

# BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email [info.bmjopen@bmj.com](mailto:info.bmjopen@bmj.com)

# BMJ Open

## Kinetics and ecology of Human Papillomavirus (HPV) genital infections in young women: the PAPCLEAR study

|                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|-------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Journal:                      | <i>BMJ Open</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Manuscript ID                 | bmjopen-2018-025129                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| Article Type:                 | Protocol                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| Date Submitted by the Author: | 06-Jul-2018                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| Complete List of Authors:     | <p>Murall, Carmen Lia; Centre National de la Recherche Scientifique (CNRS), MIVEGEC<br/> Rahmoun, Massilva; Centre National de la Recherche Scientifique (CNRS), MIVEGEC<br/> Selinger, Christian; Centre National de la Recherche Scientifique (CNRS), MIVEGEC<br/> Bernat, Claire; Centre National de la Recherche Scientifique (CNRS), MIVEGEC<br/> Buisson, Mathilde; Centre Hospitalier Regional Universitaire de Montpellier, Direction Recherche Innovation<br/> Christophe, Guillaume; Centre Hospitalier Regional Universitaire de Montpellier, CeGIDD<br/> D'Auria, Giuseppe; Fundacio per al Foment de la Investigacio Sanitaria i Biomedica, Sequencing and Bioinformatics Service; Centro de Investigacion Biomedica en Red de Epidemiologia y Salud Publica De Taroni, Florence; Centre Hospitalier Regional Universitaire de Montpellier, CeGIDD<br/> Froissart, Rémy; Centre National de la Recherche Scientifique (CNRS), MIVEGEC<br/> Hirtz, Christophe; Centre Hospitalier Regional Universitaire de Montpellier, LBPC/PPC, IRMB<br/> Jausent, Audrey; Centre Hospitalier Regional Universitaire de Montpellier, Département de l'Information Médicale<br/> Lajoie, Julie; University of Manitoba College of Medicine, Department of Medical microbiology<br/> Lorcy, Frédérique; Centre Hospitalier Regional Universitaire de Montpellier, Laboratoire d'anatomie et cytologie pathologiques<br/> Picot, Eric; Centre Hospitalier Regional Universitaire de Montpellier, CeGIDD<br/> PICOT, Marie-Christine; Centre Hospitalier Regional Universitaire de Montpellier, Département d'Information Médicale<br/> Ravel, Jacques; University of Maryland School of Medicine, Institute for Genome Sciences<br/> Reynes, Jacques; Centre Hospitalier Regional Universitaire de Montpellier, Département des Maladies Infectieuses et Tropicales<br/> Rousset, Thérèse; Centre Hospitalier Regional Universitaire de Montpellier, Laboratoire d'anatomie et cytologie pathologiques<br/> Seddiqi, Aziza; Centre Hospitalier Regional Universitaire de Montpellier, Direction Recherche Innovation<br/> Teirlinck, Martine; Centre Hospitalier Regional Universitaire de Montpellier, CeGIDD</p> |

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

|           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|-----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|           | <p>Tribout, Vincent; Centre Hospitalier Regional Universitaire de Montpellier, CeGIDD</p> <p>Tuaille, Edouard; Centre Hospitalier Regional Universitaire de Montpellier, Pathogenesis and Control of Chronic Infections</p> <p>Waterboer, Tim; Deutsches Krebsforschungszentrum, Infections and Cancer Epidemiology</p> <p>Jacobs, Nathalie; Universite de Liege Faculte des Sciences, GIGA-Research, Cellular and molecular immunology</p> <p>Bravo, Ignacio; Centre National de la Recherche Scientifique (CNRS), MIVEGEC</p> <p>Segondy, Michel; Centre Hospitalier Regional Universitaire de Montpellier, Pathogenesis and Control of Chronic Infections</p> <p>Boule, Nathalie; Centre Hospitalier Regional Universitaire de Montpellier, Pathogenesis and Control of Chronic Infections</p> <p>Alizon, Samuel; Centre National de la Recherche Scientifique (CNRS), MIVEGEC</p> |
| Keywords: | VIROLOGY, IMMUNOLOGY, Epidemiology < INFECTIOUS DISEASES                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |



## STUDY PROTOCOL

# Kinetics and ecology of Human Papillomavirus (HPV) genital infections in young women: the PAPCLEAR study

Carmen Lía Murall<sup>1</sup>, Massilva Rahmoun<sup>1</sup>, Christian Selinger<sup>1</sup>, Claire Bernat<sup>1</sup>, Mathilde Buisson<sup>2</sup>, Guillaume Christophe<sup>3</sup>, Giuseppe D'Auria<sup>4,13</sup>, Florence De Taroni<sup>3</sup>, Rémy Froissart<sup>1</sup>, Christophe Hirtz<sup>5</sup>, Audrey Jaussent<sup>6</sup>, Julie Lajoie<sup>7</sup>, Frédérique Lorcy<sup>14</sup>, Eric Picot<sup>3</sup>, Marie-Christine Picot<sup>6</sup>, Jacques Ravel<sup>9</sup>, Jacques Reynes<sup>10</sup>, Thérèse Rousset<sup>14</sup>, Azziza Seddiki<sup>2</sup>, Martine Teirlinck<sup>3</sup>, Vincent Tribout<sup>3</sup>, Édouard Tuaille<sup>8</sup>, Tim Waterboer<sup>11</sup>, Nathalie Jacobs<sup>12</sup>, Ignacio G Bravo<sup>1</sup>, Michel Segondy<sup>8</sup>, Nathalie Boule<sup>8</sup>, Samuel Alizon<sup>1\*</sup>

Word count: 4860 words, excluding title page, abstract, references, figures and tables.

\* Corresponding author: [samuel.alizon@cnr.fr](mailto:samuel.alizon@cnr.fr), phone: +33 6 10 65 49 02

1 Laboratoire MIVEGEC (UMR 5290 CNRS, IRD, UM), 911, avenue Agropolis, BP 64501, 34394 Montpellier, France

2 Direction Recherche Innovation, Centre Hospitalier Universitaire de Montpellier, Montpellier, France

3 CeGIDD, Centre Hospitalier Universitaire de Montpellier, Montpellier, France

4 Sequencing and Bioinformatics Service, Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunidad Valenciana (FISABIO-Salud Pública), 46020 Valencia, Spain

5 University of Montpellier, LBPC/PPC- IRMB, CHU de Montpellier, 80 rue Augustin Fliche, Montpellier, France

6 Département de l'Information Médicale, Centre Hospitalier Universitaire de Montpellier, Montpellier, France

7 Department of Medical microbiology, University of Manitoba, 745 Bannatyne, Winnipeg, Canada

8 Pathogenesis and Control of Chronic Infections, INSERM, CHU, University of Montpellier, Montpellier, France

9 Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, USA

10 Département des Maladies Infectieuses et Tropicales, Centre Hospitalier Universitaire de Montpellier, Montpellier, France

11 German Cancer Research Center (DKFZ), Infections and Cancer Epidemiology, Im Neuenheimer Feld 280, Heidelberg, Germany

12 GIGA-Research, Cellular and molecular immunology, University of Liège, 3 Avenue de l'Hôpital, 4000 Liège, Belgium

13 CIBER en Epidemiología y Salud Pública (CIBEResp), Madrid, Spain

14 Laboratoire d'anatomie et cytologie pathologiques, Hôpital Gui de Chauliac, Centre Hospitalier Universitaire de Montpellier, 80 avenue Augustin Fliche, 34295 Montpellier, France

For peer review only

## ABSTRACT

### Introduction

Human papillomaviruses (HPVs) are responsible for one third of infection-induced cancers. However, most HPV studies focus on chronic infections and cancers, and we know little about the early stages of the viral infection. In particular, the roles of the immune system, the microbiota, the virus genetics and of the host genetics on infection clearance or persistence remains poorly understood.

### Methods and Analysis

We follow 150 women aged 18-25 longitudinally to monitor immune response features (cytokines and immune cells in the genital tract, circulating anti-HPV antibodies), HPV virus load and vaginal microbiota composition. This is complemented by virus and human genetics and behavioural data. To increase the statistical power for the epidemiological framework, an additional 150 women are screened cross-sectionally.

### Ethics and Dissemination

This study will provide us with one of the most detailed follow-up studies of acute HPV infections and their interactions with the host and the vaginal microbiota. It will also allow us to investigate related issues regarding HPV intra-host evolution and diversity, vaginal microbiota dynamics and sexually transmitted infections. The trial has been registered to ClinicalTrials.gov on 27 Oct 2016 with ID number NCT02946346.

## ARTICLE SUMMARY

### STRENGTHS AND LIMITATIONS OF THIS STUDY

- We set up a longitudinal study to investigate the natural history of HPV genital infections in N=150 young women.
- The follow-up is dense (visit every 2 month for infected women) and long (up to 24 months).
- At each visit, the goal is to estimate virus load, cytokine densities, immune cell counts in the cervical area and vaginal microbiota composition.
- Clinical data will be combined to population dynamics models to perform parameter estimation and model comparison.
- The longitudinal study is combined with a cross-sectional study of N=150 women to allow for epidemiological analyses.

Keywords: HPV; acute infection; persistence; virus load; immunity; microbiota; population dynamics

## INTRODUCTION

### Epidemiology of HPV genital infections in young adults and public health implications

Infections by Human Papillomaviruses (HPVs) are probably the most common sexually transmitted infections (STI) globally. It is often estimated that  $\approx 20\%$  of men and women in European countries are currently infected by HPVs and that, worldwide, 75% of the individuals will be infected at some point in their life by HPV [1]. In France, a recent study performed in the Paris area estimated prevalences of genital HPV of  $\approx 16\%$  in women, and  $\approx 25\%$  in women below 25 years of age [2]. In the area of Montpellier, prevalence of oncogenic HPVs (often referred to as 'high-risk', HR) in pregnant women aged 16 to 42 was close to 20% [3].

Fortunately, the vast majority of HPVs infections are asymptomatic and benign. Even for HPV16, probably the most oncogenic biologic agent to humans, only a minority (less than 10%) of infections becomes persistent [4], and again a minority of these (12%) will progress into cancer if untreated [1, 5]. Indeed, it is estimated that approximately 70 to 100% of HPV infections are cleared within 12 to 24 months, even for the most oncogenic HPVs (such as HPV16 and 18) that are responsible for the majority of HPV-induced cancers [1, 4, 6, 7]. Yet, we currently know little about the biology of these very prevalent non-persisting infections [8].

Our lack of knowledge partly comes from the fact that hitherto studies interested in persistent infections follow participants every six months for several years (e.g. a median 50.4 months in [9]). This is indeed sufficient to assess the time to clearance (or to persistence) but it is clearly not precise enough to understand the kinetics of infections that last on average between six to 24 months. After 24 months of infection, an infection is often considered as being persistent [10].

