Transmission Reduction and Prevention with HPV Vaccination (TRAP-HPV) study: A Randomized Controlled Trial of the Efficacy of HPV Vaccination in Preventing Transmission of HPV Infection in Heterosexual Couples

Trial Registration Number: NCT01824537
Date: September 2022

Version number 1 and date of the study protocol: May 17, 2012 (revisions and submission: March 27, 2014; March 18, 2014; January 26, 2015; July 08, 2015; January 28, 2016; July 5, 2016; March 13, 2017; May 8, 2017; June 12, 2018; May 2022; September 2022)

History of protocol amendments approved by McGill University's Research Ethics Board (Refer to Appendix 1)**:**
- Recruitment age increased from 18-26 years to 18-40 years (approved April 14, 2014).
- Maximum duration of relationship increased from 3 to 6 months (approved April 30, 2014).
- Compensation increased from CAD 350 to CAD 500 per couple (approved February 9, 2015).
The above protocol changes were made to improve recruitment and/or simplify the logistics of the study.
- Gardasil 9 as intervention vaccine: The additional amendment relating to Gardasil 9 will enhance the health benefit of the study to participants while making this protocol truly cutting-edge with respect to the state of HPV science (approved July 08, 2015).
- Recruitment age increased from 18-40 years to 18-45 years (approved February 16, 2016).
- Discontinuing anal sampling: potential and current participants have confirmed our suspicions that troubling recruitment rates are due partially to the embarrassing and uncomfortable nature of this procedure (approved July 14, 2016).
- Adding a recruitment site in New York to increase the rate of accrual of participants (approved April 3, 2017).
- Compensation increase from CAD 500 to CAD 1000 per couple (approved May 8, 2017)
- Switch the placebo vaccine from Havrix to Avaxim (requested June 4, 2018, approved June 12, 2018).
- Second Oral Tube Collected: Julie transfers the sample to a freezer tube with tampon (she calls it "TE") - Julie removes the residues of the mouthwash and re-suspends it in the tampon. It's all kept in the freezer at Dr. Coutlee's lab (information collected with Julie Guenoun on February 11, 2022).
- Close study recruitment at 188 couples instead of the original 500 couples (requested on March 31, 2022, approved on May 26, 2022)
- Use of a different assay for serology testing (requested on September 8, 2022, approved on September 12, 2022) (Refer to Appendix 2)

1. The Need for a Trial

1.1 What is the problem to be addressed?

Two prophylactic vaccines (Gardasil, a quadrivalent vaccine by Merck, and Cervarix, a bivalent one by GlaxoSmithKline [GSK]) have been proven in randomized controlled trials (RCT) to be highly effective in preventing infection with the vaccine target HPV types (HPV-6/11/16/18, for Gardasil, and HPV-16/18, for Cervarix) and the cervical precancerous lesions caused by them[1,2]. These vaccines have shifted the paradigm of prevention measures and are expected to have a major impact in reducing the burden of cervical cancer and other HPV-associated malignancies, such as vulvar, vaginal, penile, anal, and oropharyngeal cancers, as well as benign HPV-associated conditions (in the case of Gardasil), such as anogenital warts and respiratory papillomatosis[3-5].

Vaccination programs aim to be cost-effective by reaching sufficiently high coverage to reduce transmission to levels that are low enough to permit protection to extend also to susceptible, unvaccinated individuals, a concept broadly designated as herd immunity. Given the long timescales involved, mathematical models have played an important role in predicting herd immunity thresholds and informing policy decisions regarding HPV vaccination strategies. The results of published health economic models on the impact of HPV vaccination have consistently shown that delivery of HPV vaccination is cost-effective[6-9]. Studies of the impact of HPV vaccination of 12-year-old girls in North America estimated a cost-effective ratio ranging from $31,000 - $43,600 per quality-adjusted life-year gained, compared with current screening[6,9]. The added benefits of vaccinating boys have been also evaluated with mixed results[10,11]. Most recently, we concluded that the potential incremental gains of vaccinating boys may be limited because of the predicted herd immunity impact of vaccinating girls under moderate to high vaccine coverage[12]. However, differences in model structure and assumptions have made it difficult to compare the findings across studies and apply them to policy evaluations. HPV transmission dynamics have been identified as a key source of heterogeneity in health economic studies of the impact of HPV vaccination[13]. In summary, whether or not provinces should implement vaccination of boys has to rely on such health economic models, but the latter require knowledge of empirically-derived estimates of how vaccination prevents transmission between partners. No vaccination study has yet derived these estimates.

1.2 What is/are the principal research question(s) to be addressed?

Much remains to be understood on the effects of HPV vaccination in preventing transmission of target HPV types to sexual partners of vaccinated individuals and its impact on herd immunity. Does vaccination of unexposed persons against HPV infection effectively protect their sexual partners from HPV infection? The published RCT data has been unequivocal in that vaccination seems to be exclusively prophylactic, i.e., it does not help to clear preexisting HPV infections in an individual. However, it is not known whether or not such a previously infected individual, once vaccinated, would be less infective to her or his sexual partner. Likewise, does vaccination protect against HPV infection with the target types in mucosal sites other than the cervix? Apart from the knowledge from Gardasil RCTs that focused on the female lower genital tract, no information is available concerning whether pan-mucosal protection (via exudation of neutralizing antibodies) leads to decreased HPV transmission risk.

Would the efficacy of vaccination in preventing transmission of HPV 6/11/16/18 warrant a two-gender vaccination strategy? This question has been the focus of much research on mathematical modeling of the cost-effectiveness of HPV vaccination and policy discussions[6-13]. Thus far, there is no empirical evidence for answering the above question, other than the knowledge that the vaccine is efficacious in women and also in men[14].

The objective of this study (TRAP-HPV) is to shed light into the effect of an HPV vaccine (Gardasil) in preventing transmission of target HPV types to heterosexual partners among young sexually active couples in Montreal. Specifically, TRAP-HPV aims to determine the efficacy of an HPV vaccine in reducing transmission of genital, anal, and oral HPV infection in sexual partners of vaccinated individuals.

1.3 Why is a trial needed now? Evidence from the literature - see 1.4 below, professional and consumer consensus and pilot studies should be cited if available.

This proposal describes an RCT that aims at measuring the impact of vaccination in preventing HPV transmission to sexual partners of young, sexually-active university students in Montreal. Valuable data will be collected on HPV transmission dynamics of both women and men with either incident or prevalent infections of HPV 6/11/16/18 at multiple infection sites. The information provided from this study could potentially have large public health implications by providing proof of concept and empirical data to inform new effective policies and vaccination programs aimed a population level.

1.4 Give references to any relevant systematic review(s) and discuss the need for your trial in the light of the(se) review(s). If you believe that no relevant previous trials have been done, give details of your search strategy for existing trials.

On 8 August, 2012, we searched the PubMed online database with keywords 'HPV' AND 'vaccine' AND 'transmission'. This search returned with only two references[15,16]. One study[16] reviews the importance of using condoms in reducing STD transmission. The second study[15] tests the immunogenicity and protective efficacy of a vaccine to reduce the transmission of both HPV and the Human Immunodeficiency Virus (HIV) among macaques. No clinical trials on this topic are currently registered in the clinical trials registries (ClinicalTrials.gov and ISRCTN). Our inquiries to Merck and GSK indicates that no such study is being done or planned, as these two companies would have to be involved to provide vaccines. Finally, we have scanned all abstracts submitted to the past 6 International Papillomavirus Conferences (and actually attended them); no study has sought to obtain proof of concept for a transmission prevention effect consequent to HPV vaccination. Obtaining such proof would provide valuable insights and empirical data to inform the mathematical modeling of HPV vaccination strategies.

1.5 How will the results of this trial be used? E.g. inform decision making/improve understanding.

At this time there is no way to treat cervical HPV infections. As described above, we do not know whether HPV vaccination is effective in blocking transmission, or preventing the acquisition of HPV infection in anatomical sites other than the cervix. If any additional effect for the HPV vaccine is proven, this will be used as new empirical evidence for mathematical models of vaccination that are used by policymakers in Canada and elsewhere. Our own CIHR Team (CRN-83320) includes a health economics component (led by Dr. Marc Brisson [appended letter]), which will enable us to quickly move to a knowledge translation activity when the results become

available. So far, models that gauge the potential impact of vaccinating boys have not taken into account the impact of the vaccine in reducing transmission between partners and, within the same partner, to other mucosal sites.

