use by virtue of their sexual behaviour. In fact, all sexually active males interviewed as part of our feasibility study reported using sexual lubricants (see appendix).

This study is a phase IIb RCT. Jointly with our CIHR-funded CATCH (Carrageenan-gel Against Transmission of Cervical HPV) study, this is the first exploration of the efficacy of carrageenan as a topical microbicide for preventing HPV acquisition. We launched the CATCH study in January 2013, focusing only on cervical HPV infection in 18-29 year-old women. Though results are not yet available, the intervention has been positively received with no serious adverse events (AE) (i.e., out of 110 participants, only three have reported mild or moderate AE). Our study physician evaluated these participants and the intervention was not regarded as the cause of these AEs. The proposed study would be the first to test carrageenan against anal HPV infections. If efficacy is demonstrated, we intend to launch a larger phase III trial using our extensive HPV and HIV research network. Adequate proof of concept will help accurately predict future effect size and protocol adherence, and devise more accessible means of delivering the gel, e.g., condoms pre-lubricated with carrageenan microbicide.

Scientific basis for carrageenan: An anionic polymer derived from red algae**,** carrageenan has a long history of human use as a stabilizer and emulsifier in many industries. All three major classes of carrageenan act as extremely potent HPV inhibitors and block HPV infection by binding to the viral capsid, thus preventing attachment to the appropriate cell-surface heparan sulfate proteoglycans (HSPG) receptors.[59,63] The length of interaction is sufficient to allow natural inactivation of the pathogen by the synergistic action of the innate and adaptive immune systems within the genital tract. Carrageenan also exerts a secondary, HSPG-independent inhibitory effect by interfering with virion surface proteins necessary for infection of cells.[59] Lastly, in vitro studies have elucidated that HPV capsids bind sperm cells at two distinct sites along their heads, promoting dispersal and mucosal penetration of HPV.[64-69] Carrageenan has been demonstrated in vitro to bind to sperm cells and prevent this means of HPV infection.[69] The existence of multiple inhibition mechanisms increases the chances that carrageenan may be effective as a topical microbicide against all mucosotropic HPV types.

Safety and acceptability of carrageenan: Carraguard® is the λ-carrageenan derived microbicide gel employed in a large $40 million HIV prevention trial funded by the Bill and Melinda Gates Foundation.[59,70,71] Safety and acceptability trials of Carraguard included participants from the U.S., Thailand, Chile, Australia, Dominican Republic, and South Africa. The gel was found to be pleasant or neutral in feel.[72,73] Since the carrageenan-based gel proposed for our trial is almost identical to Carraguard, we expect that it will also be well received by study participants. Unsuccessful against HIV transmission in heterosexual couples, the inhibitory effect of carrageenan is 1000 times stronger for HPV than for HIV, reinforcing our expectation that a carrageenan-based intervention is likely to prevent HPV infections in MSM.[59,71] Safety and acceptability trials also report no increased risk in other (non-HPV) reproductive tract infections,[71] and direct application to the penis was not associated with any irritation.[74] Finally, Carraguard was not associated with other anogenital complications.[74]

It is important to inform unvaccinated individuals that Gardasil has now been approved for men between 9 and 26 years of age; however, we should also remind them that protection is exclusively prophylactic and restricted to the 4 vaccine-target types. Participating MSMs will likely be highly sexually active and already exposed to the vaccine target types. The potential benefits of vaccination as such are limited, especially knowing that vaccination of women has little impact on the establishment of herd immunity in the MSM community. Not benefitting from the full impact of HPV vaccination, supplementary means of protection are greatly needed for MSMs.

In addition to the required intervention gel, we will recommend condom use to all participants for the prevention of HIV and other STIs. Condoms will be easily accessible: our participating clinics and many community organizations in Montreal such as REZO already provide condoms free of charge as a public health intervention. Although we cannot enforce condom use, our nurses will counsel participants on their importance during all acts of intercourse. Unfortunately, current evidence does not suggest high adherence to this method of protection. A New York City study in a mixed group of MSMs found that 43% of them either used condoms inconsistently or not at all.[75] This reinforces the need for a practical alternative, such as a personal lubricant that protects against all HPV types.