We know some factors that correlate with persistence (e.g. immunosuppression, smoking, and co-infection with other STIs [11]) but we do not know how these play out in the within-host dynamics of infections. Also, there are hypothesized changes in viral-immunity interactions that appear related to persistence and disease progression [12–15] but, again, we do not know the



1 underlying interactions between the viruses, the host target cells and the immune response in  
2 acute infections [8]. Finally, it has been argued that the vaginal microbiota may differ between  
3 HPV-infected and HPV-uninfected women [16] and that specific microbiota composition may  
4 interact with HPV detection [17]. However, it is difficult to disentangle the cause and the  
5 consequence. For instance, does the microbiota composition change after the establishment of  
6 an HPV infection, or do certain microbiota compositions increase susceptibility to HPV  
7 infection?  
8  
9  
10  
11  
12  
13  
14

15 A better understanding of the kinetics of HPV infections and of the determinants of clearance  
16 and persistence of viral infection is particularly important in the context of vaccination [18–21].  
17 Indeed, the long-term efficacy of the vaccine at the population level will largely depend on the  
18 virus within-host dynamics. Furthermore, a better understanding of acute HPV infections can  
19 shed a new light on issues related to latency, fertility, or immunotherapies [8].  
20  
21  
22  
23  
24  
25  
26

## 27 **Prevention strategies and treatment**

### 30 Treatment

31  
32 Since the majority of HPV infections are benign in young adults and clear within six to 24  
33 months, the current standard of care is to avoid over-treatment [22]. Clinical interventions  
34 (colposcopies, biopsies and treatment) are performed less in young women (< 25) and only for  
35 high-grade (pre-cancerous) lesions (cervical intraepithelial neoplasia grade 2, CIN-2, or more).  
36 Low-grade lesions (CIN-1) are not systematically treated but rather followed (approximately  
37 every twelve months) to detect any progression to high-grade lesions.  
38  
39  
40  
41  
42  
43  
44  
45

46 Genital warts caused by non-oncogenic HPVs (often referred to as 'low-risk' (LR) HPVs) can be  
47 removed by surgery or treated with bi- and trichloroacetic acid, cryotherapy or other treatments  
48  
49  
50  
51 [23].  
52  
53

### 54 HPV vaccination

55  
56  
57 There are currently three licensed vaccines: a bivalent vaccine (Cervarix<sup>®</sup>) targeting HPV16  
58 and HPV18 (the most oncogenic and the most prevalent HPVs), a quadrivalent vaccine  
59  
60

(Gardasil<sup>□</sup>) that additionally targets HPV6 and HPV11 (non-oncogenic, but highly prevalent and associated to benign proliferative lesions) and, since 2014, a nonavalent vaccine (Gardasil 9<sup>□</sup>) that targets five more oncogenic types, HPV31, HPV33, HPV45, HPV52, and HPV58. These vaccines succeed in eliciting a protective immune response against new infections by the targeted viruses, and are used throughout the world with wide variation in coverage (for reviews, see e.g. [24, 25]).

In France, vaccination started in 2006 but with limited coverage: it was 28.5% in 2008 [26] and has been decreasing since then [27]. The vaccine is recommended for girls from 11 to 14 (current vaccination scheme is two doses with a six months interval), and with a catch-up for girls aged 15-19 (three doses). It is reimbursed by the social security but not mandatory. It is also recommended for men who have sex with men (MSM) and for immunocompromised people [27]. Vaccination is now the primary prevention strategy against cervical cancers.

### Screening

In France, the secondary prevention strategy against cervical cancer is routine individual cytology-based screening for pre-cancerous and cancerous cervical lesions in women between 25 and 65 years. Cytologies can also be performed in younger women if they report risk factors for cervical cancer (multiple partners, chronic STIs or HIV status [27]). Detection of oncogenic HPVs is proposed for triage in case of abnormal cytology (i.e. ASCUS, or Atypical Squamous Cells of Undetermined Significance).

### Primary objectives

The first objective is to decipher the kinetics and ecology of HPVs cervical infections, i.e. the population dynamics of the virus, target epithelial cells and immune effectors in healthy young women.

The second objective is to determine the prevalence of genital HPVs in young women in the region of Montpellier, in relationship with lifestyle, vaginal microbiota and human genetics.

## Secondary objectives

1  
2 A secondary objective is to characterize the acquisition and clearance dynamics of cervical  
3  
4 HPVs infections as a function of viral diversity, host immunity, vaginal microbiota and human  
5  
6 genetics.  
7

8  
9 Finally, another secondary objective is to investigate the genetic diversity of HPVs during  
10  
11 cervical infections.  
12

## 13 14 15 16 17 18 **METHODS AND ANALYSIS**

### 19 20 21 **Participants**

22  
23 The study population is young women aged 18 to 25 at risk of HPV infection. The estimated  
24  
25 prevalence in this age class is approximately 25% [2], and it decreases to 15% in women older  
26  
27 than 25.  
28

29  
30  
31 The composition of the population visiting the Montpellier STI detection centre (CeGIDD) has  
32  
33 already been documented in an earlier study [28]. In total, the centre is visited by approximately  
34  
35 3,000 women per year, who tend to be less than 25 years old (80%). Approximately 40% of the  
36  
37 attendants report three or more partners over the last twelve months and approximately 50%  
38  
39 report using adopting adequate behaviour for prevention against HIV. Overall, in terms of STI  
40  
41 exposure, the centre is equally visited by people with high-risk and low-risk behaviours.  
42  
43  
44

### 45 46 **Inclusion criteria**

47  
48 Participants are women aged 18 to 25. They must be sexually active with at least one new  
49  
50 partner over the last 12 months. This criteria is fixed to maximise the incidence of new HPVs  
51  
52 infections. As in any clinical study, participants must be able to and willing to give written  
53  
54 informed consent: they must sign and informed consent, understand the requirements for the  
55  
56 study and be affiliated to a social security scheme.  
57  
58  
59

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Women cannot be included in the study if they have a history of cervical pathology (genital warts or cervical lesions), if they are pregnant or intending to become pregnant in the coming year, infected by HIV, undergoing (or planning to undergo) heavy treatment (biotherapy, chemotherapy, immunosuppression), planning on moving outside the Montpellier metropole within the next 18 months, in a dependency or employment with the sponsor or the investigator, if they participated in a clinical trial involving administration of drugs within the last four weeks or if they belong to a vulnerable group (guardianship).

## Design/setting

Our study has a longitudinal component aimed at deciphering within-host kinetics and a cross-sectional component, aimed at understanding the epidemiology of HPVs infections in young adults in the area of Montpellier, France. The general structure of the study is shown in Figure [1](#).

If participants fit the inclusion criteria, they go through an inclusion visit ( $V_1$ ) with a gynaecologist, at the CeGIDD. During this visit, they fill out health and lifestyle questionnaires and undergo a medical consultation during which a number of samples are collected.

Participants are given self-sample swabs for collecting microbiome samples until the next visit and are instructed on how to fill out weekly questionnaires through an online form (these are performed throughout the study).

An appointment is scheduled four weeks later for the results visit ( $V_2$ ), where the cervical cytology results are communicated. We collect some samples and provide more self-sample swabs for home collection.

The next return visits ( $V_i$ , where  $i > 2$ ) are as follows:

- Participants infected by an *Alphapapillomavirus* at  $V_1$  join the HPV positive (HPV+) arm of the study with return visits scheduled every 2 months.
- Participants who were not infected by an *Alphapapillomavirus* at  $V_1$  join the HPV negative (HPV-) arm with return visits scheduled every 4 months.

- HPV- participants who become infected by HPV move to the HPV+ arm.

Participants in the HPV- arm will be followed until month 26 of the study.

Participants in the HPV+ arm will be followed until they clear the infection or until they have been infected for 24 months (after which we consider that the infection is persistent). Clearance is defined as being negative two visits in a row for the first HPV type detected in the follow-up.

In between these visits to the CeGIDD, participants are asked to perform regular (every week for HPV+ and every second week for HPV-) self-samples using vaginal swabs, along with a measure of vaginal pH and filling a short questionnaire.

### **Patients and public involvement**

Participants are all healthy and are therefore referred to as such rather than patients. They will be mainly recruited amongst the people visiting the CeGIDD via poster, leaflet and direct contact with the Clinical Research Technician (TRC) or the clinicians. To maximise inclusion, recruitment will also target students from the various Universities in Montpellier. Finally, a social media page will be set up.

Participants did not play a role in the design of this study.

Results of the study will be disseminated to study participants via email.

### **Visits**

Inclusion visit (V1)

This visit takes place at the CeGIDD and is scheduled by the Clinical Research Technician (TEC) via phone or email.

Women meet a physician investigator, who explains the study goal and requirements. The

1 physician also checks that the inclusion criteria are met. If so, after a general discussion, the  
2 informed consent forms are signed.  
3

4 The physician first performs a general exam, before performing a gynaecological exam during  
5 which the following samples will be taken:  
6  
7

- 8
- 9
- 10 • vaginal pH cotton swab (EcoCare™)
- 11
- 12 • vaginal swab (Copan ESwab™) in 1mL Amies liquid for DNA extraction (microbiota  
13 analysis)
- 14
- 15 • vaginal swab (Copan ESwab™) in 1mL of RNA preservation medium
- 16
- 17 • ophthalmic sponge (Weck-cel<sup>□</sup>) to collect cervical secretions (cytokine analysis)
- 18
- 19 • cervical smear in 20mL of Thinprep<sup>□</sup> Preservcyt<sup>□</sup> fixation liquid (HPV and HSV assays,  
20 and cytology evaluation)  
21  
22  
23  
24  
25  
26

27 Following the gynaecological consult, the participant meets with a nurse to measure body  
28 temperature, blood pressure and draw 20mL of blood: a 5mL tube for SNPs sequencing, a  
29 10mL tube for immunophenotyping and a 5mL tube for HPV antibody titration. For the  
30 longitudinal study, the nurse provides the participant with 3 self-sampling kits, 3 pH strips, a  
31 freezer box to bring back to the next visit, and instructions on how to perform the home  
32 sampling.  
33  
34  
35  
36  
37  
38  
39

40  
41 If the participant has not been tested for a STI in the last 3 months, the nurse will draw an  
42 additional blood tube of 5mL to test for STIs (HIV, HCV, HBV) and ask for self-samples for  
43 chlamydiae and gonorrhoea detection. Syphilis testing was also prescribed based on the STI  
44 clinic's standards.  
45  
46  
47  
48

49  
50 Finally, the participant meets with the TEC to fill in questionnaires #1 (inclusion visit) and #3  
51 (home). The TEC answers any remaining questions, explains how to fill the home  
52 questionnaires (#3) and sets an appointment for the results visit.  
53  
54  
55  
56  
57  
58  
59  
60

## Results visit (V2)

1  
2 During this visit, the participants are informed if the analysis of the liquid cytology indicated a  
3 cervical lesion (ASCUS, LSIL or HSIL). Participants with a high grade lesion (HSIL) exit the  
4 study and are referred to the gynaecology service of the CHU of Montpellier.  
5  
6  
7

8  
9 During this visit, the gynaecologist collects additional samples: 2 vaginal swabs for DNA and  
10 RNA analysis, and a cervical smear in 10mL of PBS to confirm HPV status and perform flow  
11 cytometry analyses (FACS).  
12  
13  
14

15  
16  
17 The participant fills in questionnaires #2 (for return visits) and #3 (home). An appointment for  
18 the next visit is set and additional home self-samples are given.  
19  
20  
21

## Return visits (Vi)

22  
23 These visits only occur in the longitudinal study.  
24  
25  
26

27  
28 *HPV- arm* Participants uninfected by HPV visit the **clinic** every 4 months until month 26. During  
29 these visits, the same samples as in the inclusion visit (V1) are collected by the gynaecologist.  
30  
31  
32

33  
34 The nurse only draws blood if a screening test for other STIs than HPV is required. The  
35 participant then fills in questionnaires #2 and #3 and an appointment is set for the next visit in  
36 16 weeks.  
37  
38  
39

40  
41 If an HPV infection is detected based on the samples collected during this visit, the TEC will  
42 contact the participant to move the appointment forward.  
43  
44  
45

46  
47 *HPV+ arm* Participants infected by HPV visit the clinic every 2 months until clearance or chronic  
48 infection. During these visits, the same samples as in the inclusion visit (V0) are collected during  
49 the gynaecological exam.  
50  
51  
52

53  
54 Then the nurse draws 5mL of blood for HPV antibody titrating. If this is the first HPV+ visit  
55 following an HPV- visit, the nurse draws an additional 10mL of blood for immunophenotyping. If  
56 a test for additional STIs is needed, the nurse draws 5mL more of blood and asks for a self-  
57  
58  
59

sample for STI detection.