1.6 Describe any risks to the safety of participants involved in the trial.

Gardasil was approved in Canada for women aged 9-45 years and for men aged 9-26 years[17]. Its safety was studied in over 29,000 males and females, in several trials before licensure in 2006[18]. The U.S. Centers for Diseases Control and Prevention (CDC) and the U.S Food and Drug Administration (FDA) maintain a sentinel public health system called Vaccine Adverse Event Reporting System (VAERS). By 15 September 2011, 40 million doses of the Gardasil vaccine had been distributed in the US. The rates of adverse events following Gardasil in VAERS are comparable to those for other vaccines, except for syncope and venous thromboembolic events[19]. Another study[20] by a network of managed care organizations in the US found no statistically significant increases in risk of adverse events, except for venous thromboembolism. In all such cases risk factors were present (obesity, oral contraceptive use, smoking, recent hospitalization, or coagulation disorders). The Public Health Agency of Canada states that both HPV vaccines available in Canada are safe; the most common side effect being pain at the site of injection[17].

2. The Proposed Trial

2.1 What is the proposed trial design? E.g. Open-label, double or single blinded, etc.

TRAP-HPV will be a randomized, placebo-controlled, double-blinded controlled trial to investigate the impact of Gardasil vaccination on the transmission of HPV infection to sexual partners in heterosexual couples ages 18-40 years living in Montreal.

2.2 What are the planned trial interventions? Both experimental and control.

Once recruited, both individuals in a couple will be randomized independently to treatment or placebo (table 1). Treatment is defined as vaccination with Gardasil (Merck). *This vaccine was chosen because it allows for the observation of 4 HPV outcomes (HPV 6, 11, 16, & 18)* (the other available vaccine, Cervarix, protects against HPVs 16 and 18, only). The existing public HPV vaccination program in Quebec does not include women targeted by our study (aged 18-40) or men of any ages. Therefore, we expect low uptake of opportunistic HPV vaccination in the subjects eligible to be enrolled in this study; rates of Gardasil immunization among these subjects will be very low.

The placebo vaccine will be a hepatitis A vaccine, Havrix (GSK). This control vaccine was chosen because hepatitis A immunization provides a similar health prevention incentive as HPV vaccination to study participants while preserving the scientific cogency of a “placebo” comparator. Gardasil requires administration of 3 doses, while Havrix only requires 2 doses. For this reason, we will add a placebo injection (saline solution) in between the Havrix vaccination regimen. Consequently, both treatment and control vaccines will have similar regimens, i.e., study entry, 2 months, and 6 months. As detailed in table 1, four comparison groups will be formed via a 2-by-2 design where partners will both have been vaccinated with Gardasil or with Havrix or will have unequal vaccination treatments. Comparing combinations of these 4 groups will yield valuable information on the effect of vaccination on HPV transmission blockage.

Table 1: Four vaccination comparison groups in 2x2 factorial design

Female (F) vaccination	Male (M) vaccination	
	HPV (Gardasil) (T)	**Placebo (Havrix) (P)**
HPV (Gardasil) (T)	M^TF^T	M^PF^T
Placebo (Havrix) (P)	M^TF^P	M^PF^P

In order to ensure that all study participants gain similar health benefits and incentives in keeping with ethical values, we will use a crossover of interventions at the end of the study by offering Gardasil to all control subjects and Havrix to all HPV-immunized subjects. The benefit of HPV vaccination would not be significantly delayed for control subjects since the study is relatively short (12 months). Furthermore, the control subjects at risk of HPV infection will benefit from our intensive follow-up protocol which would provide substantial protection and surveillance of HPV infection that extend beyond those given by standard care. All women with persistent HPV infections with oncogenic HPV types will be offered a Pap test. At the end of TRAP-HPV, an exit Pap test will be given to all female participants. Since there are no guidelines for screening men, only male subjects who appear to have visible lesions will be referred for clinical examination.

2.3 What are the proposed practical arrangements for allocating participants to trial groups? E.g. Randomization method. If stratification or minimization is to be used, give reasons and factors to be included.

Assignment of the vaccine will be done by the coordination centre at McGill by computer-assisted block randomization with randomly variable block sizes. Please refer to section 3.1 for more information regarding the arrangements for enrolment and randomization.

2.4 What are the proposed methods for protecting against sources of bias? E.g. blinding or masking. If blinding is not possible please explain why and give details of alternative methods proposed, or implications for interpretation of the trial's results.

Participant blinding is assured because both vaccines (HPV and hepatitis A) and their syringes will look identical (previously drawn from blinded vials in a separate room by the nurse). Furthermore, injection pain is expected to be the same for both vaccines. To maximize retention, every effort will be made to enrol only eligible and motivated couples. Issues regarding recruitment, compliance and loss to follow up are described in more detail in sections 2.12-2.14.

2.5 What are the planned inclusion/exclusion criteria?

Recruitment of university students will occur at multiple centres. Volunteer couples must (1) not have been vaccinated against HPV (both partners); (2) plan on remaining in Montreal for at least 1 year; (3) be in a new relationship that started no more than six months prior to study entry; (4) plan on having continued sexual contact with partner; (5) have no history of cervical, penile, oral or anal cancers; (6) not be pregnant or plan on immediately becoming pregnant, and (7) be willing to comply with study procedures. Study forms will be based on the co-applicants' experience with their previous molecular epidemiologic studies of HPV infection (please see section 2.11 for details). Concerning requirements 3 and 4 above, we will screen couples based on the Dyadic Adjustment Scale (DAS) (please see appendix) as a validated instrument to measure the stability of couples' relationships[21-23]. It includes 4 scale questions that gauge the degree of confidence in

the partner, likelihood of a separation, and overall satisfaction with the relationship. Couples in which one of the partners scores at less than 80% of the maximum score will not be enrolled.

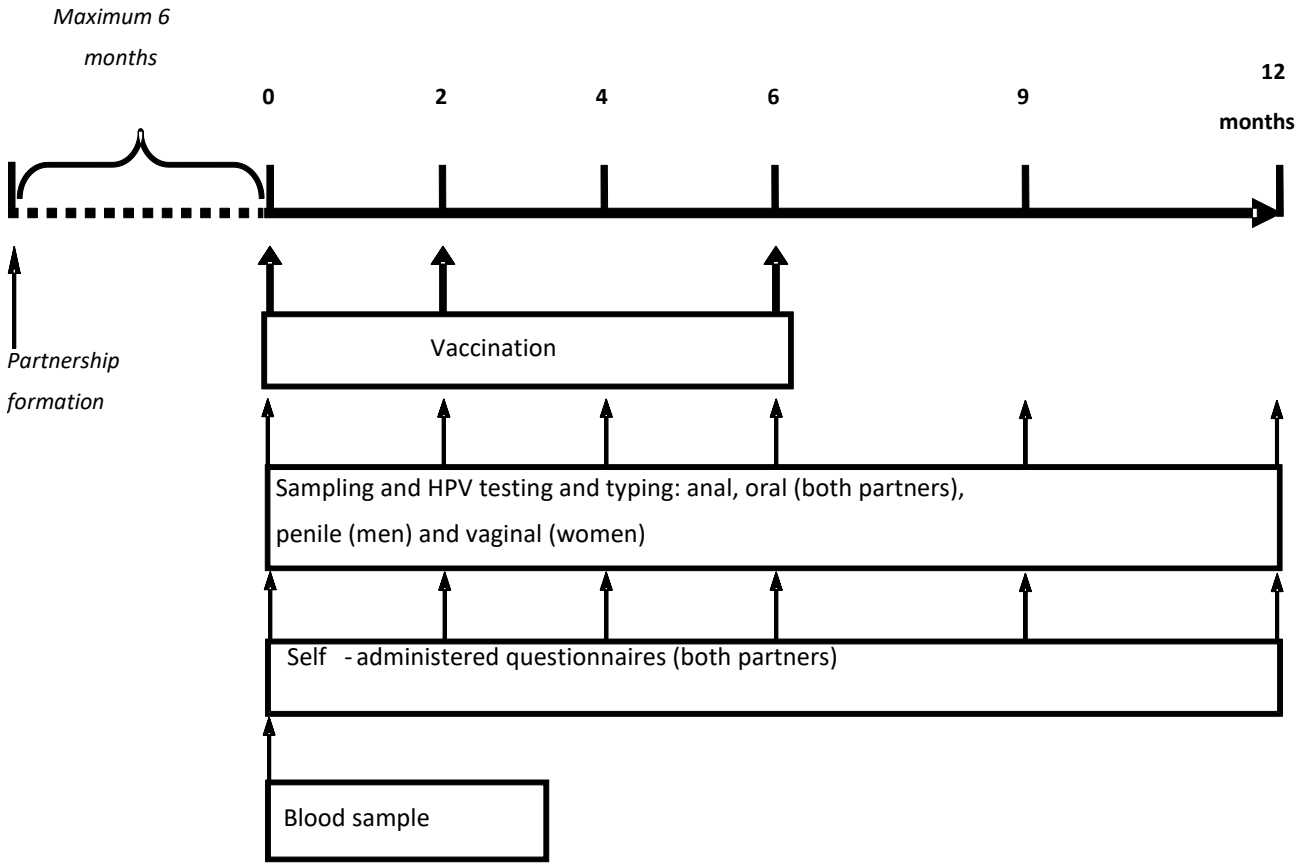
2.6 What is the proposed duration of treatment period?