It is particularly important that individuals at high risk for infection (MSMs, and especially those with HIV) be included in the trial so that carrageenan's efficacy may be evaluated in this group. Considering that carrageenan's primary mechanism of action against HPV relies on innate and adaptive immune responses, it is relevant to understand if its efficacy is similar in men with and without HIV. Other immune independent mechanisms of action do occur, and as carrageenan is extremely potent against HPV, there may actually be no difference in efficacy between the two groups. Since no other known microbicide exists to prevent or treat HPV infection, and there exists clinical equipoise, i.e., genuine uncertainty among the medical community, on whether or not carrageenan can prevent HPV, it is not

only ethical, but necessary to randomize subjects, including those with HIV, to receive carrageenan or placebo gel (as done in the CATCH trial), to obtain strong evidence to inform public health practice.

STUDY DESIGN AND METHODS

Primary aim: To evaluate the efficacy of carrageenan in reducing type-specific anal HPV incidence, i.e., in preventing infections by new HPV types in sexually active MSM.

Secondary aims: 1) to evaluate the efficacy of carrageenan in reducing type-specific anal HPV prevalence, i.e., in accelerating clearance of existing infections in sexually active MSM; 2) to compare the efficacy of carrageenan for type-specific prevention and clearance of anal HPV infections among MSM with and without HIV, i.e., to evaluate whether carrageenan is equally effective among these subgroups; and 3) to assess the safety and tolerability of the proposed gel and patient adherence to the intervention, i.e., the parameters important for future clinical use.

Trial Intervention

We propose to conduct a placebo-controlled, double-blinded RCT to evaluate the effect of a carrageenan-based lubricant on anal HPV infections in MSM. [redacted] The differentiating feature is that one gel contains carrageenan (intervention) and the other does not (control). 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 anal intercourse during the entire study period. A study nurse will provide instructions on how to use the gels. Around 15 ml of the personal lubricant will be dispensed into the hand and applied directly to the genital, anal, and condom surfaces prior to and as needed during anal sex. When sexual activity ceases, the water-based formulation of the gel allows it to be easily removed with lukewarm water. To ensure blinding, the two gels and their containers will look and feel almost identical. The success of blinding will be 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. In our feasibility study, most participants (18/20) were unable to determine the identity of the provided gel sample (appendix).

Enrolment Strategy

In a similar fashion as for the HIPVIRG study,[39] we will be recruiting subjects living with HIV through 5 HIV/AIDS outpatient clinics in Montreal: Clinique Médicale du Quartier-Latin, Clinique L'Actuel, Clinique OPUS, Unité d'Hospitalisation de Recherche et d'Enseignement sur les Soins du SIDA (UHRESS) of the Centre Hospitalier de L'Université de Montréal (CHUM) and Chronic Viral Illnesses Service of McGill University Health Centre (MUHC). For the HIV negative group, our recruitment strategy will emulate that of the EXPLORE trial.[14] We will advertise at bars, sex and health clubs, in various media, and the abovementioned clinics—with the addition of the McGill University Student Health Services. Subject recruitment resources are already in place from prior studies (e.g., CATCH and HIPVIRG) and have dedicated collaborators at each site (collaborator letters in appendix). Therefore, we do not anticipate any issues and expect to recruit all 380 participants within 3 years. In the HIPVIRG study, which required longer visits with invasive procedures (high resolution anoscopies with biopsies), an average of 84 subjects (all MSMs with HIV) were recruited per year.[39] We plan to achieve greater accrual rate on the basis of our noninvasive intervention and the size of the available subject pool—MSM without HIV outnumber those with HIV—and over 70% of MSMs recruited to

participate in the current trial will be HIV negative. In the recent feasibility study conducted to assist with the planning of the current trial, we recruited all 20 subjects, both HIV positive and negative in less than four weeks.