1  
2  
3 Importantly, if the participant has been infected by a HR-HPV for more than 12 months and a  
4 cytology has not been performed within the last 12 months, the cervical smear is put in  
5 Thinprep<sup>□</sup> fixation medium, instead of PBS, for cytological analysis (cervical lesion screening).  
6  
7  
8  
9

10 Finally, the participant will fill in questionnaires #2 and #3, receive self-samples for home  
11 collection and an appointment is set for the next visit in 8 weeks.  
12  
13  
14

## 15 Endpoints

16  
17  
18 The primary endpoint for the study is the kinetics of the HPV virus load, associated to local  
19 cytokines profile, local immune cells and cervical smear cytology.  
20  
21  
22

23  
24 Secondary endpoints are the interaction between the course of the infection (e.g. duration), the  
25 HPV type(s), the bacterial, fungal and viral communities in vaginal microbiota, human genetics  
26 (SNPs) and basal immunological status.  
27  
28  
29  
30

## 31 Technical procedures

### 32 DNA extraction

33  
34  
35  
36 DNA extraction from cervical smears will be performed using Nuclisens EasyMAG from  
37 Biomerieux or an equivalent protocol. For the microbiota analyses, special kits involving  
38 physical (via beads) and/or enzymatic breaking of the cellular barrier will be favoured following  
39 standard protocols to study the vaginal microbiome [29], e.g. the MagAttract<sup>□</sup>  
40  
41  
42  
43 PowerMicrobiome<sup>□</sup> DNA/RNA kit from Qiagen. Detection will be based on 16S RNA loci for the  
44 bacteria and ITS loci for fungi. We anticipate that the bacteria should belong to the OTU  
45 described in the five community state types [30, 31].  
46  
47  
48  
49  
50  
51

### 52 HPV detection, typing and quantification

53  
54  
55 The participants' infection status (HPV+ or HPV-) will be assessed using the DEIA test, which is  
56 based on a PCR of the short SPF10 amplicon [32] and detects all *Alphapapillomaviruses* with  
57  
58  
59  
60  
61  
62  
63  
64  
65  
66  
67  
68  
69  
70  
71  
72  
73  
74  
75  
76  
77  
78  
79  
80  
81  
82  
83  
84  
85  
86  
87  
88  
89  
90  
91  
92  
93  
94  
95  
96  
97  
98  
99  
100  
101  
102  
103  
104  
105  
106  
107  
108  
109  
110  
111  
112  
113  
114  
115  
116  
117  
118  
119  
120  
121  
122  
123  
124  
125  
126  
127  
128  
129  
130  
131  
132  
133  
134  
135  
136  
137  
138  
139  
140  
141  
142  
143  
144  
145  
146  
147  
148  
149  
150  
151  
152  
153  
154  
155  
156  
157  
158  
159  
160  
161  
162  
163  
164  
165  
166  
167  
168  
169  
170  
171  
172  
173  
174  
175  
176  
177  
178  
179  
180  
181  
182  
183  
184  
185  
186  
187  
188  
189  
190  
191  
192  
193  
194  
195  
196  
197  
198  
199  
200  
201  
202  
203  
204  
205  
206  
207  
208  
209  
210  
211  
212  
213  
214  
215  
216  
217  
218  
219  
220  
221  
222  
223  
224  
225  
226  
227  
228  
229  
230  
231  
232  
233  
234  
235  
236  
237  
238  
239  
240  
241  
242  
243  
244  
245  
246  
247  
248  
249  
250  
251  
252  
253  
254  
255  
256  
257  
258  
259  
260  
261  
262  
263  
264  
265  
266  
267  
268  
269  
270  
271  
272  
273  
274  
275  
276  
277  
278  
279  
280  
281  
282  
283  
284  
285  
286  
287  
288  
289  
290  
291  
292  
293  
294  
295  
296  
297  
298  
299  
300  
301  
302  
303  
304  
305  
306  
307  
308  
309  
310  
311  
312  
313  
314  
315  
316  
317  
318  
319  
320  
321  
322  
323  
324  
325  
326  
327  
328  
329  
330  
331  
332  
333  
334  
335  
336  
337  
338  
339  
340  
341  
342  
343  
344  
345  
346  
347  
348  
349  
350  
351  
352  
353  
354  
355  
356  
357  
358  
359  
360  
361  
362  
363  
364  
365  
366  
367  
368  
369  
370  
371  
372  
373  
374  
375  
376  
377  
378  
379  
380  
381  
382  
383  
384  
385  
386  
387  
388  
389  
390  
391  
392  
393  
394  
395  
396  
397  
398  
399  
400  
401  
402  
403  
404  
405  
406  
407  
408  
409  
410  
411  
412  
413  
414  
415  
416  
417  
418  
419  
420  
421  
422  
423  
424  
425  
426  
427  
428  
429  
430  
431  
432  
433  
434  
435  
436  
437  
438  
439  
440  
441  
442  
443  
444  
445  
446  
447  
448  
449  
450  
451  
452  
453  
454  
455  
456  
457  
458  
459  
460  
461  
462  
463  
464  
465  
466  
467  
468  
469  
470  
471  
472  
473  
474  
475  
476  
477  
478  
479  
480  
481  
482  
483  
484  
485  
486  
487  
488  
489  
490  
491  
492  
493  
494  
495  
496  
497  
498  
499  
500  
501  
502  
503  
504  
505  
506  
507  
508  
509  
510  
511  
512  
513  
514  
515  
516  
517  
518  
519  
520  
521  
522  
523  
524  
525  
526  
527  
528  
529  
530  
531  
532  
533  
534  
535  
536  
537  
538  
539  
540  
541  
542  
543  
544  
545  
546  
547  
548  
549  
550  
551  
552  
553  
554  
555  
556  
557  
558  
559  
560  
561  
562  
563  
564  
565  
566  
567  
568  
569  
570  
571  
572  
573  
574  
575  
576  
577  
578  
579  
580  
581  
582  
583  
584  
585  
586  
587  
588  
589  
590  
591  
592  
593  
594  
595  
596  
597  
598  
599  
600  
601  
602  
603  
604  
605  
606  
607  
608  
609  
610  
611  
612  
613  
614  
615  
616  
617  
618  
619  
620  
621  
622  
623  
624  
625  
626  
627  
628  
629  
630  
631  
632  
633  
634  
635  
636  
637  
638  
639  
640  
641  
642  
643  
644  
645  
646  
647  
648  
649  
650  
651  
652  
653  
654  
655  
656  
657  
658  
659  
660  
661  
662  
663  
664  
665  
666  
667  
668  
669  
670  
671  
672  
673  
674  
675  
676  
677  
678  
679  
680  
681  
682  
683  
684  
685  
686  
687  
688  
689  
690  
691  
692  
693  
694  
695  
696  
697  
698  
699  
700  
701  
702  
703  
704  
705  
706  
707  
708  
709  
710  
711  
712  
713  
714  
715  
716  
717  
718  
719  
720  
721  
722  
723  
724  
725  
726  
727  
728  
729  
730  
731  
732  
733  
734  
735  
736  
737  
738  
739  
740  
741  
742  
743  
744  
745  
746  
747  
748  
749  
750  
751  
752  
753  
754  
755  
756  
757  
758  
759  
760  
761  
762  
763  
764  
765  
766  
767  
768  
769  
770  
771  
772  
773  
774  
775  
776  
777  
778  
779  
780  
781  
782  
783  
784  
785  
786  
787  
788  
789  
790  
791  
792  
793  
794  
795  
796  
797  
798  
799  
800  
801  
802  
803  
804  
805  
806  
807  
808  
809  
810  
811  
812  
813  
814  
815  
816  
817  
818  
819  
820  
821  
822  
823  
824  
825  
826  
827  
828  
829  
830  
831  
832  
833  
834  
835  
836  
837  
838  
839  
840  
841  
842  
843  
844  
845  
846  
847  
848  
849  
850  
851  
852  
853  
854  
855  
856  
857  
858  
859  
860  
861  
862  
863  
864  
865  
866  
867  
868  
869  
870  
871  
872  
873  
874  
875  
876  
877  
878  
879  
880  
881  
882  
883  
884  
885  
886  
887  
888  
889  
890  
891  
892  
893  
894  
895  
896  
897  
898  
899  
900  
901  
902  
903  
904  
905  
906  
907  
908  
909  
910  
911  
912  
913  
914  
915  
916  
917  
918  
919  
920  
921  
922  
923  
924  
925  
926  
927  
928  
929  
930  
931  
932  
933  
934  
935  
936  
937  
938  
939  
940  
941  
942  
943  
944  
945  
946  
947  
948  
949  
950  
951  
952  
953  
954  
955  
956  
957  
958  
959  
960  
961  
962  
963  
964  
965  
966  
967  
968  
969  
970  
971  
972  
973  
974  
975  
976  
977  
978  
979  
980  
981  
982  
983  
984  
985  
986  
987  
988  
989  
990  
991  
992  
993  
994  
995  
996  
997  
998  
999  
1000



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

If the DEIA test is positive, the present viruses will be typed using the LiPA25 technique, which is based on the same SPF10-PCR, and argued to have a lower detection threshold than other hybridisation-based typing methods [33].

The reason for basing the detection on the DEIA rather than the LiPA25 is that some *Alphapapillomavirus* may not be detected by DEIA but not genotyped by LiPA and also that the DEIA is more sensitive than the LiPA. If the DEIA is positive and the LiPA25, we will sequence part of the HPV genome using a PCR targeting another region of the genome than the SPF10 (e.g. PGMY09/11 [34]) to determine which type it belongs to

The quantification of HPV DNA genome copy number in the samples will be performed using the protocol set up by [35].

#### Cytokine titration

Cervical sponges will be centrifuged after the addition of 200 $\mu$ L of PBS. Cervical secretions will be analyzed for a set of 5 to 6 cytokines levels using the Meso Scale Discovery (MSD) Multiplex ELISA platform, which allows a low detection threshold and a slowly saturating dose-response curve. A large spectrum of cytokines will be explored first to choose the most relevant ones (see also [36, 37]).

#### Flow cytometry

Analysing immune cells via flow cytometry is extremely challenging on cells as fragile as the ones from cervical smears. However, several studies suggest that this is feasible [36–38]. Here, we will follow the protocol described in [39].

Stainings will be performed using a Duraclone custom mix targeting CD45, CD3, CD4, CD8, CD16, CD56, CD69, CD161 and TCR $\gamma\delta$ . The last marker, Live&Dead will test for cellular viability. Samples will be acquired using a Navios flow cytometer (Beckman Coulter, three-laser configuration).

## Sequencing

Sequencing will be performed for microbiota profiling. It will involve PCR amplification of 16S RNA (for bacteria) and ITS (for fungi). The virome will also be explored using shotgun sequencing. Human genetics will be explored using chip sequencing for SNPs.

## Statistical analyses

### Times series analyses

The core results of the study will come from the longitudinal follow-up of infected women, which will generate time series, i.e. a set of values collected from the same individual over time (Figure 2). There will be several time series per individual (virus load, number of immune cells, cytokine and antibody levels). These time series will be used to fit mathematical population dynamics models that describe the interaction between viruses, host target cells (here, in the case of HPV, keratinocytes) and the immune response. These models are commonly developed for viral infections [40–42] and are being developed for HPV [43].

We will use non-linear mixed effect models [44] to jointly analyse time series from all participants. More precisely, we will rely on *R* packages such as nlme [45] or lme4 [45]. Note that in addition to estimating model parameters (e.g. life-expectancy of infected cells or virion production rate of infected cells), this approach can also allow us to compare biological models using statistical tools based on model likelihood such as Akaike Information Criterion. For an example of such analysis in the case of HIV, see [41].

### Microbiota dynamics

The composition of the vaginal microbiota has already been described and shown to exhibit much less diversity than the gut microbiota for instance [30]. The dynamics of this microbiota has also been studied and shown to closely follow menstrual cycles [31].

We will use the time series of OTU abundances (measured via 16S RNA sequencing and qPCR) to infer interaction parameters by assuming an underlying Lotka-Volterra competition model [46]. This work will include time series analysis techniques (e.g. auto-correlation or local

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

similarity analysis) and statistical inference methods in order to infer community structure and interactions from the NGS datasets [47]. Finally, statistical methods from ecology will also be used to study community diversity (e.g diversity indices) and community assembly, such as cluster and ordination analyses [48].

### Genome Wide Association Studies

In our analysis, we will use human single nucleotide polymorphisms (SNPs) inferred by chip sequencing to look for genetic determinants of key traits (e.g. microbiota composition, HPV infection duration). This is classically done by performing a Genome Wide Association Study (GWAS), which is a complex regression method designed for situations where there are many explanatory variables (here millions of SNPs) for a single trait of interest. GWAS will be performed using classical methods [49]. Earlier GWAS studies have been applied to HPV infections for instance to test for determinants to the ability to seroconvert following infection [50] and cervical cancer (see [51] for a review).