Participants will be provided three doses of Gardasil or Havrix at enrolment (time 0), and at 2 and 6 months. After month 12, a crossover of the intervention will take place, as well.

2.7 What is the proposed frequency and duration of follow up?

HPV infection status will be measured at enrolment (time 0), and at 2, 4, 6, 9, and 12 months (Figure 1). A one-year follow-up period was chosen to allow sufficient opportunity for HPV infection transmission (in our HITCH cohort study of couples most transmission events occur within that period). Longer follow-up would likely result in substantial attrition.

Figure 1: Study design and schedule of procedures



2.8 What are the proposed primary and secondary outcome measures?

The primary outcome will be the reduction of HPV DNA positivity for the target HPV vaccine types (i.e., HPVs 6, 11, 16, and 18) in multiple anatomic sites in Havrix-treated sexual partners of persons who received Gardasil. These are the combinations M^TF^P and M^PF^T in table 1. The main control group for unhindered HPV transmission will be group M^PF^P and the full protection control group is represented by the combination M^TF^T.

We will measure prevalence and incidence of HPV 6, 11, 16, and 18 infections (via DNA detection by the PCR protocol described below) in all three samples (vaginal, anal, and oral, for women, and penile, anal, and oral, for men) for the above time points among all subjects. Specimens for all three sites will be collected during all six clinic visits.

We will not exclude subjects if they are positive for HPV DNA of HPVs 6, 11, 16, and 18 because we intend to verify whether protection conferred by vaccination (both self and partner's) extends to mucosal sites that have not yet been infected by the above target types. Actually, prevalent infections at enrolment will be informative because they could be the source of a transmission event to a partner.

Reduction in HPV type concordance (for the four target types) will be the main outcome evaluable as per the above group contrasts. These comparisons will be done with due attention to the enrolment virological status of the individuals. For instance, we expect that a Havrix-treated woman who is positive for HPV 6 in the anal specimen but negative for this type in the vaginal specimen may derive benefit if her partner receives Gardasil, even if he is HPV-6 positive in the penile sample. Our assumption is that protection via vaccination is pan-mucosal, via transudation of neutralizing antibodies; this protection may mediate transmission.

2.9 How will the outcome measures be measured at follow up?

Data collection and follow-up

Figure 1 shows the study design and schedule of procedures. Assessing pre-enrolment humoral immune response to HPV infection will be done by withdrawing 10 ml of blood from all study participants and testing serum samples with a competitive Luminex immunoassay (CLIA) for detecting any antibody response and quantifying it[24,25]. CLIA testing will be done by Merck Research Laboratory at Merck's Headquarters in Pennsylvania, USA, at no cost to the study. CLIA testing will measure neutralizing antibodies and thus will provide valuable insights to help us understand initial transmission events. We will also maintain serum samples at -70°C for future serological studies with polyclonal anti-HPV assays. Funding for such studies will be sought at the renewal stage of this investigation so as to avoid unduly increasing costs for the current budget.

The most important outcome is measurement of HPV infection status (via HPV DNA testing) in both partners. They will be done at 0, 2, 4, 6, 9, and 12 months. Samples will be collected at three sites: penile/vaginal, anal, and oral for men and women at every visit. Anal and vaginal samples will be self-collected at the clinic, after properly instructing participants. The rest of the samples will be collected by specially trained study nurses, at every visit. The nurse will collect a sample of exfoliated cells from representative sites in the oral cavity using a toothbrush and a mouthwash. This method has been shown to yield good quantities of DNA for testing. Oral samples will be immersed and suspended in phosphate-buffered saline.

Previous research on the method of penile skin swabbing to detect HPV DNA has proven this method to be reliable[26]. All samples will be placed in a –20ºC freezer pending transfer to Dr. François Coutlée's laboratory where they will be stored at –70ºC until being tested. HPV DNA testing will be done by the PGMY linear array PCR method[27], which permits typing and has been extensively validated in our molecular epidemiologic studies of HPV infection[28-30].

Collection of vaginal specimens

Self-collected vaginal specimens will be obtained at months 0, 2, 4, 6, 9 and 12, resulting in six specimens in total (Figure 1). Women would be asked to abstain from intercourse a minimum of 48 hours[31] before collection of the specimen. This will minimize the risk of contamination with residual male epithelial cells, urethral secretions, and or/semen[32].

Self-collection methods have been shown to be valid for research and clinical purposes, and acceptable to women[33-35]. Feasibility studies done in our group prior to the HITCH study and during the summer 2012 (appendix) showed that although participants prefer to have their specimens collected by the nurse, they were also comfortable with the self-collection approach, if taught by the study nurse. They also found that self-collection was more practical for multiple specimens collected during the course of the study.

The instructions for self-collection of vaginal specimens will follow those of the validated protocol of Gravitt et al.[35]. Women will be instructed to remove clothes from the waist down, remove the swab from the wrapping, and choose a comfortable position (either standing with one foot on a stool, toilet, or bathtub, or standing with legs apart and knees slightly bent). They will be told to relax and insert the cotton tip of the swab into the vagina, without touching the labia or urethra. The swab is to be gently pushed up into the vagina until physically it cannot go any further. This will be followed by a motion using the thumb and two fingers to remove the swab halfway out of the vagina, and then re-inserting it. The swab is to be rotated 3 times inside the vagina, while kept as far into the vagina as possible. The subject will then withdraw the swab holding the lips of the labia apart taking care not to touch other portions of the genitals. The swab will be placed directly into the plastic vial with the liquid preservative (Universal Collection Medium [UCM], Qiagen®, Gaithersburg, MD) that will be provided. UCM adequately preserves exfoliated specimens for DNA, RNA, and protein analyses.

Collection of Male penile skin swabs

Men will provide penile skin swab specimens for HPV testing on all aforementioned six study visits (Figure 1). Previous research and our own experience in the HITCH study has indicated that men are very willing to submit to painless penile skin swabbing and that this method of sampling yields adequate material for HPV DNA detection compared to urine and urethral specimens[26]. In the feasibility study, 64% (9/14) said that this procedure would not prevent them from participating. Men will be asked to abstain from sexual intercourse for 48 hours[31] preceding collection to reduce the possibility of detecting HPV carriage from residual female secretions[36].

The research nurse will conduct an external examination of the genital area to note circumcision status, and the presence of any relevant clinical findings (e.g., warts, lesions, erythema, abrasions, inflammation, discharges, tenderness, adenopathy). Exfoliated cells will be collected by swabbing vigorously with a Dacron™ applicator moistened with sterile normal saline. The nurse will use a new wet swab and sweep 360° around the coronal sulcus and then another 360° around the glans penis. The nurse will use a new wet swab to sample the entire skin surface of each of the quadrants

of the shaft of the penis (left and right ventral, and left and right dorsal). Each swab will be placed into an individual UCM-containing collection vial and labelled according to the anatomic source.