Inclusion/Exclusion Criteria

We plan to recruit males who are (i) aged 18 or older; (ii) live in Montreal and plan to remain in the city for the next 12 months; (iii) have 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; (iv) understand French or English; (v) expect to have more than 2 but less than 50 DIFFERENT partners this year, based on their experience during the previous years; and (vi) are willing to follow study instructions and comply with follow-ups for 12 months. Eligible men must not be receiving treatment for anal or perianal condylomas or AIN during the trial, or have a known allergy or hypersensitivity to any of the ingredients in either gels. Participants will not be excluded if they are participating in other concurrent trials, unless these other trials also focus on prevention or treatment of HPV or HPV-related diseases. We will not exclude men who have detectable HPV infections upon enrolment or who have been vaccinated with Gardasil, as baseline prevalence for any HPV type is high in this population[11] and our assay will allow the detection of 37 different HPV types. We will also not attempt to recruit MSMs who are HPV negative at baseline. Adopting a 'must be HPV-negative' criterion would restrict the study to MSMs with minimal HPV exposure, which would impair the generalizability of the findings and compromise our ability to investigate the prevention effect during the follow-up period. MSMs with and without HIV will be recruited. For those with HIV, a chart review will be performed at enrolment to collect information on CD4+ count, viral load, HAART status, year of HIV diagnosis, and nadir CD4+ count. HIV testing will also be performed on MSMs without HIV to verify their status. A volunteer sample also maximizes internal validity due to improved protocol adherence and provision of more accurate information on risk factors and sexual histories.

Outcome Measures and Measurement Methods

The primary outcome is the presence of a newly detected anal infection of a specific HPV type in someone who was negative for that HPV type at enrolment. Detection of 37 different HPV types will allow for the assessment of new HPV types even among those already infected. Since most men may be infected with some HPV types at baseline, this point is critical to understanding why we expect to have sufficient statistical power to evaluate our primary objective. The secondary outcome is clearance of anal type-specific HPV infections found at baseline. HPV DNA genotyping of anal samples will be done by a validated assay. Other secondary outcomes will be measured via questionnaires (e.g., patient adherence), review of patient adverse event reports, and involve separate analyses to compare efficacy according to HIV status. Study participants will be asked to continue using the assigned gel for the entire 12 months of follow-up, independent of other methods of protection against STIs (e.g., condoms). With the high frequency of new sex partners among MSMs in a similar study by our group,[39] 1-year follow-up should be sufficient to allow HPV exposure opportunity and to evaluate compliance.

HPV infection status will be measured using anal specimens at baseline (enrolment/time 0), and at all follow-up clinic visits (1, 2, 3, 6, 9 and 12 months). *The initial visit with the nurse will take approximately 30 minutes, while all subsequent follow-up visits will require only about 20 minutes.* Men will be asked to abstain from receptive anal sex and gel use 48 hours before specimen collection in order to minimize the risk of contamination.[76] Daily measurement of HPV status would produce the most reliable estimation of time of infection and detect transient infections. However, the need for precise measurement must be offset by the need to minimize participant burden and financial costs.

HPV incidence will consequently be interval-censored (i.e., infection date will be placed sometime between the last negative and the first positive test).

Our trained study nurses will collect specimens according to the Protocol for Anal Swab Collection (appendix).[39] *A Dacron™ swab will be inserted 3cm into the anal canal of the participant, and removed in a twirling motion while applying gentle pressure on the walls of the canal to ensure exfoliation of anal epithelial cells.[40] The swab will then be agitated in a plastic vial containing liquid preservative (Preservcyt™, Hologic Co, MA), pressed against the side of the vial to express any remaining fluid, and then discarded.* PreservCyt adequately preserves cell specimens for DNA, RNA, and protein analyses. The swab sample in PreservCyt will be kept at 4°C pending transfer to the laboratory. Samples will be batched and transported every 45 days or sooner if there are a large number of sample vials. After centrifugation at 13,000*g* for 15 min at 22°C, the supernatant will be discarded, the pellet resuspended in 300µL of 20mmol/L Tris buffer (pH 8.3). Finally, DNA will be purified using a Master-Pure Kit (Epicentre) and tested in each PCR assay.[77]