### Additional analyses

For all collected variables, descriptive statistics will be calculated according to the level of measurement. For metric variables this contains mean and standard deviation as well as median and range of the data. In case of categorical variables group proportions and contingency tables are prepared.

Univariate inferential statistics will follow the descriptive analysis. Generally, parametric testing procedures are preferred to non-parametric tests, as the former have higher power. That is why, for metric variables, a check whether the data can be assumed normally distributed will be first conducted. For normally distributed variables, ANOVA statistics will be done to detect differences between the groups. In case of significance, post-hoc analysis (Tukey test) are planned to reveal pairwise differences. If the data are not normally distributed or ordinally scaled, non-parametric analysis will be used. This contains the Kruskal-Wallis test and the Wilcoxon test as a post-hoc test with an appropriate correction of the significance level. Since the cell counts are expected to be small, Fisher's exact test will be performed for contingency

tables instead of the asymptotic chi-square test for categorical variables.

### Sample size calculation

The study will enrol a total of  $N = 300$  women, with  $N = 150$  in a longitudinal study and  $N = 150$  in a cross-sectional study. The goal of the longitudinal study is to follow approximately 75 and at least 40 women longitudinally, preferentially before they are infected (see above).

For this, 150 participants will be enrolled longitudinally in the study. Enrolment will stop if 75 infected women are being followed. Dropouts of infected women during the enrolment period (i.e. until month 22) will be replaced. For the following calculations, we assumed a high percentage of lost during follow-up (30%).

With 150 enrolments and considering that the prevalence of HPV infection in the young women is  $\approx 60\%$  (based on our preliminary data) and 30% of lost to follow-up, we expect to detect (and successfully follow) 63 infections at inclusion [CI95: 51–75], using a 95% confidence interval assuming a binomial distribution.

Among the uninfected women at the first visit and considering the yearly incidence being close to 30% [52] and 30% of lost to follow-up, we expect 12 [CI95: 6–20] to be infected during the first year of follow-up.

In the end, with 150 enrolments and assuming a high percentage of lost to follow-up (30%), we expect to successfully follow 75 [CI95: 56–95] women infected at different stages of HPV infection: beginning, during and end.

Note that this will be made possible by the probability of transmission of HPV, which is extremely high without condom use ( $\approx 90\%$ ) and still high with condom use ( $\approx 40\%$ ) [53]. Moreover, only  $\approx 50\%$  of the target population at the CeGIDD reported adopting safe-sex prevention measures [28].

Finally, regarding potential interference with the HPV vaccines, we do not anticipate any significant problem for two reasons. First, as mentioned above, the vaccine coverage is low in

France. Second, and more importantly, the vaccines only target few HPV types, thus leaving open the possibility of infection by dozens of types. Furthermore, studying the kinetics of a non-vaccine HPV type in a vaccinated woman will be extremely informative.

To run cross-sectional analyses (especially on the microbiota and human genetics), we will enrol  $N = 150$  women who will only perform the inclusion and the results visit. This sample size is based on that of some earlier GWAS studies [51].

## **Trial governance**

### **Sponsor**

This study is sponsored by the Centre Hospitalier Universitaire (CHU) of Montpellier. The CHU is involved in the implementation of the trial, legal/ethical submissions and implementing the database (eCRF), which is hosted by Ennov-Clinical (ClinSight). The CHU is not involved in the analysis or interpretation of the data. The CHU of Montpellier performs regular quality control assessments. A clinical research assistant will visit the CeGIDD every 4 months to ensure that implementation is in accordance with the protocol. The CHU has taken out insurance from the Société hospitalière d'assurances mutuelles, 18, rue Edouard Rochet-6 9372 Lyon cedex 08 (contract number 138983) through the full research period, covering its own civil liability and that of any agent (doctor or research staff), in accordance with article L.1121-10 of the French Public Health Code.

### **Scientific committee**

The scientific committee comprises the study investigators, clinicians, scientific experts and representatives of the sponsor. It will meet yearly. It will be responsible for following research progress, monitoring compliance with good clinical practice and patient safety. It will also be able to decide relevant modification of the protocol. Request from third parties to access data collected during the study will be evaluated by the committee.

## Monitoring

Monitoring will be performed during the whole study at CeGIDD according to the sponsor specific SOP. Routine monitoring visits will be made by the monitors designated by the sponsor to check compliance with the protocol, the completeness, accuracy and consistency of the data, and adherence to GCP. The principal investigator must ensure that eCRFs are completed in a timely manner and must allow periodical access to eCRFs, patient records, drug logs and all other study-related documents and materials. The frequency of monitoring visits will be determined by factors such as study design and the site enrolment requirements but visits will normally occur at least once every 4 months.

## Trial registration

The trial has been registered to ClinicalTrials.gov on 27 Oct 2016 with ID number NCT02946346.

## DISCUSSION

### Expected results

HPVs acute infections have been put into the spotlight because vaccination is much more efficient when it occurs before primo-infection. However, we currently know very little about the early stages of HPV infections. This clinical study will give us an unprecedented level of detail on the kinetics of HPV infections in young women. Variations in virus load have been studied but in the context of cervical cancer in older women [54]. In addition to variations in virus load, we will also have access to the description and the dynamics of parts of the immune response (local immune cells and cytokines, circulating anti-HPV antibodies) and of the vaginal microbiota. Beyond these kinetics, we will also have access to other informations regarding the

infection such as clearance or not in 24 months, presence of more than one HPV type or coinfection by other STIs.

To analyse these data, we will have access to numerous cofactors. One of the most important will be human genetics, with the sequencing of millions of SNPs. Others will be related to the sexual behaviour (number of partners, contraception methods, sexual practices) and general life. We therefore expect general insights regarding sexual health in young women.

### **Practical and operational issues**

Practically, one of the main challenges resides in the analysis of cervical smears by flow cytometry. Indeed, the tissues are known to be fragile, adhesive and auto-fluorescent. Even though standard protocols now exist [39], they require the process of fresh samples in less than 2 hours.

Another potential issue has to do with contaminations, which are frequent in the HPV field due to the robustness of the virions and the sensitivity of the tests. To certify our ability to control for these, we have entered the 2017 GLOBAL HPV DNA Proficiency Panel from the WHO HPV LabNet [55].

Regarding the enrolment of the participants, given the number of visitors of the centre who fit the inclusion criteria (more than 3,000 per year) and given earlier high participation rates in the same population ([28] enrolled 1381 participants in 5 months for their study) we do not expect to meet any problems to enrol 150 women in 22 months for the longitudinal study and 150 for the cross-sectional study.

As in any longitudinal study, ensuring participant commitment will be challenging. To achieve this goal, we have set up a compensation of 40 EUR per visit and an additional 10 EUR times the total number of visits in case of a complete follow-up. Furthermore, participants who have answered a sufficient number of questionnaires and brought back a sufficient number of self samples will get a 100 EUR bonus at the end. Overall, a participant performing 12 return visits would gain a total compensation of 650 EUR.

Concerning the follow-up, the high incidence rate of HPV can also lead to transient carriage, i.e. women who are positive for a type only at a single visit. This has been observed for instance in longitudinal studies with a tight follow-up interval [17]. To control for this, we will run the HPV detection test on the cells from the cervical smear after washing with RPMI.

For peer review only



Table 1: **Summary of the visits schedules and sample taken.** The cross-sectional study only includes the first two columns (V1 and V2). The · indicate samples taken at visits. □ participants infected by a HR-HPV for 12 month will have one PBS smear replaced by a Thinprep□ smear to perform a cytology and check for lesions. □ this sample is only taken at the first HPV+ visit of a formerly HPV- participant. □ STI detection will be performed at inclusion unless the participant has been tested within the last 3 months and during the study every 6 months if a new partner has been reported or upon request.

For peer review only

|                                                        | Inclusion<br>( $V_1$ ) | Results<br>( $V_2$ ) | Return<br>( $V_i$ , with $i > 2$ ) |            |
|--------------------------------------------------------|------------------------|----------------------|------------------------------------|------------|
| Participants                                           | all                    | all                  | HPV+                               | HPV-       |
| Time                                                   | day 0                  | + 4 weeks            | + 8 weeks                          | + 16 weeks |
| Eligibility                                            | .                      |                      |                                    |            |
| Consent                                                | .                      |                      |                                    |            |
| Gynecological consult                                  | .                      | .                    | .                                  | .          |
| Vaginal pH cotton swab                                 | .                      | .                    | .                                  | .          |
| 2 vaginal swab samples (Copan<br>ESwab <sup>TM</sup> ) | .                      | .                    | .                                  | .          |
| 1 ophthalmological sponge sample                       | .                      |                      | .                                  | .          |
| 1 cervical smear in Thinprep <sup>□</sup> (cytology)   | .                      |                      | □                                  |            |
| 1 cervical smear in PBS                                |                        | .                    | □                                  | .          |
| Blood sampling (HPV antibodies)                        | .                      |                      | .                                  |            |
| Blood sampling (sequencing)                            | .                      |                      |                                    |            |
| Blood sampling (immunophenotyping)                     | .                      |                      | □                                  |            |
| Other STI detection                                    | □                      | □                    | □                                  | □          |
| Questionnaire #1 (inclusion)                           | .                      |                      |                                    |            |
| Questionnaire #2 (visit)                               |                        | .                    | .                                  | .          |
| Questionnaire #3 (home)                                | .                      | .                    | .                                  | .          |
| Returning self-sampling samples                        |                        | .                    | .                                  | .          |
| Serious Adverse Event collection                       |                        | .                    | .                                  | .          |

## Abbreviations

1  
2 ANOVA: Analysis of variance,  
3

4 ASC-US: Atypical squamous cells of undetermined significance,  
5

6 CD: Cluster of differentiation,  
7

8 CI95: 95% Confidence interval,  
9

10 CeGIDD: :Centre Gratuit d'Information de Dépistage et de Diagnostic,  
11

12 CHU: Centre Hospitalier Universitaire,  
13

14 CIN: Cervical intraepithelial Neoplasia,  
15

16 ELISA: enzyme-linked immunosorbent assay,  
17

18 GWAS: Genome Wide Association Study,  
19

20 HIV: Human Immunodeficiency Virus,  
21

22 HPV: Human Papillomavirus,  
23

24 HR: high-risk,  
25

26 ITS: Internal Transcribed Spacer,  
27

28 HSIL: High grade Squamous Intraepithelial Lesion,  
29

30 LR: low-risk,  
31

32 LSIL: Low grade Squamous Intraepithelial Lesion,  
33

34 OTU: Operational Taxonomic Unit,  
35

36 PBMC: Peripheral Blood Mononuclear Cell,  
37

38 PBS: Phosphate Buffered Saline,  
39

40 RPMI: Roswell Park Memorial Institute medium,  
41

42 SNP: Single Nucleotide Polymorphism,  
43

44 TCR: T-cell receptor,  
45

46 WHO: World Health Organisation.  
47  
48  
49  
50  
51  
52  
53  
54  
55

## TRIAL STATUS

56  
57  
58  
59 The study began on Oct 1, 2016, and the first inclusion was on Nov 3, 2016. On Jun 23, 2018,  
60

89 participants have been included in the longitudinal study. Inclusions in the longitudinal study will continue until Dec 2018 and the study is expected to last until Nov 2020.

## CONFLICTS OF INTERESTS

The authors have read and understood BMJ policy on declaration of interests and declare that they have no competing interests.

## FUNDING

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement No 648963 to SA). SA acknowledges further support from the Centre National de la Recherche Scientifique (CNRS) and the Institute de Recherche pour le Développement (IRD).

## DATA STATEMENT

All personal and identifying information collected from participants are kept in a secure place at the CeGIDD during the duration of the trial and will be destroyed at the end of the study. The final raw dataset will be accessible only by the sponsor (CHU) and the chief scientist's (SA) team. Anonymous data will be available to external parties upon approval of both the sponsor and the scientific committee. All publications will be made green or gold open access and the corresponding data will be provided as supplementary material or via a public repository (e.g. Dryad), provided that there is no conflict with ethical guidelines.

## AUTHOR CONTRIBUTIONS

SA, CLM and MR were the major contributors in the conception of the protocol. All authors were involved in the conception of the protocol or in the implementation of the trial. SA wrote the

initial version of the manuscript and NB, CB, JL, MR, CLM and CS further edited it. All authors read and approved the final manuscript.