Collection of anal swabs (Added 09-June-2017: refer to the first page of the protocol and to appendix 1 for justification of dropping anal sampling)

All study participants (both genders) will provide anal swab specimens for HPV testing on all six study visits (Figure 1). Lampinen et al. developed feasible[37], simple illustrated instructions[38] for self-collecting anal swabs. Study participants will be instructed to remove clothes from the waist down, remove the swab from the wrapping, and choose a comfortable position, e.g. by standing with one foot on a stool, to facilitate access to the anus. The subject will be asked to hold one buttock to one side. Then the subject will hold the swab 3-4 cm from the tip. They will have to hold the swab firmly and gently insert it into their anus (3-4 cm) until the tips of their fingers touch the outside of their anus. They will need to release their hold on the shaft, then take hold of the swab again, about halfway down the shaft. The swab is to be moved once in a large circle, pressing gently against the inside of their anus. Then the swab will be, slowly and gently, removed from their anus and turned slowly as they remove it. The swab will then be placed into a UCM-containing collection vial.

Self-administered web-based questionnaire (please see appendix)

We will use a secure, confidential study-designated Internet site to provide participants with protected access to the web-based questionnaire by assigned login names and passwords. At induction, males and female subjects will learn to use the web-based system using computers at the clinic sites. At that time they will complete their enrolment questionnaire. Subsequently, all participants will complete five follow-up questionnaires (months 2, 4, 6, 9 and 12).

Randomization Strategy

Eligible couples will be recruited into each centre and treatment assignment will apply to a couple as a unit. Randomization of the study participants will be done via a computer-assisted block randomization with randomly variable block sizes at the coordination centre at McGill University. Treatment assignment will be given to the study coordinator at a centre via a secure web-based program at the time the couple is enrolled. To ensure participant blinding, vaccine vials will be masked and look identical so that both the participant and the nurse administering the vaccine will be unaware of their content. This will also ensure that both partners will not know whether they have received Gardasil or Havrix.

2.10 Will health service research issues be addressed? Justify inclusion/exclusion of health economics and quality of life measures. If these measures are to be included full details should be given including power calculations.

Health service research issues will not be directly addressed by the proposed study. However, should our trial demonstrate efficacy, its findings will be instrumental in orienting initial estimates of cost-effectiveness by our team or others. Our CIHR Team on HPV-associated diseases includes a health economist, Dr. Marc Brisson, at Laval University. The results of this trial will assist in future analyses by Dr. Brisson. Detailed information to inform future cost-utility analyses will be collected during the trial and will be made available to Dr. Brisson (please see his letter).

2.11 What is the proposed sample size and what is the justification for the assumptions underlying the power calculations? It is important to give the justification for the size of the difference that the trial is powered to detect. Does the sample size calculation take into account the anticipated rates of non-compliance and loss to follow-up given below?

Statistical power

Empirically-based estimates of parameters for sample size calculation were obtained from our unit's molecular epidemiologic investigations of HPV infection of female university students, i.e., the McGill-Concordia cohort study[28] and the "HPV Infection and Transmission among Couples through Heterosexual activity" (HITCH) cohort study[29-31], both funded by CIHR. The HITCH study is the same recruitment platform that will serve TRAP-HPV. Prevalence of the four Gardasil preventable HPV types (6,11,16, & 18) at enrolment was 23% and comparable between genders. The rate of acquisition of any of these types was 3.8 per 100 subject-months, which translates to approximately 40% cumulative incidence for these types in the unvaccinated HITCH population. The latter is the most empirically valid expectation for the rate of transmission events in cell M^PF^P in table 1. Therefore, the latter group provides the baseline comparison for the two groups in table 1 with vaccination-discordant regimens, M^TF^P and M^PF^T, whereas the concordant group with both partners receiving Gardasil (M^TF^T) serves as a secondary control group where minimal transmission is expected to occur.

We used the Bernstein and Lagakos[39] approach to derive sample size projections for different levels of power (80% and 90%), with type one error of 0.05, and one-sided hypothesis for reductions in the above rate of transmission varying between 30% and 70%, i.e., the expected magnitude of the protective effect in the discordant vaccination groups where the partner receiving placebo is the one to be protected. We also assumed gender equivalent rates of transmission as per our findings in the HITCH study[31]. At 80% power we would require 97 couples per group (cells in table 1) to detect a significant 40% reduction in transmission of vaccine-targeted types. A group size of 80 couples per group would be required to attain 90% power to detect a 50% reduction. The latter can be statistically detected with as little as 59 couples per group if we relax power to 80%. Evidently, larger effect sizes will have reduced requirements. There are no published empirical estimates of the expected level of transmission reduction, but our informal consultation with experts in the field, including Dr. Alex Ferenczy, a member of our Steering Committee (see below), indicates that 40%-50% reduction in transmission is a conservative estimate for the protective effect to a partner of a vaccinated individual.

HITCH, the closest recruitment platform that TRAP-HPV will be based on, has a cumulative 16% loss to follow-up at month 12, which translates to a per-visit attrition rate of 2.7%. By applying the same expectation of losses to follow-up and using the most stringent definition of projected sample size (at 90% power and 40% effect size), we therefore propose to enroll 125 couples per group for a total of 500 couples. It is noteworthy that vaccine efficacy against HPV infection can occur with only two and even one dose[40]. As we have observed in HITCH, the prevalence of vaccine-preventable types at enrolment is 23% and most transmission events occur early. Therefore, analyses of prevention of transmission events will begin to be informative from the second (2 months) visit and beyond.

2.12 What is the planned recruitment rate? How will the recruitment be organized? Over what time period will recruitment take place? What evidence is there that the planned recruitment rate is achievable?

Our team has developed considerable experience in recruiting female and male university students via our multiple epidemiological studies of HPV infection in Montreal[28-31,41-43]. We are confident that based on our past rate of accrual (obtained with the HITCH study, which has the same characteristics of TRAP-HPV and just completed accrual of 500 couples very successfully) we can recruit 125-135 couples per year, which would permit accruing the complete sample of 500 couples in 4 years. Based on our previous epidemiological studies, such as HITCH, we expect excellent retention in our study (per visit attrition of 2.7%). Participants will also be given a monetary incentive at every visit to ensure their continued participation in our study. Individuals will be given $500 ($1000 per couple) as a cash incentive for their participation, if all study visits were completed. Remuneration will be distributed as follows: $120 at enrollment visit, $60 for each of the three subsequent visits, $80 at visit 5, and $120 at the last visit.

Recruitment: Our base for recruitment will be the McGill and Concordia University Health Services Clinics, which provide medical care year-round to students. Our team has conducted three studies among women attending these clinics[28-31,41-43] since the late 90's. We have developed an excellent collaboration with the directors and staff of the student health services of both universities and we have been given ample office and clinic space there to manage our study subjects. We have a team of enthusiastic interns who serve as recruiters and have developed a good knowledge of campus life to identify study volunteers. Recruitment will be bolstered through campus-wide appeals, including posted notices, e-mails to student lists, etc. Additional efforts include mail-outs to students living in residence, presentations to students in professional schools, and information booths at student activities. Word-of-mouth will also be used for 'snowball' sampling. Also, given our 13-year experience recruiting subjects in university settings and that recruitment resources are already in place, we do not anticipate any major issues with recruitment.

2.13 Are there likely to be any problems with compliance? On what evidence are the compliance figures based?

Although TRAP-HPV is complex in design and number of procedures, we are confident that there will be high compliance because of our inclusion and exclusion criteria, which will select only highly motivated couples (see section 2.5). Management will be done by a team that is highly experienced in conducting studies of HPV infection in couples. Participants will be administered the intervention at the clinic and attend follow-up visits much like the ones in our previous studies, such as the most recent HITCH study, which was successful in terms of retention and in maintaining constant contact with the couples. In any case, our sample size calculations took the anticipated losses into consideration.