HPV DNA detection and typing: HPV detection and typing will be done via the well-established PGMY PCR protocol coupled with the linear array method, commercially available from Roche. This test permits testing and typing for 37 different genital types of HPV (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, 89).[78] HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 will be considered of high oncogenic risk, whereas the remaining types will be considered low-risk, i.e., non-oncogenic.[79] All assays will be done in Dr. Coutlée's laboratory, a WHO-accredited HPV diagnostics centre. He was one of the lead investigators that validated Roche's PGMY linear array technique for clinical use.

Computerized questionnaire: Participants will complete a self-administered baseline questionnaire during the enrolment visit, and follow-up questionnaires during all other visits. The shorter follow-up questionnaires are intended to evaluate recent sexual behaviours and to corroborate the responses given during the baseline visit. These questionnaires will measure HPV risk factors, compliance, and monitor safety and tolerability of the gels. Between follow-up visits, participants will be 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 AEs. To minimize recall bias, information can only be updated for the past 7 days (incomplete surveys will 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.[80] This ensures high compliance and improves the quality of data. Responses will be employed to evaluate adherence and assist us in developing future studies. A similar web-based diary is used in our CATCH trial with high compliance and no known issues.

HIV testing: Our trained research nurse will test all HIV negative participants with a rapid HIV test at baseline, and 12 months (as is standard of care in high risk populations) (appendix). If the test is positive, a blood test will be taken for standard HIV serology, the site investigator will perform counselling, and a follow-up appointment will be scheduled for 2-4 weeks later. For HIV-positive participants, a brief chart review will be done at 0, 6 & 12 mths to collect information on their CD4 count, HIV viral load, and current ART regimen.

Adverse event reporting: In order to gauge the severity of any AEs related to the study intervention, we will refer to the Rectal Genital Grading Table for Use in Microbicide Studies and the Male Genital Grading Table for Use in Microbicide Studies (appendix). If a stable, chronic condition is noted in the enrolment medical history questionnaire, but does not exacerbate during the trial, the symptoms will be recorded in the AE report but not attributed to the gel. Subjects will be advised to promptly notify the research nurse of any AEs; the event will be documented in the patient's Case Report Form and the

patient triaged and treated at the discretion of the study physician. However, should a subject fail to immediately report an AE, they will also be asked about any recent medical visits/AEs at each follow-up visit.

Data Handling Procedures and Randomization

Intervention assignment will occur at the McGill Division of Cancer Epidemiology (MDCE) via computer-assisted block randomization with randomly variable block sizes. Dr. Agnihotram Ramanakumar will conduct data management and randomization. Joseph Tota, an epidemiology PhD candidate under the supervision of the PI, will coordinate the study. Mr. Tota has an MSc in epidemiology and over 7 years of experience in cancer research. He assisted in the development of the CATCH trial and has performed analysis of cross-sectional and longitudinal epidemiologic data.

Enrolment and randomization: Individuals will be screened for eligibility over the telephone or in person and eligible men will attend an enrolment visit, where the nurse will obtain informed consent and instruct the participant on gel use. They will receive a one month's supply of gel and provide the first specimen. Random number sets will be assigned to the treatment and control gel. Each participant will be assigned an individual code, which will be used to match him to the study arm. Lastly, the nurse will provide details about HPV infection and advice about condom use and sexual health.

Data Handling

Study and data management will be facilitated through the use of a secure, password-protected web-based database to record and manage study procedures. The database will be used to record participant and clinic visit information, review due, overdue and completed clinic visits and surveys, and for exporting data. It will only be accessible from specific IP addresses. Confidentiality will be of utmost importance. A coded alphanumeric system will be used to identify subjects and online interactions will be password protected. All study data, including but not limited to records, case report forms, and laboratory results will remain confidential and stored in a secure location. Research staff will be the only individuals with access to these personal documents. These will be available to the study sponsor or participating regulatory agencies upon request.