## **ETHICS APPROVAL AND CONSENT TO PARTICIPATE**

The PAPCLEAR trial obtained favourable opinions from the Comité de Protection des Personnes (CPP) Sud Méditerranée I on May 11, 2016 (CPP number 16 42, reference number ID RCB 2016-A00712-49); from the Comité Consultatif sur le Traitement de l'Information en matière de Recherche dans le domaine de la Santé (CCTIRS) on July 12, 2016 (reference number 16.504); and from the Commission Nationale Informatique et Libertés (CNIL) on Dec 16, 2016 (reference number MMS/ABD/AR1612278, decision number DR-2016-488). This trial was authorised by the Agence Nationale de Sécurité du Médicament et des Produits de Santé (ANSM) on July 20, 2016 (reference 20160072000007).

The protocol has been modified since its initial version and the modification was submitted to the CPP on Jan 29, 2018. In case the protocol needs to be further modified, the investigator-coordinator will submit a request to the CPP and send an information note to all the investigators.

All participants in the study will sign an informed consent form prior to participation.

## **ACKNOWLEDGEMENTS**

We thank all the study participants and the CeGIDD staff. The EVOLPROOF advisory committee is composed of Giuseppe d'Auria, Michael Blum, Ignacio G Bravo, Marc Choisy, Anne Cori, Elisabeth Delarocque-Astagneau, Christophe Depuydt, Rémy Froissart, Jérémie Guedj, Robert D Holt, Nathalie Jacobs, Julie Lajoie, Dorothée Missé, Lulla Opatowski, Vincent Pedergrana, José-Miguel Ponciano, Jean-Luc Prétet, Jacques Ravel, François Renaud, Jacques Reynes, Silvia de San José, Catherine Tamalet, Javier Tamames, Muriel Thomas, Édouard Tuillon, Tim Waterboer

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only

## REFERENCES

1. Tota, J.E., Chevarie-Davis, M., Richardson, L.A., Devries, M., Franco, E.L.: Epidemiology and burden of HPV infection and related diseases: implications for prevention strategies. *Prev Med* **53 Suppl 1**, 12–21 (2011). doi:[10.1016/j.ypmed.2011.08.017](https://doi.org/10.1016/j.ypmed.2011.08.017)
2. Monsonego, J., Zerat, L., Syrjänen, K., Zerat, J.C., Smith, J.S., Halfon, P.: Prevalence of genotype-specific HPV infection among women in France: implications for screening and vaccination. *Gynecol Obstet Fertil* **41**(5), 305–313 (2013). doi:[10.1016/j.gyobfe.2013.03.003](https://doi.org/10.1016/j.gyobfe.2013.03.003)
3. Brun-Micaleff, E., Coffy, A., Rey, V., Didelot, M.-N., Combecal, J., Doutre, S., Daurès, J.-P., Segondy, M., Boulle, N.: Cervical cancer screening by cytology and human papillomavirus testing during pregnancy in french women with poor adhesion to regular cervical screening. *J Med Virol* **86**(3), 536–45 (2014). doi:[10.1002/jmv.23764](https://doi.org/10.1002/jmv.23764)
4. Insinga, R.P., Dasbach, E.J., Elbasha, E.H., Liaw, K.-L., Barr, E.: Incidence and duration of cervical human papillomavirus 6, 11, 16, and 18 infections in young women: an evaluation from multiple analytic perspectives. *Cancer Epidemiol Biomarkers Prev* **16**(4), 709–15 (2007). doi:[10.1158/1055-9965.EPI-06-0846](https://doi.org/10.1158/1055-9965.EPI-06-0846)
5. Woodman, C.B.J., Collins, S.I., Young, L.S.: The natural history of cervical HPV infection: unresolved issues. *Nat Rev Cancer* **7**(1), 11–22 (2007). doi:[10.1038/nrc2050](https://doi.org/10.1038/nrc2050)
6. Rodríguez, A.C., Schiffman, M., Herrero, R., Wacholder, S., Hildesheim, A., Castle, P.E., Solomon, D., Burk, R., Proyecto Epidemiológico Guanacaste Group: Rapid clearance of human papillomavirus and implications for clinical focus on persistent infections. *J Natl Cancer Inst* **100**(7), 513–7 (2008). doi:[10.1093/jnci/djn044](https://doi.org/10.1093/jnci/djn044)
7. Trottier, H., Mahmud, S., Prado, J.C.M., Sobrinho, J.S., Costa, M.C., Rohan, T.E., Villa, L.L., Franco, E.L.: Type-Specific Duration of Human Papillomavirus Infection: Implications for Human Papillomavirus Screening and Vaccination. *J Infect Dis* **197**(10), 1436–1447 (2008). doi:[10.1086/587698](https://doi.org/10.1086/587698)

8. Alizon, S., Murall, C.L., Bravo, I.G.: Why Human Papillomavirus Acute Infections Matter. *Viruses* **9**(10), 293 (2017). doi:[10.3390/v9100293](https://doi.org/10.3390/v9100293)
9. Herrero, R., Wacholder, S., Rodríguez, A.C., Solomon, D., González, P., Kreimer, A.R., Porras, C., Schussler, J., Jiménez, S., Sherman, M.E., Quint, W., Schiller, J.T., Lowy, D.R., Schiffman, M., Hildesheim, A., Costa Rica Vaccine Trial Group: Prevention of persistent human papillomavirus infection by an HPV16/18 vaccine: a community-based randomized clinical trial in Guanacaste, Costa Rica. *Cancer Discov* **1**(5), 408–19 (2011). doi:[10.1158/2159-8290.CD-11-0131](https://doi.org/10.1158/2159-8290.CD-11-0131)
10. Stanley, M.: Immune responses to human papillomavirus. *Vaccine* **24 Suppl 1**, 16–22 (2006). doi:[10.1016/j.vaccine.2005.09.002](https://doi.org/10.1016/j.vaccine.2005.09.002)
11. Ferenczy, A., Franco, E.: Persistent human papillomavirus infection and cervical neoplasia. *Lancet Oncol* **3**(1), 11–6 (2002)
12. zur Hausen, H.: Review: Papillomaviruses — to Vaccination and Beyond. *Biochemistry* **73**(5), 498–503 (2008). doi:[10.1134/S0006297908050027](https://doi.org/10.1134/S0006297908050027)
13. Einstein, M.H., Schiller, J.T., Viscidi, R.P., Strickler, H.D., Coursaget, P., Tan, T., Halsey, N., Jenkins, D.: Clinician’s guide to human papillomavirus immunology: knowns and unknowns. *Lancet Infect Dis* **9**(6), 347–56 (2009). doi:[10.1016/S1473-3099\(09\)70108-2](https://doi.org/10.1016/S1473-3099(09)70108-2)
14. Van Hede, D., Langers, I., Delvenne, P., Jacobs, N.: Origin and immunoescape of uterine cervical cancer. *Presse Med* **43**(12P2), 413–421 (2014). doi:[10.1016/j.lpm.2014.09.005](https://doi.org/10.1016/j.lpm.2014.09.005)
15. Stanley, M.: Immunology of HPV infection. *Curr Obstet Gynecol Rep* **4**(4), 195–200 (2015). doi:[10.1007/s13669-015-0134-y](https://doi.org/10.1007/s13669-015-0134-y). Accessed 2017-03-20
16. Gao, W., Weng, J., Gao, Y., Chen, X.: Comparison of the vaginal microbiota diversity of women with and without human papillomavirus infection: a cross-sectional study. *BMC Infect Dis* **13**, 271 (2013). doi:[10.1186/1471-2334-13-271](https://doi.org/10.1186/1471-2334-13-271)



17. Brotman, R.M., Shardell, M.D., Gajer, P., Tracy, J.K., Zenilman, J.M., Ravel, J., Gravitt, P.E.: Interplay between the temporal dynamics of the vaginal microbiota and human papillomavirus detection. *J Infect Dis* **210**(11), 1723–33 (2014). doi:[10.1093/infdis/jiu330](https://doi.org/10.1093/infdis/jiu330)
18. Koutsky, L.A., Ault, K.A., Wheeler, C.M., Brown, D.R., Barr, E., Alvarez, F.B., Chiacchierini, L.M., Jansen, K.U., Proof of Principle Study Investigators: A controlled trial of a human papillomavirus type 16 vaccine. *N Engl J Med* **347**(21), 1645–51 (2002). doi:[10.1056/NEJMoa020586](https://doi.org/10.1056/NEJMoa020586)
19. Riethmuller, D., Jacquard, A.-C., Lacau St Guily, J., Aubin, F., Carcopino, X., Pradat, P., Dahlab, A., Pr etet, J.-L.: Potential impact of a nonavalent hpv vaccine on the occurrence of hpv-related diseases in france. *BMC Public Health* **15**, 453 (2015). doi:[10.1186/s12889-015-1779-1](https://doi.org/10.1186/s12889-015-1779-1)
20. Joura, E.A., Giuliano, A.R., Iversen, O.-E., Bouchard, C., Mao, C., Mehlsen, J., Moreira, E.D. Jr, Ngan, Y., Petersen, L.K., Lazcano-Ponce, E., Pitisuttithum, P., Restrepo, J.A., Stuart, G., Woelber, L., Yang, Y.C., Cuzick, J., Garland, S.M., Huh, W., Kjaer, S.K., Bautista, O.M., Chan, I.S.F., Chen, J., Gesser, R., Moeller, E., Ritter, M., Vuocolo, S., Luxembourg, A., Broad Spectrum HPV Vaccine Study: A 9-valent HPV vaccine against infection and intraepithelial neoplasia in women. *N Engl J Med* **372**(8), 711–23 (2015). doi:[10.1056/NEJMoa1405044](https://doi.org/10.1056/NEJMoa1405044)
21. Murall, C.L., Bauch, C.T., Day, T.: Could the human papillomavirus vaccines drive virulence evolution? *Proc Biol Sci* **282**, 20141069 (2015). doi:[10.1098/rspb.2014.1069](https://doi.org/10.1098/rspb.2014.1069)
22. Moscicki, A.-B., Ma, Y., Wibbelsman, C., Darragh, T.M., Powers, A., Farhat, S., Shiboski, S.: Rate of and Risks for Regression of CIN-2 in adolescents and young women. *Obstet Gynecol* **116**(6), 1373–1380 (2010). doi:[10.1097/AOG.0b013e3181fe777f](https://doi.org/10.1097/AOG.0b013e3181fe777f)
23. Buck Jr., H.W.: Warts (genital). *BMJ Clin Evid* **2015**, 1602 (2015)
24. Herrero, R., Gonz alez, P., Markowitz, L.E.: Present status of human papillomavirus vaccine development and implementation. *Lancet Oncol* **16**(5), 206–16 (2015). doi:[10.1016/S1470-2045\(14\)70481-4](https://doi.org/10.1016/S1470-2045(14)70481-4)