2.14 What is the likely rate of loss to follow up? On what evidence is the loss to follow-up rate based?

Data from our HITCH couples' cohort, which has just completed accrual of 502 couples and is approaching completion of all follow-up visits, indicates an average per-visit loss to follow-up of 2.7% for a cumulative 16% attrition after 6 visits in one year. TRAP-HPV will be based on the same source population and will use the same team and comparable methodology and we have incorporated these estimates into our sample size calculation. Since we plan to offer participants a

generous incentive to remain in the study, the rate of loss to follow-up is expected to be just as low as it was in HITCH of even lower (we did not screen couples for their stability in HITCH).

2.15 How many centres will be involved?

The Student Health Services of McGill and Concordia Universities in Montreal will serve as recruitment centres. Study oversight and data management will be carried out at McGill's Division of Cancer Epidemiology (MDCE), directed by the PI. This unit has all the resources necessary to carry out large molecular epidemiological investigations. The MDCE is the central hub for a network of research activities related to the epidemiology and prevention of HPV infection and its associated diseases that involves epidemiologists, lab scientists, and clinicians in Montreal, in other Canadian provinces, and internationally.

2.16 What is the proposed type of analyses?

Statistical Analysis

We hypothesize that HPV vaccination is effective in reducing the risk of HPV transmission to their sexual partners. The above plan takes advantage of the opportunity for measuring transmission events in multiple mucosal sites for multiple HPV types, over multiple clinic visits, and in both directions (i.e., male-to-female and female-to-male). We will take advantage of advanced regression methods as framework for measuring the effects expected via HPV vaccination.

In the simplest core formulation (e.g. analyzing a single HPV type and a single mucosal site), we will use the Kaplan-Meier technique to plot the cumulative probability of HPV infection in sexual partners of vaccinated versus unvaccinated individuals against follow-up time. Using the layout in table 1, this implies comparing the HPV infection histories of women in the $M^{T}F^{P}$ and $M^{P}F^{P}$ groups and of men in the $M^{P}F^{T}$ and $M^{P}F^{P}$ groups. Expectedly, protection is also likely to occur via cumulative effects observable via HPV DNA detection in multiple mucosal sites. Similar time-to-event analyses will be conducted to examine these pan-mucosal site effects.

Statistical comparisons in the HPV transmission between vaccine and control groups will be determined using the log-rank test. The Cox proportional hazard regression model will be used to estimate the effect of vaccination on transmission of HPV to sexual partners. Time to HPV infection will be defined in days as the time from study enrolment to the date of infection. Hazard ratios (HR) and their respective 95% confidence intervals will be computed. Statistical analyses will follow an intention-to-treat approach but additional regression models will examine the role of several candidate determinants in mediating transmission and the protective effects.

Cumulative risk models may be fitted with type-specific transmission as an outcome. GEE models can be used to incorporate data across multiple types of HPV[44], which is an alternative approach for incorporating repeated HPV prevalence data across HPV types and across visits involving the same woman). In a single Generalized Estimating Equations (GEE) logistic regression model, we can treat type-specific infection events for each of the four different HPV types as separate transmission endpoints and then estimate the exposure effect on selected HPVs as a group, as we have published recently[29,30]. Mixed-effects models will also be adopted for incorporating repeated HPV transmission data across HPV types and across visits involving the same subject[45].

Although they will be screened as per the likelihood of their stability, if couples break-up and end their relationship before their last visit, they will be censored of the study while still contributing follow-up time and visits with informative specimens. As mentioned above, vaccination protection is expected to begin as early as the first dose, thus even with a subset of visits for a few couples, we can expect to analyze reductions in transmission before the full set of visits is completed.

2.17 What is the proposed frequency of analyses?

TRAP-HPV is an RCT that aims at obtaining empirically valid estimates of vaccine protection against transmission of preventable HPV types to partners of vaccinated individuals and to the same individuals in different mucosal sites. It is not designed to derive a therapeutic or preventive effect to assist regulatory approval of a new intended use of a vaccine. TRAP-HPV should thus be more appropriately classified as a controlled epidemiologic study whose data collection is completed in multiple visits within one year of each subject's participation. This time span is too short to be of any concern with respect to risks that may require medical intervention. Furthermore, after 12 months there will be a crossover of vaccination regimens so that all subjects will receive the vaccine that they did not receive at enrolment. Therefore, typical design safeguards for early termination are not as important. However, to decide whether or not the trial should terminate early, the available data will be analyzed annually. An independent data safety monitoring board will review the interim analysis results. We will seek advice to McGill University's Research Ethics Board (REB) concerning the composition of this board. Members will likely be nominated by the REB, outside of our purview. The type 1 error for concluding efficacy will be controlled by the Lan-Demets spending function[46] with O'Brien and Fleming type boundaries[47]. The Lan-Demets method offers us flexibility to analyze the data either sporadically, or at equal intervals.

2.18 Are there any planned subgroup analyses?

Although the primary outcome is the reduction of transmission via vaccination, we will also have the opportunity to measure the protective effect of Gardasil for the vaccine recipient her- or himself. Pan-mucosal effects due to HPV vaccination have not yet been measured by the industry-sponsored RCTs. We expect that this study will provide valuable insights as to whether protection may exist for a recipient in preventing infection in a site in which a target type has not yet established infection (since we will not exclude participants who are HPV DNA positive at enrolment).

2.19 Has any pilot study been carried out using this design?

No, to our knowledge this will be the first study to test the potential efficacy of the HPV vaccine in blocking transmission.

3. Trial Management

3.1 What are the arrangements for day to day management of the trial? E.g. Randomization, data handling, and who will be responsible for coordination.

Figure 1 shows the study procedures according to study visit. Assignment of the intervention will be done at the MDCE coordination centre. Randomization was carried out by Dr. Agnihotram Ramanakumar, the previous MDCE biostatistician. Data management, and project coordination will be carried out by Dr. Mariam El-Zein (the current MDCE epidemiologist). She holds a PhD in

Occupational Health from the Department of Epidemiology, Biostatistics and Occupational Health, McGill University. Dr. El-Zein carried out her postdoctoral training at the INRS Institut-Armand Frappier, Laval, Canada. She has extensive work experience in cancer epidemiology and statistical analysis of longitudinal epidemiologic data.

3.2 What will be the role of each principal applicant and co-applicant proposed?

Principal applicant: Dr. Eduardo Franco (MDCE Director) is the PI. He will provide oversight for all aspects of study conduct and report preparation. He will work closely with the Study Director (Dr. El-Zein), support personnel, and with laboratory co-investigators to ensure close adherence to the protocol. Dr. Franco has a successful track record in conducting influential molecular epidemiologic studies of HPV infection and transmission, most notably, the Ludwig-McGill[48], McGill-Concordia cohort studies[28,42,43] and the HITCH study of transmission in couples[29-31]. His team is also experienced in conducting RCTs of cancer screening, having carried out the only North American RCT of HPV versus Pap testing for cervical cancer screening (a CIHR-funded study known by the acronym of CCCaST[49]).

Co-applicants: Dr. Francois Coutlée: Professor of Microbiology at the University of Montreal and adjunct Professor at McGill University. He 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. Drs. Coutlée and Franco have been close research associates in several studies in Montreal since the mid-90's.

Dr. Ann Burchell, Project Director of the HITCH cohort study at McGill and an Adjunct Professor at Dr. Franco's unit. She will provide advice on day-to-day conduct and statistical analyses.

Dr. Agnihotram Ramanakumar (Assistant Professor at Department of Oncology) will be responsible for the statistical data analysis. He has a PhD level training in both qualitative and quantitative research methods and has worked in the field of cancer research over a decade. He has extensive experience in HPV and cervical cancer epidemiology.

Dr. Pierre Tellier (Associate Professor in Family Medicine at McGill University and Director of McGill's Student Health Service) has worked with the PI as the main clinical collaborator in the aforementioned 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.