Sample Size Calculations

Data to inform our sample size calculations were derived from our Montreal HIPVIRG cohort study of MSMs living with HIV,[39] and a meta-analysis that included results from different sites representing both MSM subgroups.[11] Although no other study besides HIPVIRG has reported HPV incidence for MSMs with HIV, the reported prevalence in the HIPVIRG population[39] was very similar to studies conducted outside of Montreal.[11] This gave us confidence that adopting incidence data for MSMs without HIV (from settings outside of Montreal) may be appropriate. Calculations used the technique of Dupont and Plummer[81] and the hazard rate estimates of acquisition. This approach is consistent with the aim of studying outcomes at different time points via time-to-event analysis (i.e., log-rank test and Cox proportional hazards regression). Notwithstanding the strong inhibitory properties of carrageenan,[59,61] and the expert opinion of Dr. John Schiller, the investigator who discovered carrageenan's inhibitory properties (appended letter),[59] we were conservative in the estimated preventive effect size of 50% among MSMs without HIV. As the primary inhibition mechanism relies on the immune response, the anticipated effect size among MSMs with HIV is lower at 30%. We separately tailored our power calculations to satisfy our primary endpoint in each of these MSM populations; however, we will consider pooling results to improve our precision if they are found to be homogeneous across groups. As additional parameters, we specified 80% power to evaluate our primary objective with a type 1 error of 0.05 and 2-sided hypothesis. Assuming an incidence proportion of HPV infection at 12 months of 85% (among HIV positive MSMs)[39] and accounting for 10% loss to follow-up, the estimated sample

size required for effect sizes of 30%, 40%, and 50% was calculated to be 107, 66 and 45. Similarly, assuming an incidence proportion at 12 months of 30% (among HIV negative MSMs)[11] and accounting for 10% loss to follow-up, the sample size required for effect sizes of 50%, 60%, and 70% was calculated to be 270, 178 and 124. To permit verification of the study's objectives with sufficient power at the end of the one-year follow-up period, we propose to recruit 380 subjects (110 HIV+ and 270 HIV-). Tables 2 and 3 reveal the effect that varying parameter estimates and effect sizes has on the sample size required for the study's primary aim in each of the two groups.

Data Analysis

Primary aim 1 (prevention): Calculation of carrageenan's efficacy will be done by testing the null hypothesis of no difference in time to type-specific HPV infection between treatment groups with the log rank test. All analyses will be performed separately according to HIV status at baseline and later pooled if appropriate. In our primary analysis, HPV infection date will be considered the midpoint between the first visit that a new HPV type is detected and the last visit when the new HPV type was not present. Time to HPV infection will be defined as the difference in days between the calculated HPV infection date and the enrolment date plus one. We also plan to compare our results applying a discrete hazard approach.[82] We will use Cox proportional hazards regression to estimate the hazard ratio and 95%CI of HPV infection for treatment versus placebo. Proportional hazards assumptions will be verified to ensure their appropriate use. If the proportionality assumption is not met or the hazard ratio changes over time, then we will fit a discrete-time hazards model.[83] To evaluate effectiveness in our study population we will conduct our analyses according to the intent-to-treat approach (i.e., including all participants who were randomized and received at least one-month's supply of gel). To evaluate efficacy we will use the according-to-protocol approach (i.e., including only "adherent" participants who complied with the protocol). A participant will be considered adherent if he reported gel use as recommended >50% of the time. Since very few subjects may be expected to use the gel they were assigned as recommended (prior to every act of intercourse), we plan to perform additional analyses focusing on those who were compliant most of the time. Our intent-to-treat analysis will provide us with an unbiased estimate of population effectiveness, whereas our per-protocol analysis may provide a better sense of whether carrageenan actually works in preventing HPV infection among those who use it as recommended, i.e., efficacy of the intervention. Since our per-protocol analysis may introduce some bias, we plan to report results from our intent-to-treat analysis in our main results. In our analyses, we will allow for time-varying adherence, defined as adherence since the last questionnaire. Dosage efficacy of the gel will not be investigated due to design limitations. Measurement of gel bottles at follow up visits will assist in assessing compliance.