25. Maver, P.J., Poljak, M.: Progress in prophylactic human papillomavirus (HPV) vaccination in 2016: A literature review. *Vaccine* (2018). doi:[10.1016/j.vaccine.2017.07.113](https://doi.org/10.1016/j.vaccine.2017.07.113)
26. Fagot, J.-P., Boutrelle, A., Ricordeau, P., Weill, A., Allemand, H.: HPV vaccination in France: uptake, costs and issues for the National Health Insurance. *Vaccine* **29**(19), 3610–6 (2011). doi:[10.1016/j.vaccine.2011.02.064](https://doi.org/10.1016/j.vaccine.2011.02.064)
27. YAHIA, M.-B.B.H., DERVAUX, B., DUPORT, N., FLORET, D., GAILLOT, J., HEARD, I., JACQUET, A., GOASTER, C.L., LEVY-BRUHL, D., MORER, I., du CHATELET, I.P., PEIGUE-LAFEUILLE, H., RUMEAU-PICHON, C.: Vaccination contre les infections à papilloamvirus. Technical report, Haut Conseil de la Santé Publique, Paris, France (2014). <https://www.hcsp.fr/Explore.cgi/avisrapportsdomaine?clefr=454>
28. Clarivet, B., Picot, E., Marchandin, H., Tribout, V., Rachedi, N., Schwartzentruber, E., Ledésert, B., Dereure, O., Guillot, B., Picot, M.-C.: Prevalence of Chlamydia trachomatis, Neisseria gonorrhoeae and Mycoplasma genitalium in asymptomatic patients under 30 years of age screened in a French sexually transmitted infections clinic. *Eur J Dermatol* **24**(5), 611–6 (2014). doi:[10.1684/ejd.2014.2413](https://doi.org/10.1684/ejd.2014.2413)
29. Ravel, J., Brotman, R.M., Gajer, P., Ma, B., Nandy, M., Fadrosch, D.W., Sakamoto, J., Koenig, S.S., Fu, L., Zhou, X., Hickey, R.J., Schwebke, J.R., Forney, L.J.: Daily temporal dynamics of vaginal microbiota before, during and after episodes of bacterial vaginosis. *Microbiome* **1**(1), 29 (2013). doi:[10.1186/2049-2618-1-29](https://doi.org/10.1186/2049-2618-1-29)
30. Ravel, J., Gajer, P., Abdo, Z., Schneider, G.M., Koenig, S.S.K., McCulle, S.L., Karlebach, S., Gorle, R., Russell, J., Tacket, C.O., Brotman, R.M., Davis, C.C., Ault, K., Peralta, L., Forney, L.J.: Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci USA* **108**, 4680–7 (2011). doi:[10.1073/pnas.1002611107](https://doi.org/10.1073/pnas.1002611107)
31. Gajer, P., Brotman, R.M., Bai, G., Sakamoto, J., Schütte, U.M.E., Zhong, X., Koenig, S.S.K., Fu, L., Ma, Z.S., Zhou, X., Abdo, Z., Forney, L.J., Ravel, J.: Temporal dynamics of the

human vaginal microbiota. *Sci Transl Med* **4**(132), 132–52 (2012).

doi:[10.1126/scitranslmed.3003605](https://doi.org/10.1126/scitranslmed.3003605)

32. Kleter, B., van Doorn, L.-J., ter Schegget, J., Schrauwen, L., van Krimpen, K., Burger, M., ter Harmsel, B., Quint, W.: Novel Short-Fragment PCR Assay for Highly Sensitive Broad-Spectrum Detection of Anogenital Human Papillomaviruses. *Am J Pathol* **153**(6), 1731–1739 (1998). doi:[10.1016/S0002-9440\(10\)65688-X](https://doi.org/10.1016/S0002-9440(10)65688-X)

33. Geraets, D.T., Struijk, L., Kleter, B., Molijn, A., van Doorn, L.-J., Quint, W.G.V., Colau, B.: The original SPF10 LiPA25 algorithm is more sensitive and suitable for epidemiologic HPV research than the SPF10 INNO-LiPA Extra. *J Virol Meth* **215-216**, 22–29 (2015).

doi:[10.1016/j.jviromet.2015.01.001](https://doi.org/10.1016/j.jviromet.2015.01.001)

34. Gravitt, P.E., Peyton, C.L., Alessi, T.Q., Wheeler, C.M., Coutlée, F., Hildesheim, A., Schiffman, M.H., Scott, D.R., Apple, R.J.: Improved amplification of genital Human Papillomaviruses. *J Clin Microbiol* **38**(1), 357–361 (2000)

35. Micalessi, I.M., Boulet, G.A.V., Bogers, J.J., Benoy, I.H., Depuydt, C.E.: High-throughput detection, genotyping and quantification of the human papillomavirus using real-time PCR. *Clin Chem Lab Med* **50**(4), 655–61 (2012). doi:[10.1515/cclm.2011.835](https://doi.org/10.1515/cclm.2011.835)

36. Hunter, P.J., Sheikh, S., David, A.L., Peebles, D.M., Klein, N.: Cervical leukocytes and spontaneous preterm birth. *Journal of Reproductive Immunology* **113**, 42–49 (2016).

doi:[10.1016/j.jri.2015.11.002](https://doi.org/10.1016/j.jri.2015.11.002)

37. Shannon, B., Yi, T.J., Perusini, S., Gajer, P., Ma, B., Humphrys, M.S., Thomas-Pavanel, J., Chieza, L., Janakiram, P., Saunders, M., Tharao, W., Huibner, S., Shahabi, K., Ravel, J., Rebbapragada, A., Kaul, R.: Association of HPV infection and clearance with cervicovaginal immunology and the vaginal microbiota. *Mucosal Immunology* **10**(5), 1310–1319 (2017).

doi:[10.1038/mi.2016.129](https://doi.org/10.1038/mi.2016.129)

38. Lajoie, J., Juno, J., Burgener, A., Rahman, S., Mogk, K., Wachihi, C., Mwanjewe, J.,

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
- Plummer, F.A., Kimani, J., Ball, T.B., Fowke, K.R.: A distinct cytokine and chemokine profile at the genital mucosa is associated with HIV-1 protection among HIV-exposed seronegative commercial sex workers. *Mucosal Immunol* **5**(3), 277–287 (2012). doi:[10.1038/mi.2012.7](https://doi.org/10.1038/mi.2012.7)
39. Juno, J.A., Boily-Larouche, G., Lajoie, J., Fowke, K.R.: Collection, isolation, and flow cytometric analysis of human endocervical samples. *J Vis Exp* **89**, 51906 (2014). doi:[10.3791/51906](https://doi.org/10.3791/51906)
40. Nowak, M.A., May, R.M.: *Virus Dynamics: Mathematical Principles of Immunology and Virology*. Oxford University Press, Oxford, USA (2000)
41. Stafford, M.A., Corey, L., Cao, Y., Daar, E.S., Ho, D.D., Perelson, A.S.: Modeling plasma virus concentration during primary HIV infection. *J. theor. Biol.* **203**(3), 285–301 (2000). doi:[10.1006/jtbi.2000.1076](https://doi.org/10.1006/jtbi.2000.1076)
42. Perelson, A.S.: Modelling viral and immune system dynamics. *Nat. Rev. Immunol.* **2**(1), 28–36 (2002). doi:[10.1038/nri700](https://doi.org/10.1038/nri700)
43. Murall, C.L., Jackson, R., Zehbe, I., Boulle, N., Segondy, M., Alizon, S.: Epithelial stratification shapes infection dynamics. *bioRxiv*, 231985 (2017). doi:[10.1101/231985](https://doi.org/10.1101/231985)
44. Steimer, J.L., Vozeh, S., Racine Poon, A., Holford, N., O’Neil, R.: The population approach: rationale, methods and applications in clinical pharmacology and drug development. In: Balant, P.G.W..L. (ed.) *Handbook of Experimental Pharmacology*, vol. 110, pp. 405–451. Springer, Berlin (1994)
45. Bates, D., Mächler, M., Bolker, B., Walker, S.: Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**(1) (2015). doi:[10.18637/jss.v067.i01](https://doi.org/10.18637/jss.v067.i01)
46. Bucci, V., Tzen, B., Li, N., Simmons, M., Tanoue, T., Bogart, E., Deng, L., Yeliseyev, V., Delaney, M.L., Liu, Q., Olle, B., Stein, R.R., Honda, K., Bry, L., Gerber, G.K.: MDSINE: Microbial dynamical systems INference engine for microbiome time-series analyses. *Genome*

Biology **17**, 121 (2016). doi:[10.1186/s13059-016-0980-6](https://doi.org/10.1186/s13059-016-0980-6). Accessed 2017-03-09

1  
2  
3 47. Faust, K., Lahti, L., Gonze, D., de Vos, W.M., Raes, J.: Metagenomics meets time series  
4 analysis: unraveling microbial community dynamics. *Curr Opin Microbiol* **25**, 56–66 (2015).

5  
6  
7 doi:[10.1016/j.mib.2015.04.004](https://doi.org/10.1016/j.mib.2015.04.004)  
8  
9

10 48. Fox, G.A., Negrete-Yankelevich, S., Sosa, V.J.: *Ecological Statistics: Contemporary*  
11 *Theory and Application*. Oxford University Press, Oxford, USA (2015)  
12  
13

14  
15 49. Shi, Y., Li, L., Hu, Z., Li, S., Wang, S., Liu, J., Wu, C., He, L., Zhou, J., Li, Z., Hu, T., Chen,  
16 Y., Jia, Y., Wang, S., Wu, L., Cheng, X., Yang, Z., Yang, R., Li, X., Huang, K., Zhang, Q., Zhou,  
17 H., Tang, F., Chen, Z., Shen, J., Jiang, J., Ding, H., Xing, H., Zhang, S., Qu, P., Song, X., Lin,  
18 Z., Deng, D., Xi, L., Lv, W., Han, X., Tao, G., Yan, L., Han, Z., Li, Z., Miao, X., Pan, S., Shen, Y.,  
19 Wang, H., Liu, D., Gong, E., Li, Z., Zhou, L., Luan, X., Wang, C., Song, Q., Wu, S., Xu, H.,  
20 Shen, J., Qiang, F., Ma, G., Liu, L., Chen, X., Liu, J., Wu, J., Shen, Y., Wen, Y., Chu, M., Yu, J.,  
21 Hu, X., Fan, Y., He, H., Jiang, Y., Lei, Z., Liu, C., Chen, J., Zhang, Y., Yi, C., Chen, S., Li, W.,  
22 Wang, D., Wang, Z., Di, W., Shen, K., Lin, D., Shen, H., Feng, Y., Xie, X., Ma, D.: A genome-  
23 wide association study identifies two new cervical cancer susceptibility loci at 4q12 and 17q12.  
24 *Nat Genet* **45**(8), 918–22 (2013). doi:[10.1038/ng.2687](https://doi.org/10.1038/ng.2687)  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34

35  
36  
37

38 50. Chen, D., Gaborieau, V., Zhao, Y., Chabrier, A., Wang, H., Waterboer, T., Zaridze, D.,  
39 Lissowska, J., Rudnai, P., Fabianova, E., Bencko, V., Janout, V., Foretova, L., Mates, I.N.,  
40 Szeszenia-Dabrowska, N., Boffetta, P., Pawlita, M., Lathrop, M., Gyllensten, U., Brennan, P.,  
41 McKay, J.D.: A systematic investigation of the contribution of genetic variation within the MHC  
42 region to HPV seropositivity. *Hum Mol Genet* **24**(9), 2681–2688 (2015).  
43  
44  
45  
46  
47  
48

49 doi:[10.1093/hmg/ddv015](https://doi.org/10.1093/hmg/ddv015)  
50  
51

52 51. Chen, D., Gyllensten, U.: Lessons and implications from association studies and post-  
53 GWAS analyses of cervical cancer. *Trends Genet* **31**(1), 41–54 (2015).  
54  
55

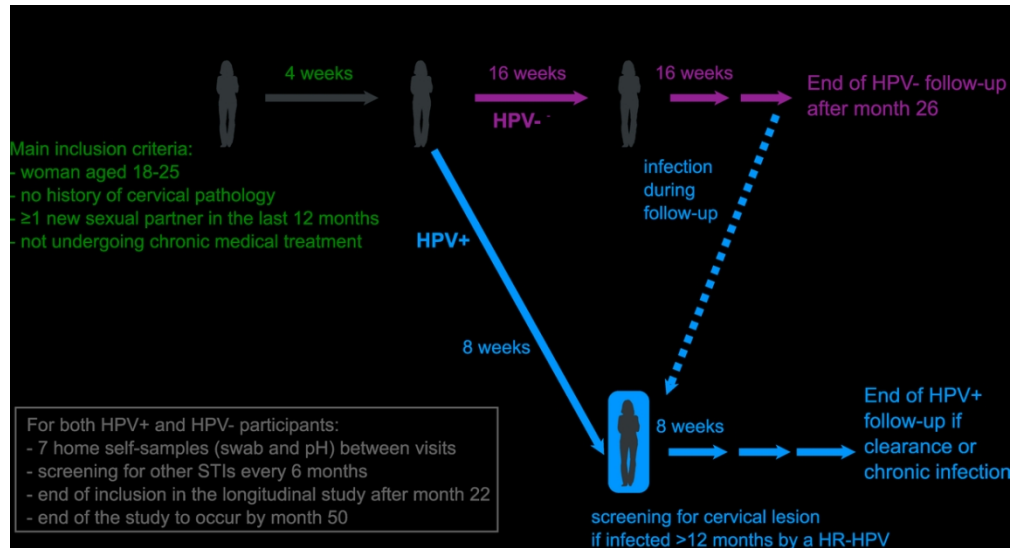
56  
57 doi:[10.1016/j.tig.2014.10.005](https://doi.org/10.1016/j.tig.2014.10.005)  
58  
59