3.3 Describe the trial steering committee and if relevant the data safety and monitoring committee.

This committee will provide overall supervision of the trial, will ensure that it is being conducted in accordance with the principles of good clinical practice and advise on possible protocol amendments. Its members (letters attached) are: 1) Dr. Alex Ferenczy, Professor of Gynaecologic Pathology at McGill University and one of the world's leading experts on HPV-associated diseases. He brings considerable expertise as previous consultant to industry-sponsored HPV vaccine trials. He has substantial experience on the definition of HPV prevention endpoints and other outcome measures. 2) Dr. Zeev Rosberger, Associate Professor of Psychiatry and Oncology, and Director of the Psychosocial Oncology Program at McGill. He has over ten years of experience with oncology-related RCTs. 3) Ms. Penny Chipman, manager of the clinical research

program at the McGill's Centre for Clinical Research in Oncology. Ms. Chipman has managed hundreds of clinical trials over the last 20 years and is one of the most experienced managers of oncology RCTs in Canada. The above three members contribute substantial expertise and experience on the substantive and methodological aspects of conducting RCTs.

Additional members include the PI (Dr. Franco) and the Study Director (Dr. El-Zein). This committee will meet twice a year to review progress and issues related to protocol adherence, endpoints, and statistical analysis.

***Data Safety and Monitoring Committee*:** This committee will review the accruing trial data and make recommendations regarding safety issues or reasons that may require trial termination. Members of this committee will be independent of the trial. We will seek the McGill REB's recommendation with respect to the possible committee membership. Members will have expertise in statistics, HPV prevention, RCTs, and ethics/law.

Appendix 1. Justifications for previous amendments

Age range for eligible couples was increased from 18-26 to 18-40 years

The National Advisory Committee on Immunization (NACI) recommends the use of Gardasil in females aged 9-45 as well as males aged 9-26 and males who have sex with men (MSM) aged ≥9 years with no age cut-off. Studies have shown the vaccine to be safe and efficacious in older females and males (specifically MSM).[1,2] Although females <25 are at the highest risk of HPV, those older than 25 remain at risk and the risk among males is not associated with age but remains high throughout life.[3] Based on this, increasing the age range for eligible couples is safe and will further improve our potential for recruitment.

References

1- Castellsague X, Munoz N, Pitisuttithum P, et al. End-of-study safety, immunogenicity, and efficacy of quadrivalent HPV (types 6, 11, 16, 18) recombinant vaccine in adult women 24–45 years of age. Br J Cancer. 2011; 105: 28 – 37.

2- Swedish KA, Factor SH, Goldstone SE. Prevention of Recurrent High-Grade Anal Neoplasia With Quadrivalent Human Papillomavirus Vaccination of Men Who Have Sex With Men: A Nonconcurrent Cohort Study. Clin Infect Dis. 2012; 54 (7): 891-898.

3- Moscicki AB, Schiffman M, Burchell A, et al. Updating the Natural History of Human Papillomavirus and Anogenital Cancers. Vaccine. 2012; 30S: F24– F33.

Maximum duration of relationship for eligible couples was increased from 3 to 6 months

Although the likelihood that transmission of HPV has already occurred becomes greater with longer duration of relationship, evidence from a recent couple's study conducted by our division (HITCH study) indicates that new HPV infections are still very common among young couples reporting being involved in a sexual relationship for up to 6 months.

Remuneration of couples was increased from 350$ to 500$ *(if all study visits were to be completed)*

The TRAP-HPV study began enrollment on January 2014 and included, as of January 26, 2015, 10 couples recruited from student populations out of the 500 targeted. In an effort to improve potential recruitment, we proposed the following:

	Enrollment	Follow-up					Total
	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	All visits
Current remuneration (CDN$)							
Per Person	50	25	25	25	25	25	175
Per Couple	100	50	50	50	50	50	350
Amended remuneration (CDN$)							
Per Person	60	30	30	30	40	60	250
Per Couple	120	60	60	60	80	120	500

Incorporating Gardasil 9 as intervention vaccine, July 08, 2015

Gardasil (quadrivalent vaccine) will be replaced with Gardasil 9 (nonavalent vaccine) (hereinafter designated G4 and G9, respectively). The indications are the same as for G4. There are no negative implications in switching from G4 to G9 but there are many advantages for the study and its participants. These are explained below.

1) Improved protection to participants

The benefit here is self-evident. G9 induces comparable immunogenicity to HPVs 6, 11, 16, and 18 and extends protection to HPVs 31, 33, 45, 52, and 58. Currently, participants are receiving G4 and thus they understand that protection from vaccination exists only for HPVs 6, 11, 16, and 18. Female participants will likely appreciate having access to the expanded protection from G9. Conceivably, their male partners may not be as keen because of the lesser importance of the extra 5 HPV types regarding HPV disease in men. However, few men are as technically well informed about the nuances in gender-specific importance of different HPV types in disease causation and, most importantly, they will want for their female partners the best possible benefit that the study can give them.

2) Improved recruitment

A corollary of the above argument is the opportunity to make the study more attractive to participants, which should improve the rate of accrual. A related benefit is that switching to G9 will obviate the serious concern we have that the pool of potential eligible subjects to TRAP-HPV will soon decrease because the G4-vaccinated cohorts of young women are approaching the age of eligibility in TRAP-HPV (18 years). Obviously, a couple in which the woman received HPV vaccination in the past (at school or clinic) is not eligible in our study. Our statistics from screening participants indicate that a prior receipt of HPV vaccination is among the most common reasons for our staff to turn away interested couples. Since G9 has not yet been implemented broadly and it provides expanded protection, we could eliminate the criterion of no prior HPV vaccination because a previous G4- or Cervarix-vaccinated woman would be at risk for the extra 5 types in a new couple (or 7 types, in the case of prior Cervarix receipt). Of course, we will track prior receipt of a first-generation HPV vaccine to guide statistical analysis.

3) Greater study power and better precision for estimates of transmission protection

TRAP-HPV's target sample size of 500 couples was based on the experience of our two previous epidemiologic studies in the same population of university students, including the recent HITCH cohort study, which is co-funded by Merck. When we designed TRAP-HPV we obviously focused our sample size calculations on the 4 types in G4 to study an impact on infection transmission. Not

surprisingly, HPV16 is the most common type in HITCH, both in incidence and prevalence. The joint contribution of the remainder 3 types, HPV6, HPV18, and HPV11, is slightly more than half of the HPV16 prevalence in the couple dyads in HITCH. Ranking the 9 types present in G9 we get: HPV16 (#1), HPV52 (#10), HPV31 (#15), HPV6 (#16), HPV18 (#18), HPV58 (#19), HPV33

(#24), HPV45 (#25), HPV11 (#31), out of 33 individual Alphapapillomavirus (i.e., mucosotropic) types thus far detected via PCR in genital samples from HITCH couples. The combined dyad prevalence (a surrogate of incidence) of Gardasil (G4) types is 35.36%, whereas that of Gardasil 9 (G9) types is 61.22%, which represents a gain of 73.1% in frequency of outcomes that are amenable to a statistical analysis of transmission reduction.

The statistical power underlying the ability of TRAP-HPV to test a hypothesis that HPV vaccination reduces the sexual transmission of different HPV types hinges on examining protection for individual HPV types targeted by the vaccine, not as a combined group of HPV infections. Therefore, the above projections based on the extra number of types in G9 should directly translate into reduced sample size requirements to study the impact of G9 (versus that of G4) in transmission reduction.

Moreover, TRAP-HPV examines not only genital transmission but also anal and oral, which will permit additional anatomical sites to evaluate transmission reduction by HPV vaccination, thus further enhancing the incremental gain in power that should come from switching from G4 to G9.

4) Enhanced scientific value to HPV epidemiology and vaccinology

TRAP-HPV's primary goal is in assessing the impact of vaccination on transmission reduction. However, switching from G4 to G9 is also beneficial in the sense of providing a separate investigator-initiated and managed study of G9 efficacy to those receiving vaccination. TRAP-HPV will (i) add to the knowledge base of G9's efficacy in preventing virological outcomes, and (ii) have the extra objective of assessing protection to different mucosal sites (anus and mouth).