Secondary aim 1 (clearance): We will also use time-to-event analysis techniques to measure type-specific clearance of HPV infections present at enrolment according to the intervention. Time to clearance and hazard ratios of clearance will be calculated as above. Because of randomization, the rates of type-specific HPV infections will be comparable between study arms at enrolment. Analyses will use intention-to-treat and according-to-protocol approaches.

Secondary aim 2 (HIV Status): The aforementioned statistical calculations will be conducted separately among MSMs with and without HIV (baseline status) for the study endpoints.

Secondary aim 3 (Safety, tolerability, and adherence): We will use the chi-square test to compare adherence between intervention and control groups and for all participants combined at each follow-up visit (adherence since the last questionnaire), as well as overall adherence (adherence from month 0 to

12). Safety and tolerability of the interventions will be evaluated using the AE reports from both arms of the study.

Interim Analyses

Yearly analyses will be performed separately among the HIV positive and negative subgroups to determine if early termination of the trial for either or both groups is warranted. The type 1 error for concluding efficacy will be controlled by the Lan-Demets spending function[84] with O'Brien and Fleming type boundaries.[85] The Lan-Demets method offers us flexibility to analyze the data either sporadically, or at equal intervals. An independent data safety monitoring board (DSMB) will review the results and make recommendations regarding safety or reasons that may force early trial termination (e.g., unequivocal evidence of efficacy). For instance, if carrageenan can no longer be proven effective in preventing HPV acquisition among HIV-positive patients, then early termination may be considered for this subgroup. McGill University's IRB will nominate member of this board, outside of our purview. *Members will have expertise in statistics, HPV prevention, the conduct of clinical trials, and ethics/law. The data safety and monitoring committee will meet periodically during the course of the trial to review the data.*

Compliance

We expect higher compliance in this trial than among the heterosexual females of the CATCH trial. Unlike vaginal intercourse, anal intercourse frequently requires the use of a lubricant (feasibility study results; appendix). Nevertheless, we will take measures to increase compliance, such as restricting participation to volunteers; providing written instructions on proper gel use; sending regular email or text message reminders; and by requiring them to fill out a weekly coital journal. Participants will be able to contact the nurse at any point with questions or if they require more gel before their scheduled clinic visit. To prevent trial contamination, we will discourage the use of other sexual lubricants, including those from other participants. Any outstanding gel bottles will be returned at follow-up visits and weighed to determine the volume of gel used between visits (see appendix). Data will be used as an additional (objective) measure of adherence to the protocol.

Loss to Follow-up

Our estimates of the rate of loss to follow up are based on the HIPVIRG study, where at the 12-month mark, there was only a 13.8% rate of loss to follow up.[39] This trial should present comparatively lower attrition due to the noninvasive intervention. Compensation of $25 will be dispensed per clinical visit, which reflects the general consensus of our feasibility study respondents (mean: $26.50). The free sexual lubricant is an added incentive for trial continuation. In our feasibility study, 90% of respondents used personal lubricants during every (or almost every) act of anal sex. [redacted] Assuming 2 acts of intercourse per week, and 15mL of gel per act over one year (feasibility study results), each participant would use upwards of 13 bottles, thus saving $386.26 by participating in this trial. All benefits considered, it is reasonable to expect high retention for the trial and an accordingly low loss to follow-up of 10%. Complete removal of a patient from the trial will occur if the patient: voluntarily withdraws from the trial, or has AEs, illness, or other medical conditions determined by a physician that are serious enough to terminate their involvement in the study. Loss to follow up will be described as: failure to reach the patient for follow-up visits after 6 months from randomization, or the patient has the potential to jeopardize the integrity of the study through protocol noncompliance.