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
52. Winer, R.L., Feng, Q., Hughes, J.P., O'Reilly, S., Kiviat, N.B., Koutsky, L.A.: Risk of female human papillomavirus acquisition associated with first male sex partner. *J Infect Dis* **197**(2), 279–82 (2008). doi:[10.1086/524875](https://doi.org/10.1086/524875)
53. Winer, R.L., Hughes, J.P., Feng, Q., O'Reilly, S., Kiviat, N.B., Holmes, K.K., Koutsky, L.A.: Condom use and the risk of genital human papillomavirus infection in young women. *N Engl J Med* **354**(25), 2645–54 (2006). doi:[10.1056/NEJMoa053284](https://doi.org/10.1056/NEJMoa053284)
54. Depuydt, C.E., Thys, S., Beert, J., Jonckheere, J., Salembier, G., Bogers, J.J.: Linear viral load increase of a single HPV-type in women with multiple HPV infections predicts progression to cervical cancer. *Int J Cancer* **139**(9), 2021–2032 (2016). doi:[10.1002/ijc.30238](https://doi.org/10.1002/ijc.30238)
55. WHO HPV LabNet. World Health Organization.  
[http://www.who.int/biologicals/areas/human\\_papillomavirus/WHO HPV LabNet/en/](http://www.who.int/biologicals/areas/human_papillomavirus/WHO_HP_V_LabNet/en/)

**FIGURE CAPTIONS**

1  
2  
3  
4  
5  
6 **Figure 1: General structure of the PAPCLEAR study.** For the longitudinal study, participants  
7 have an inclusion visit ( $V_1$ ), a results visit ( $V_2$ ) and then return visits ( $V_i$  with  $i > 2$ ). For the cross-  
8 sectional study, participants only have  $V_1$  and  $V_2$ .  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22

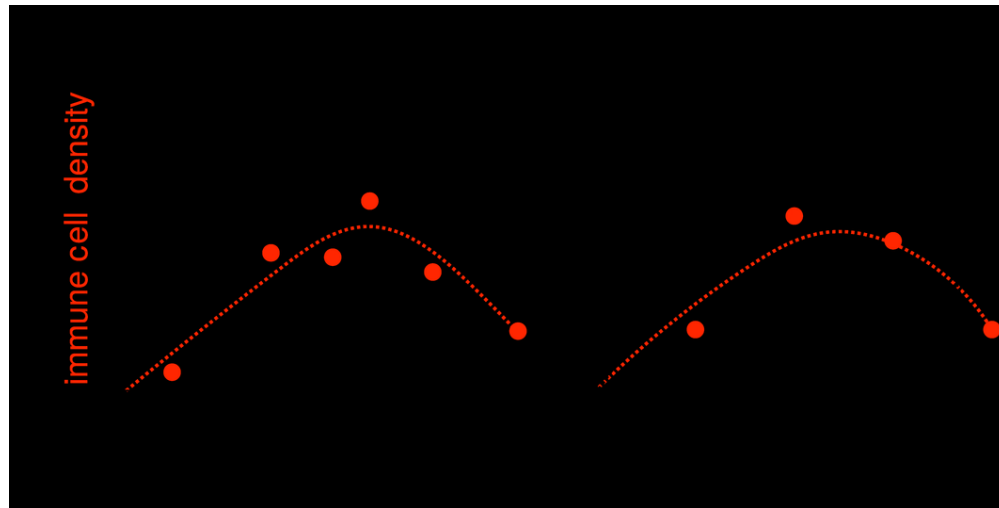
23 **Figure 2: Fitting kinetics dynamical models to within-host times series.** Dashed lines  
24 indicate a model fitted using virus load (in black) or immune cells (in red) time series. In panel A,  
25 the follow-up is bi-monthly with 2 missing visits and several delayed visits, whereas in panel B  
26 the follow-up is every 4 months without any missing or delayed visits. In spite of missing data  
27 this, the situation shown in panel A is clearly the best for inferring parameter values and for  
28 fitting the underlying dynamics.  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



Caption : General structure of the PAPCLEAR study. For the longitudinal study, participants have an inclusion visit (V1), a results visit (V2) and then return visits (Vi with  $i > 2$ ). For the cross-sectional study, participants only have V1 and V2.

103x56mm (300 x 300 DPI)





Fitting kinetics dynamical models to within-host times series. Dashed lines indicate a model fitted using virus load (in black) or immune cells (in red) time series. In panel A, the follow-up is bi-monthly with 2 missing visits and several delayed visits, whereas in panel B the follow-up is every 4 months without any missing or delayed visits. In spite of missing data this, the situation shown in panel A is clearly the best for inferring parameter values and for fitting the underlying dynamics.

90x45mm (300 x 300 DPI)

# BMJ Open

## The natural history, dynamics, and ecology of Human papillomaviruses (HPVs) in genital infections of young women: the PAPCLEAR study

|                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|-------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Journal:                      | <i>BMJ Open</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Manuscript ID                 | bmjopen-2018-025129.R1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Article Type:                 | Protocol                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Date Submitted by the Author: | 25-Feb-2019                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| Complete List of Authors:     | <p>Murall, Carmen Lia; Centre National de la Recherche Scientifique, MIVEGEC</p> <p>Rahmoun, Massilva; Centre National de la Recherche Scientifique, MIVEGEC</p> <p>Selinger, Christian; Centre National de la Recherche Scientifique, MIVEGEC</p> <p>Baldellou, Monique; Centre Hospitalier Regional Universitaire de Montpellier, CeGIDD</p> <p>Bernat, Claire; Centre National de la Recherche Scientifique, MIVEGEC</p> <p>Bonneau, Marine; Centre Hospitalier Regional Universitaire de Montpellier, Department of Obstetrics and Gynaecology</p> <p>Boué, Vanina; Centre National de la Recherche Scientifique, MIVEGEC</p> <p>Buisson, Mathilde; Centre Hospitalier Regional Universitaire de Montpellier, Department of Research and Innovation</p> <p>Christophe, Guillaume; Centre Hospitalier Regional Universitaire de Montpellier, CeGIDD</p> <p>D'Auria, Giuseppe; Fundacio per al Foment de la Investigacio Sanitaria i Biomedica, Sequencing and Bioinformatics Service; Centro de Investigacion Biomedica en Red de Epidemiologia y Salud Publica De Taroni, Florence; Centre Hospitalier Regional Universitaire de Montpellier, CeGIDD</p> <p>Foulongne, Vincent; Centre Hospitalier Regional Universitaire de Montpellier, Department of Bacteriology and Virology</p> <p>Froissart, Rémy; Centre National de la Recherche Scientifique, MIVEGEC</p> <p>Graf, Christelle; Centre Hospitalier Regional Universitaire de Montpellier, Department of Obstetrics and Gynaecology</p> <p>Grasset, Sophie; Centre Hospitalier Regional Universitaire de Montpellier, Department of Virology; Centre National de la Recherche Scientifique, MIVEGEC</p> <p>Groc, Soraya; Centre Hospitalier Regional Universitaire de Montpellier, Department of Virology; Centre National de la Recherche Scientifique, MIVEGEC</p> <p>Hirtz, Christophe; Centre Hospitalier Regional Universitaire de Montpellier, LBPC/PPC, IRMB</p> <p>Jaussent, Audrey; Centre Hospitalier Regional Universitaire de Montpellier, Department of Medical Information</p> <p>Lajoie, Julie; University of Manitoba College of Medicine, Department of Medical microbiology</p> <p>Lorcy, Frédérique; Centre Hospitalier Regional Universitaire de</p> |

|                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|---------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                 | <p>Montpellier, Laboratoire d'anatomie et cytologie pathologiques<br/> Picot, Eric; Centre Hospitalier Regional Universitaire de Montpellier, CeGIDD<br/> PICOT, Marie-Christine; Centre Hospitalier Regional Universitaire de Montpellier, Department of Medical Information<br/> Ravel, Jacques; University of Maryland School of Medicine, Institute for Genome Sciences<br/> Reynes, Jacques; Centre Hospitalier Regional Universitaire de Montpellier, Department of Infectious and Tropical Diseases<br/> Rousset, Thérèse; Centre Hospitalier Regional Universitaire de Montpellier, Department of pathology and oncobiology<br/> Seddiki, Aziza; Centre Hospitalier Regional Universitaire de Montpellier, Department of Research and Innovation<br/> Teirlinck, Martine; Centre Hospitalier Regional Universitaire de Montpellier, CeGIDD<br/> Tribout, Vincent; Centre Hospitalier Regional Universitaire de Montpellier, CeGIDD<br/> Tuaille, Edouard; Centre Hospitalier Regional Universitaire de Montpellier, Department of bacteriology and virology<br/> Waterboer, Tim; Deutsches Krebsforschungszentrum, Infections and Cancer Epidemiology<br/> Jacobs, Nathalie; Universite de Liege Faculte des Sciences, GIGA-Research, Cellular and molecular immunology<br/> Bravo, Ignacio; Centre National de la Recherche Scientifique, MIVEGEC<br/> Segondy, Michel; Centre Hospitalier Regional Universitaire de Montpellier, Department of Bacteriology and Virology<br/> Boulle, Nathalie; Centre Hospitalier Regional Universitaire de Montpellier, Department of pathology and oncobiology<br/> Alizon, Samuel; Centre National de la Recherche Scientifique, MIVEGEC</p> |
| <b>Primary Subject Heading</b>: | Infectious diseases                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| Secondary Subject Heading:      | Epidemiology, Immunology (including allergy), Genetics and genomics                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| Keywords:                       | VIROLOGY, IMMUNOLOGY, Epidemiology < INFECTIOUS DISEASES, MICROBIOLOGY, GENETICS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |

SCHOLARONE™  
Manuscripts

## STUDY PROTOCOL

# The natural history, dynamics, and ecology of Human papillomaviruses (HPVs) in genital infections of young women: the PAPCLEAR study

Carmen Lía Murall<sup>1</sup>, Massilva Rahmoun<sup>1</sup>, Christian Selinger<sup>1</sup>, Monique Baldellou<sup>2</sup>, Claire Bernat<sup>1</sup>, Marine Bonneau<sup>3</sup>, Vanina Boué<sup>1</sup>, Mathilde Buisson<sup>4</sup>, Guillaume Christophe<sup>2</sup>, Giuseppe D'Auria<sup>5,6</sup>, Florence De Taroni<sup>2</sup>, Vincent Foulongne<sup>7,8</sup>, Rémy Froissart<sup>1</sup>, Christelle Graf<sup>3</sup>, Sophie Grasset<sup>1,2</sup>, Soraya Groc<sup>1,7</sup>, Christophe Hirtz<sup>9</sup>, Audrey Jausse<sup>10</sup>, Julie Lajoie<sup>11</sup>, Frédérique Lorcy<sup>12</sup>, Eric Picot<sup>2</sup>, Marie-Christine Picot<sup>10</sup>, Jacques Ravel<sup>13</sup>, Jacques Reynes<sup>11</sup>, Thérèse Rousset<sup>12</sup>, Aziza Seddiki<sup>4</sup>, Martine Teirlinck<sup>2</sup>, Vincent Tribout<sup>2</sup>, Édouard Tuillon<sup>8</sup>, Tim Waterboer<sup>15</sup>, Nathalie Jacobs<sup>16</sup>, Ignacio G Bravo<sup>1</sup>, Michel Segondy<sup>7,8</sup>, Nathalie Boule<sup>8,12</sup> and Samuel Alizon<sup>1,\*</sup>

Word count: 5105 words, excluding title page, abstract, references, figures and tables.