5) Better ethical circumstances to scientific regulators

Ethical review of a protocol amendment asking for a switch from G4 to G9 should not pose a challenge. G9 is already approved by Health Canada, which transferred the entire set of G4 indications to G9. REB members will likely appreciate the extra protection to participants and be satisfied with the proven safety and tolerability of G9. REB members will also be sensitive to the fact that a G4-based TRAP-HPV study has serious recruitment challenges and that the switch to G9 will improve its potential for making a relevant public health contribution.

The only possible item which REB members may raise concerns is with the concrete possibility that TRAP-HPV will administer G9 to women (and some men) who may have already received G4 or Cervarix. This is certainly possible and desirable as part of my aforementioned reasoning to improve accrual by extending the study to women that already received a first-generation HPV vaccine. We will pre-empt this concern by providing data from Merck's filing with regulators about safety of giving G9 to prior G4 recipients.

6) Contingencies and preparedness

As a pragmatic point, TRAP-HPV has so far successfully recruited and retained only 17 couples (34 individuals). This is a small fraction of the targeted sample size of 500 couples. Therefore, the

requested protocol amendment cannot be considered a midstream change; we are early enough in the study that a change is possible and of obvious clinical and scientific relevance.

Switching to G9 will allow us to recruit former G4 recipients. Thus, the previous eligibility criterion (*Volunteer couples must not have been vaccinated against HPV, both partners*) will no longer hold true. Should G4 recipients be randomized to receive a placebo vaccine, it is likely that transmission events will be reduced in couples that include such individuals. However, this potential bias will be assessed in isolation because we collect enrolment blood samples in TRAP-HPV. We will perform serological assays in these samples to measure baseline titres against all 4 types in G4 and thus validate prior G4 (or Cervarix) receipt in participants and use this information to stratify the statistical analyses accordingly.

Age range for eligible couples was increased from 18-40 to 18-45 years

The National Advisory Committee on Immunization (NACI) recommends the use of Gardasil in females aged 9-45. Studies have shown the vaccine to be safe and efficacious in older females and males.[1] Although females <25 are at the highest risk of HPV, those older than 25 remain at risk and the risk among males is not associated with age but remains high throughout life.[2] Based on this, increasing the age range for eligible couples is safe and will further improve our potential for recruitment.

References

1- Castellsague X, Munoz N, Pitisuttithum P, et al. End-of-study safety, immunogenicity, and efficacy of quadrivalent HPV (types 6, 11, 16, 18) recombinant vaccine in adult women 24–45 years of age. Br J Cancer. 2011; 105: 28 – 37.

2- Moscicki AB, Schiffman M, Burchell A, et al. Updating the Natural History of Human Papillomavirus and Anogenital Cancers. Vaccine. 2012; 30S: F24– F33.

Discontinuing anal sampling

In light of the troubling recruitment rates, we propose a minor modification to increase the rate of recruitment in the TRAP-HPV study by discontinuing anal sampling. Our recruiters frequently refer to conversations with candidate participants in which they disclose feelings of embarrassment, pain or discomfort in undergoing anal sampling, as deterrents to their participation in TRAP-HPV. We are confident that if we drop this procedure, our recruitment will improve.

Adding a recruitment site in Rutgers, New York

We began enrollment on January 2014, and so far only 63 couples were recruited from student populations out of the 500 targeted. To increase the rate of recruitment, we propose recruitment of subjects in the TRAP-HPV study via Rutgers University affiliated health clinics. We will collaborate with Dr. Mark Einstein (Professor and Chair in the Department of Obstetrics, Gynecology and Women's Health, Rutgers New Jersey Medical School) and Dr. Nicolas Schlecht (Associate Professor in the Department of Epidemiology & Population Health, Albert Einstein College of Medicine in New York). Their research team will follow the original protocol as filed

with McGill's IRB. Drs. Einstein and Schlecht will lead recruitment of subjects in the TRAP-HPV study via Rutgers University affiliated health clinics.

Remuneration of couples was increased from 500$ to 1000$ *(if all study visits were to be completed)*

The TRAP-HPV study began enrollment on January 2014, and includes so far 66 couples recruited from student populations out of the 500 targeted. In an effort to improve potential recruitment, remuneration of couples will be doubled from 500$ to 1000$, if all study visits were to be completed.

We propose the following:

	Enrollment	Follow-up					Total
	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	All visits
Current remuneration (CDN$)							
Per Person	60	30	30	30	40	60	250
Per Couple	120	60	60	60	80	120	500
Amended remuneration (CDN$)							
Per Person	120	60	60	60	80	120	500
Per Couple	240	120	120	120	160	240	1000

Switch from Havrix to Vaqta

The placebo vaccine currently used is Havrix, a hepatitis A vaccine that we have been purchasing from GSK. Due to the increased cost of Havrix (~46$), we are considering a switch to Avaxim by Sanofi Pasteur (~26$), also a hepatitis A vaccine that is currently used at the McGill Student Health Services Clinic, as confirmed by our clinical collaborator Dr. Pierre-Paul Tellier (Former Director of Student Health at McGill). Avaxim will be administered to participants using the same blinded and concealed regimen as described in the original protocol. Avaxim and Havrix have been in long-term use in Canada.

Close recruitment early

Recruitment in the TRAP-HPV study has been a challenge, and more so since the start of the COVID-19 pandemic. We had to cease study-related activities (mostly occur among student populations) from March 13 to June 2020 under the COVID-19 lockdown measures announced by the Quebec Government and by extension the McGill Research and Graduate Studies Offices at the Faculty of Medicine and Health Sciences. We resumed research activities on July 28, 2020, but most student populations continued online learning. Study recruitment continued to be a challenge; only 19 new couples were recruited over the last two years.
In order to maintain the scientific value of the TRAP-HPV study as well as allocate the remaining study funds to test the collected biological samples and pay the remuneration for all pending study visits, we are:

1- Closing recruitment as we will never be able to reach the target sample within a reasonable timeframe. The last couple was recruited on February 16, 2022.
2- Completing the follow-up of existing couples. However, we are proposing to eliminate Visit 6 and stop follow-up at Visit 5 (one assessment point post-vaccination with the third dose of the vaccine). By design, six study visits are scheduled over a one-year period (at enrollment [month 0], and at 2, 4, 6, 9, and 12 months) and the vaccines are administered at three visits (0, 2, and 6 months). We anticipate by this to complete the study by

November 2022 (rather than February 2023 if we were to keep Visit 6). However, because of the usual delays for students to adhere with the expected date of appointments keeping Visit 6 will actually impose an inordinately long study duration.

For couples who will not have a visit 6, compensation will be modified to $100/person ($200/couple) instead of $80/person ($160/couple) for Visit 5.

Appendix 2. Justification for current amendment

Use a different assay for serology testing

The approved protocol indicated that the serology testing (used to measure the antibody response HPV VLPs types 6, 11, 16, 18) would be done via a competitive Luminex assay (cLIA) that uses multiplex technology to detect type-specific neutralizing antibodies against four HPV types in a single serum sample.

We will now use a more appropriate assay to test the baseline serum samples prior to vaccination for neutralizing anti-HPV seroreactivity for the vaccine-targeted types (Gardasil 9). This will be done using the Pseudovirion-Based Neutralization Assay (PBNA; 9v) which will enable us to detect type-specific neutralizing antibodies against nine HPV types.