CATCH Trial

CATCH is a similar ongoing RCT led by Dr. Eduardo Franco. The described study borrows much of its design from CATCH, but instead proposes a different population that should demonstrate higher adherence and a better evaluation of the gel's inhibitory properties against HPV. The study gels are the same ones used in CATCH and already have regulatory approval from the Natural Health Products Branch of Health Canada.

STUDY TEAM AND ROLES

Nominated Principal Applicant: Dr. Eduardo Franco (MDCE Director, and Professor and Chair of the Department of Oncology) will provide oversight for all aspects of study conduct. Dr. Franco has worked in the field of HPV-associated diseases for over 30 years and published extensively on the topic. He is the PI of several successful epidemiologic studies related to HPV in Canada, Brazil, and the Congo Republic. He has also conducted RCTs of treatment and preventive interventions in oncology and infectious diseases.[86-88] Specifically for HPV and associated diseases, he was the PI for the CIHR-funded Canadian Cervical Cancer Screening Trial (CCCaST), the first RCT in North America that compared the efficacy of HPV testing with Pap cytology,[89,90] and for the CATCH study. Finally, he has served as a consultant to GlaxoSmithKline in the design and analysis of one of the international RCTs of HPV vaccination.[51,55] Dr. Franco is the academic and research supervisor for the Study Coordinator, Mr. Joseph Tota. He will contribute 4 hrs/week to the project.

Co-applicants

Dr. Alexandra de Pokomandy, a prior MSc student of Dr Franco and an Associate Professor at McGill University's Department of Family Medicine is a practicing physician for HIV care at the MUHC with over 10 years of experience. She worked extensively on the HIPVIRG study of anal HPV and AIN in HIV-infected MSM, and was additionally the PI of a similar cohort study in women living with HIV and the co-PI of a Canadian cohort study in women living with HIV, based on community based research principles (designated CHIWOS), both CIHR-funded. Familiar with the HIV-positive population, she has valuable experience in the epidemiology of HPV infection in MSM, and is jointly responsible for the study design, collaborating with recruiting sites, and future data analysis and knowledge translation (KT) to the HIV community. She will contribute at least 4 hrs/week to the project.

Dr. François Coutlée (Professor of Microbiology at the Université de Montreal and Adjunct Professor at McGill University) will be responsible for the HPV testing and typing. He heads one of the most experienced laboratories worldwide in the field of STD testing, particularly HPV. He has made several methodological contributions on this topic. Dr. Coutlée and both PIs have been close research associates since many years in several studies in Montreal (since the mid-90's with Dr. Franco, and for more than 9 years with Dr. de Pokomandy). He will contribute 2 hrs/week to the project.

Dr. Pierre Tellier (Associate Professor in Family Medicine at McGill University and Director of McGill's Student Health Service) has worked with Dr. Franco as the main clinical collaborator in three CIHR-funded studies that served as predecessors for the present project. Dr. Tellier plays a prominent role in professional and patient education at McGill and will oversee all clinical activities related to this project. He will supervise the study nurses and advise the study team concerning issues related to subject recruitment and sexual health. He will contribute 2 hrs/week to the project.

Trial Steering Committee

This Committee will supervise the trial, and ensure that it is conducted in accordance with the principles of good clinical practice. Aside from the study team, it will include: Dr. John Schiller, a Senior Scientist with the US National Cancer Institute and the lead investigator who discovered the anti-HPV properties

of carrageenan; and Mr. Jose Sousa, who has been living with HIV since 1985 and is a member of the Community Advisory Committee of the CIHR Canadian HIV Trials Network since 1995 *and of the Ontario HIV Treatment Network for the past 5 years. He has great experience in collaborating with HIV researchers and will represent the interests of the community in the committee. We will also invite a leading Canadian biostatistician. This committee will meet twice a year to review progress and to advise on issues related to protocol adherence, endpoints, and statistical analysis.*

Study Center

There will be multiple study sites in Montreal: Concordia University clinic, McGill University Clinic, MUHC, and we may open study sites at the recruiting clinics mentioned above according to the clinic interest and rate of recruitment (Clinique Médicale du Quartier-Latin, Clinique L'Actuel, Clinique OPUS, Unité d'Hospitalisation de Recherche et d'Enseignement sur les Soins du SIDA (UHRESS) of the Centre Hospitalier de L'Université de Montréal (CHUM)). Study oversight will be carried out at the MDCE, which is directed by Dr. Franco, the PI—the hub for research related to the molecular epidemiology and prevention of HPV infection and associated diseases. It assembles epidemiologists, lab scientists, and clinicians from Montreal, nationally, and internationally. This unit has all the resources necessary to carry out large epidemiological investigations. In April 2014, the staff in the MDCE included 2 full-time professors, 11 adjunct or associate faculty members, 7 research assistants and nurses, 1 PhD-level biostatistician, 1 administrative secretary, 4 postdoctoral fellows, 2 PhD students, and 2 MSc students.

KNOWLEDGE TRANSLATION

There is presently 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 MSM community, regular application of a carrageenan-based lubricant could be a cost-effective preventive approach, especially considering that most MSMs regularly use lubricants for anal sex. *Furthermore, treatments for condylomas and AIN are costly and need to be repeated, as the rate of recurrence is very high (particularly among people with HIV)[29] and vaccination is generally only effective if administered prior to becoming sexually active.* The results of this trial are likely to have immediate KT implications. In addition to traditional end of study KT activities (e.g., peer-reviewed publication and presentations at national/international meetings), the study's community representative, multiple physician collaborators, and links to numerous sexual health clinics in Montreal and elsewhere will help us disseminate research findings directly to the target audience and impact healthcare services for the MSM community. Dr. de Pokomandy (co-applicant) is already working closely with the community of HIV-infected individuals. She can provide presentations to the community as previously done in community organisations in Montreal and Toronto. We also have the support of CATIE (Canada's source for HIV and Hepatitis C information; letter attached), to disseminate our findings in the lay media. Should the gel prove effective, it can be recommended as a component of a MSM's healthy lifestyle to reduce risk of HPV and associated diseases, such as anal cancer. The direct and indirect implications could have positive effects on the health of countless individuals around the world.

Table 1: Study procedures according to visit

	Screening (Pre-Enrolment: Days -28 to -1	Month 0 (Enrolment Visit)	Month 1 (+/- 7 days)	Month 2 (+/- 7 days)	Month 3 (+/- 7 days)	Month 6 (+/- 14 days)	Month 9 (+/- 14 days)	Month 12 (+/- 14 days)
Inclusion/Exclusion Criteria	X							
Informed Consent		X						
Enrolment Questionnaire		X						
Distribution of Gels		X	X	X	X	X	X	
HPV Typing		X	X	X	X	X	X	X
HIV testing for HIV negative MSM only		X						X
Chart review for HIV-positive men only		X				X		X
AE Reporting			X	X	X	X	X	X
Follow-up Questionnaire			X	X	X	X	X	X
Medication Counselling		X	X	X	X	X	X	X
Compliance Reinforcement		X	X	X	X	X	X	
Assessment of Blinding						X		X

Table 2: Sample size and power calculation for subgroup of MSMs living with HIV †

Incidence Rate (Control Group)	Anticipated Effect Size	Number of Subjects Required for Primary Aim‡
0.85	0.3	107
	0.4	66
	0.5	45
0.90	0.3	86
	0.4	54
	0.5	38
0.95	0.3	67
	0.4	44
	0.5	32

† Two-sided test at a significance level of α=0.05 and power of 80%

‡ Calculated sample size takes into account a 10% loss to follow-up based on reference 74.

Table 3: Sample size and power calculation for subgroup of MSMs without HIV †

Incidence Rate (Control Group)	Anticipated Effect Size	Number of Subjects Required for Primary Aim‡
0.25	0.5	340
	0.6	224
	0.7	155
0.30	0.5	270
	0.6	178
	0.7	124
0.35	0.5	220
	0.6	146
	0.7	102

† Two-sided test at a significance level of α=0.05 and power of 80%

‡ Calculated sample size takes into account a 10% loss to follow-up based on reference 74.

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