REFERENCES

1. Garland SM, Hernandez-Avila M, Wheeler CM, et al. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. N Engl J Med 2007;356:1928-43.
2. Harper DM, Franco EL, Wheeler C, et al. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. Lancet 2004;364:1757-65.
3. Monk BJ, Tewari KS. The spectrum and clinical sequelae of human papillomavirus infection. Gynecol Oncol 2007;107:S6-13.
4. Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003;348:518-27.
5. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. Lancet 2007;370:890-907.
6. Brisson M, Van de Velde N, De Wals P, Boily MC. The potential cost-effectiveness of prophylactic human papillomavirus vaccines in Canada. Vaccine 2007;25:5399-408.
7. Goldie SJ, Kohli M, Grima D, et al. Projected clinical benefits and cost-effectiveness of a human papillomavirus 16/18 vaccine. J Natl Cancer Inst 2004;96:604-15.
8. Hughes JP, Garnett GP, Koutsky L. The theoretical population-level impact of a prophylactic human papilloma virus vaccine. Epidemiology 2002;13:631-9.
9. Kim JJ, Goldie SJ. Health and economic implications of HPV vaccination in the United States. N Engl J Med 2008;359:821-32.
10. Elbasha EH, Dasbach EJ, Insinga RP. Model for assessing human papillomavirus vaccination strategies. Emerg Infect Dis 2007;13:28-41.
11. Taira AV, Neukermans CP, Sanders GD. Evaluating human papillomavirus vaccination programs. Emerg Infect Dis 2004;10:1915-23.
12. Brisson M, van de Velde N, Franco EL, Drolet M, Boily MC. Incremental impact of adding boys to current human papillomavirus vaccination programs: role of herd immunity. J Infect Dis 2011;204:372-6.
13. Ferko N, Postma M, Gallivan S, Kruzikas D, Drummond M. Evolution of the health economics of cervical cancer vaccination. Vaccine 2008;26 Suppl 5:F3-15.
14. Giuliano AR, Palefsky JM, Goldstone S, et al. Efficacy of quadrivalent HPV vaccine against HPV Infection and disease in males. N Engl J Med 2011;364:401-11.
15. Dale CJ, Liu XS, De Rose R, et al. Chimeric human papilloma virus-simian/human immunodeficiency virus virus-like-particle vaccines: immunogenicity and protective efficacy in macaques. Virology 2002;301:176-87.
16. Epstein RJ. Primary prevention of human papillomavirus-dependent neoplasia: no condom, no sex. Eur J Cancer 2005;41:2595-600.
17. Human Papillomavirus (HPV) Prevention and HPV Vaccines: Who should get the vaccine? 2011. (Accessed 15 Feb, 2012, at http://www.phac-aspc.gc.ca/std-mts/hpv-vph/hpv-vph-vaccine-eng.php#a3.)
18. Reports of health concerns following HPV vaccination. 2012. (Accessed 8 Feb, 2012, at http://www.cdc.gov/vaccinesafety/vaccines/hpv/gardasil.html.)
19. Slade BA, Leidel L, Vellozzi C, et al. Postlicensure safety surveillance for quadrivalent human papillomavirus recombinant vaccine. JAMA 2009;302:750-7.
20. Gee J, Naleway A, Shui I, et al. Monitoring the safety of quadrivalent human papillomavirus vaccine: findings from the Vaccine Safety Datalink. Vaccine 2011;29:8279-84.

21. Graham J, Liu Y, Jeziorski J. The Dyadic Adjustment Scale: A Reliability Generalization Meta-Analysis. Journal of Marriage and Family 2006;68:701 - 17.
22. Baillargeon J, Dubois G, Marineau R. Traduction francaise de l'Echelle d'ajustement dyadique. Canadian Journal of Behavioural Science 1986;18.
23. Spanier GB. Measuring dyadic adjust- ment: New scales for assessing the quality of marriage and similar dyads. Journal of Marriage and the Family 1976;38:15-28.
24. Opalka D, Lachman CE, MacMullen SA, et al. Simultaneous quantitation of antibodies to neutralizing epitopes on virus-like particles for human papillomavirus types 6, 11, 16, and 18 by a multiplexed luminex assay. Clin Diagn Lab Immunol 2003;10:108-15.
25. Dias D, Van Doren J, Schlottmann S, et al. Optimization and validation of a multiplexed luminex assay to quantify antibodies to neutralizing epitopes on human papillomaviruses 6, 11, 16, and 18. Clin Diagn Lab Immunol 2005;12:959-69.
26. Baldwin SB, Wallace DR, Papenfuss MR, et al. Human papillomavirus infection in men attending a sexually transmitted disease clinic. J Infect Dis 2003;187:1064-70.
27. Coutlee F, Rouleau D, Petignat P, et al. Enhanced detection and typing of human papillomavirus (HPV) DNA in anogenital samples with PGMY primers and the Linear array HPV genotyping test. Journal of clinical microbiology 2006;44:1998-2006.
28. Richardson H, Kelsall G, Tellier P, et al. The natural history of type-specific human papillomavirus infections in female university students. Cancer Epidemiol Biomarkers Prev 2003;12:485-90.
29. Burchell AN, Tellier PP, Hanley J, Coutlee F, Franco EL. Influence of partner's infection status on prevalent human papillomavirus among persons with a new sex partner. Sexually transmitted diseases 2010;37:34-40.
30. Burchell AN, Tellier PP, Hanley J, Coutlee F, Franco EL. Human papillomavirus infections among couples in new sexual relationships. Epidemiology 2010;21:31-7.
31. Burchell AN, Coutlee F, Tellier PP, Hanley J, Franco EL. Genital transmission of human papillomavirus in recently formed heterosexual couples. J Infect Dis 2011;204:1723-9.
32. Macaluso M, Lawson ML, Hortin G, et al. Efficacy of the female condom as a barrier to semen during intercourse. Am J Epidemiol 2003;157:289-97.
33. Wright TC, Jr., Denny L, Kuhn L, Pollack A, Lorincz A. HPV DNA testing of self-collected vaginal samples compared with cytologic screening to detect cervical cancer. JAMA 2000;283:81-6.
34. Sellors JW, Karwalajtys TL, Kaczorowski J, et al. Incidence, clearance and predictors of human papillomavirus infection in women. CMAJ 2003;168:421-5.
35. Gravitt PE, Lacey JV, Jr., Brinton LA, et al. Evaluation of self-collected cervicovaginal cell samples for human papillomavirus testing by polymerase chain reaction. Cancer Epidemiol Biomarkers Prev 2001;10:95-100.
36. Bleeker MC, Hogewoning CJ, Van Den Brule AJ, et al. Penile lesions and human papillomavirus in male sexual partners of women with cervical intraepithelial neoplasia. J Am Acad Dermatol 2002;47:351-7.
37. Lampinen TM, Miller ML, Chan K, et al. Randomized clinical evaluation of self-screening for anal cancer precursors in men who have sex with men. Cytojournal 2006;3:4.
38. Lampinen TM, Latulippe L, van Niekerk D, et al. Illustrated instructions for self-collection of anorectal swab specimens and their adequacy for cytological examination. Sex Transm Dis 2006;33:386-8.
39. Bernstein D, Lagakos S. Sample size and power determination for stratified clinical trials. The Journal of Statistical Computation and Simulation 1978;8:65-73.

40. Kreimer AR, Rodriguez AC, Hildesheim A, et al. Proof-of-principle evaluation of the efficacy of fewer than three doses of a bivalent HPV16/18 vaccine. J Natl Cancer Inst 2011;103:1444-51.
41. Burchell AN, Richardson H, Mahmud SM, et al. Modeling the sexual transmissibility of human papillomavirus infection using stochastic computer simulation and empirical data from a cohort study of young women in Montreal, Canada. Am J Epidemiol 2006;163:534-43.
42. Richardson H, Abrahamowicz M, Tellier PP, et al. Modifiable risk factors associated with clearance of type-specific cervical human papillomavirus infections in a cohort of university students. Cancer Epidemiol Biomarkers Prev 2005;14:1149-56.
43. Richardson H, Franco E, Pintos J, Bergeron J, Arella M, Tellier P. Determinants of low-risk and high-risk cervical human papillomavirus infections in Montreal University students. Sex Transm Dis 2000;27:79-86.
44. Liang K, Zeger S. Longitudinal data analysis using generalized linear models. Biometrika 1986;73:13-22.
45. Xue X, Gange SJ, Zhong Y, et al. Marginal and mixed-effects models in the analysis of human papillomavirus natural history data. Cancer Epidemiol Biomarkers Prev 2010;19:159-69.
46. Lan KK, Rosenberger WF, Lachin JM. Use of spending functions for occasional or continuous monitoring of data in clinical trials. Statistics in medicine 1993;12:2219-31.
47. O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. Biometrics 1979;35:549-56.
48. Schlecht NF, Kulaga S, Robitaille J, et al. Persistent human papillomavirus infection as a predictor of cervical intraepithelial neoplasia. JAMA 2001;286:3106-14.
49. Mayrand MH, Duarte-Franco E, Rodrigues I, et al. Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer. N Engl J Med 2007;357:1579-88.