1 Laboratoire MIVEGEC (UMR 5290 CNRS, IRD, UM), 911, avenue Agropolis, BP 64501, 34394 Montpellier, France

2 Center for Free Information, Screening and Diagnosis (CeGIDD), Centre Hospitalier Universitaire de Montpellier, Montpellier, France

3 Department of Obstetrics and Gynaecology, Centre Hospitalier Universitaire de Montpellier, Montpellier, France.

4 Department of Research and Innovation (DRI), Centre Hospitalier Universitaire de Montpellier, Montpellier, France

5 Sequencing and Bioinformatics Service, Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunidad Valenciana (FISABIO-Salud Pública), 46020 Valencia, Spain

6 CIBER en Epidemiología y Salud Pública (CIBEResp), Madrid, Spain

7 Department of Virology, Centre Hospitalier Universitaire de Montpellier, Montpellier, France

8 Pathogenesis and Control of Chronic Infections, INSERM, CHU, University of Montpellier, Montpellier, France

9 University of Montpellier, LBPC/PPC- IRMB, CHU de Montpellier, 80 rue Augustin Fliche, Montpellier, France

10 Department of Medical Information (DIM), Centre Hospitalier Universitaire de Montpellier, Montpellier, France

11 Department of Medical microbiology, University of Manitoba, 745 Bannatyne, Winnipeg, Canada

12 Department of pathology and oncobiology, Centre Hospitalier Universitaire de Montpellier, Montpellier, France

13 Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, USA

14 Department of Infectious and Tropical Diseases, Centre Hospitalier Universitaire de Montpellier, Montpellier, France

15 German Cancer Research Center (DKFZ), Infections and Cancer Epidemiology, Im Neuenheimer Feld 280, Heidelberg, Germany

16 GIGA-Research, Cellular and molecular immunology, University of Liège, 3 Avenue de l'Hôpital, 4000 Liège, Belgium

\* Author for correspondence: [samuel.alizon@cnrs.fr](mailto:samuel.alizon@cnrs.fr)

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only

## Abstract

### Introduction

Human papillomaviruses (HPVs) are responsible for one third of all cancers caused by infections. Most HPV studies focus on chronic infections and cancers, and thus, we know little about the early stages of viral infection. In particular, the effects of the dynamic interactions between the immune system, the microbiota, and the viral and host genetics on infection clearance or persistence remains poorly understood.

### Methods and Analysis

We follow 150 women, aged 18-25 years, longitudinally to monitor immune response features (cytokines and immune cells in the genital tract, circulating anti-HPV antibodies), virus load of HPVs, and vaginal microbiota composition. This is complemented by the assessment of viral and human genetics and behavioural data. To increase the statistical power of the epidemiological arm of the study, an additional 150 women are screened cross-sectionally. This study will provide one of the most detailed follow-up studies of acute HPV infections and their interactions with the host and the vaginal microbiota. It will also allow us to investigate related issues regarding HPV intra-host evolution and diversity, vaginal microbiota dynamics, and sexually transmitted infections.

### Ethics and Dissemination

This study has been approved by the Comité de Protection des Personnes Sud Méditerranée I (reference number 2016-A00712-49); by the Comité Consultatif sur le Traitement de l'Information en matière de Recherche dans le domaine de la Santé (reference number 16.504); by the Commission Nationale Informatique et Libertés (reference number MMS/ABD/AR1612278, decision number DR-2016-488) and by the Agence Nationale de Sécurité du Médicament et des Produits de Santé (reference 20160072000007). The results will be published in preprint servers, peer-reviewed journals and disseminated through conferences.

**Trial registration number:** NCT02946346

**Keywords:** HPV; acute infection; persistence; virus load; immunity; microbiota; viral kinetics

## Article summary

### Strengths and limitations of this study

- Dense follow-up (visit every two months for infected women with additional self-sampling every week) for N=150 women.
- Combination of virological (virus load), immunological (cytokine concentrations and immune cell percentages) and environmental (vaginal microbiota composition, pH) measurement at each visit.
- A limitation is that the density of the follow-up limits the number of participants, which can affect analyses at the epidemiological level.
- We complement the longitudinal study with a cross-sectional study of N=150 women to allow for epidemiological analyses.

## Introduction

### Epidemiology of HPV genital infections in young adults and public health implications

Infections by Human Papillomaviruses (HPVs) are likely the most common sexually transmitted infection (STI) globally. It is often estimated that, worldwide, 75% of the individuals will be infected at some point in their life by an HPV type [1]. In France, a study performed in 2013 in the Paris area estimated the prevalence of genital infections by HPVs at  $\approx 25\%$  of women below 25 years of age [2]. In the area of Montpellier, prevalence of oncogenic HPVs (often referred to as 'high-risk', HR) in pregnant women aged 16 to 42 years was close to 20% [3]. These numbers are consistent with worldwide estimates according to which HPVs are most prevalent in women under 25 years of age, with an estimated overall prevalence of 24% [4].

Fortunately, the vast majority of infections by HPVs are asymptomatic and benign. Even for HPV16, which is probably the most oncogenic biologic agent to humans, only a minority of infections (less than 10%) become persistent [5], and then a minority of these (12%) progress to cancer if untreated [1, 6]. Indeed, it is estimated that approximately 70 to 100% of infections by HPVs are cleared within 12 to 24 months, with strong differences between virus types [5, 7–9]. Recent studies suggest that primo-infections could be shorter in young girls [10] but, in general, there are many unknowns about the biology of non-persisting infections [11].

Our lack of knowledge partly comes from the fact that in vaccine trials, from which most of the data on infection duration originate, participants are followed every six months for several years [5, 7, 9, 12]. This frequency is sufficient to estimate the time to clearance (or to persistence) but it is not precise enough to understand the within-host dynamics, often referred to as 'kinetics' [13], of infections that last on average 6 to 24 months. Arbitrarily, after 24 months of infection, an infection is often considered as being persistent [14].

Some factors have been shown to correlate with persistence (e.g. immunosuppression, smoking, and co-infection with other STIs [15]) but we do not know how these affect viral



kinetics. Also, some changes in viral-immunity interactions appear to be related to persistence and disease progression [16–19] but, again, we do not know the underlying interactions between the viruses, the host target cells, and the immune response in acute infections [11]. Finally, it has been argued that the vaginal microbiota may differ between HPV-infected and HPV-uninfected women [20] and that specific microbiota composition may interact with HPV detection [21]. However, it is difficult to disentangle the cause and the consequence. For instance, does the microbiota composition change after the establishment of an HPV infection, or do certain microbiota compositions increase susceptibility to HPV infection?

A better understanding of the within-host infection dynamics and of the determinants of clearance and persistence of viral infection is particularly important in the context of vaccination [22–25]. Indeed, the long-term efficacy of the anti-HPVs vaccines at the population level will largely depend on the within-host viral dynamics because, ultimately, most selective pressures on viral populations occur via the immune response [26]. Furthermore, a better understanding of acute HPV infections can shed a new light on issues related to latency, fertility, or immunotherapies [11].

## **Prevention strategies and treatment**

### **Treatment**

Since most infections by HPVs are benign in young adults and clear within six to 24 months, the current standard of care is to avoid over-treatment, even in the presence of cervical lesions [27]. Clinical interventions (colposcopies, biopsies, and surgery) are less often performed with young women (< 25 years) and only for high-grade (pre-cancerous) lesions (cervical intraepithelial neoplasia grade 2, CIN-2, or more). Low-grade lesions (CIN-1) are not systematically treated but rather monitored yearly to detect any progression to high-grade lesions.

Genital warts caused by non-oncogenic HPVs (often referred to as ‘low-risk’, LR, HPVs) can be removed by surgery or treated with bi- and trichloroacetic acid, cryotherapy or other treatments [28].

## HPV vaccination

1  
2 There are currently three licensed vaccines: a bivalent vaccine (Cervarix<sup>®</sup>) targeting HPV16  
3  
4 and HPV18 (together accounting for 70% of cervical cancers [1]), a quadrivalent vaccine  
5  
6 (Gardasil<sup>®</sup>) that additionally targets HPV6 and HPV11 (non-oncogenic, but highly prevalent and  
7  
8 associated to benign proliferative lesions) and, since 2014, a nonavalent vaccine (Gardasil 9<sup>®</sup>)  
9  
10 that targets five more oncogenic types (HPV31, HPV33, HPV45, HPV52, and HPV58, which  
11  
12 altogether account for 20% of cervical cancers [24]). These vaccines succeed in eliciting a  
13  
14 protective immune response against new infections by the targeted viruses, and are used  
15  
16 throughout the world, albeit with wide variation in coverage (for reviews, see e.g. [29, 30]).  
17  
18  
19  
20

21  
22 Vaccination campaigns in France started in 2006 but with limited coverage: it reached 28.5% in  
23  
24 2008 [31] and has been decreasing ever since [32]. The vaccine is recommended for girls from  
25  
26 11 to 14 years of age, currently with a vaccination scheme of two doses with a six months  
27  
28 interval. A catch-up is organised for girls aged 15-19 years, with a three-doses vaccination  
29  
30 scheme. Vaccination is reimbursed by the French Social Security but is not mandatory. It is also  
31  
32 recommended for men who have sex with men (MSM) as well as for immuno-compromised  
33  
34 people [32]. Vaccination is now the primary prevention strategy against cervical cancers.  
35  
36  
37  
38

## Screening

39  
40 In France, the secondary prevention strategy against cervical cancer is routine individual  
41  
42 cytology-based screening for pre-cancerous and cancerous cervical lesions in women between  
43  
44 25 and 65 years. Cytology can also be performed in younger women if they report risk factors  
45  
46 for cervical cancer (multiple partners, chronic STIs or HIV infection [32]). Detection of oncogenic  
47  
48 HPVs is proposed for triage in case of abnormal cytology (i.e. high-grade or low-grade  
49  
50 squamous intraepithelial lesion, HSIL and LSIL respectively, or Atypical Squamous Cells of  
51  
52 Undetermined Significance, ASCUS).  
53  
54  
55  
56  
57

## Primary objectives

58  
59 The first primary objective is to decipher the kinetics and ecology of cervical HPV infections in  
60  
healthy young women, i.e. follow the population dynamics of the virus, the target epithelial cells,  
For peer review only - <http://bmjopen.bmj.com/site/about/guidelines.xhtml>

and the immune effectors.

The second primary objective is to characterise the diversity of genital HPVs in young women in the region of Montpellier in relationship with their lifestyle, vaccination status, vaginal microbiota, and human genetics.

### **Secondary objectives**

A secondary objective is to characterise the acquisition and clearance dynamics of cervical HPV infections as a function of viral diversity, host immunity, vaginal microbiota and human genetics.

A final objective is to investigate variations in genetic diversity of HPVs during cervical infections.

## **Methods and analysis**

### **Participants**

The study population is composed of young women at risk of HPV infection. The age class was chosen because the prevalence of HPV is the highest (24% worldwide [4] and is approximately 25% in France [2]). Inclusion of younger women would have raised technical issues because of the requirement for parental consent.

Women are recruited through a social media page, and through posters and leaflets distributed at the Universities in Montpellier and at the Montpellier STI screening centre (Centre Gratuit d'Information de Dépistage et de Diagnostic, CeGIDD). The composition of the population visiting the CeGIDD has already been documented in an earlier study [33]. In total, the centre is visited by approximately 3,000 women per year, the majority of which are under 25 years of age (80%). Approximately 40% of the attendants report three or more partners over the last twelve months and approximately 50% report using adequate behaviour for prevention against HIV.

**Inclusion criteria** Participants are women from 18 to 25 years old living in the metropole of Montpellier. They must be sexually active with at least one new partner over the last 12 months. This criteria is fixed to maximise the incidence of new HPV infections. As in any clinical study, participants must be able to and willing to give written informed consent: they must sign an informed consent form, understand the requirements for the study, and be affiliated to a French social security scheme (which is a state requirement).

Women cannot be included in the study if they have a history of HPV-associated pathology (genital warts or cervical lesions), if they are pregnant or intending to become pregnant in the coming year, infected by HIV, undergoing (or planning to undergo) intense medical treatment (biotherapy, chemotherapy, immunosuppression), planning on moving outside the Montpellier metropolitan area within the next 18 months, in a dependency or employment with the sponsor or the investigator, if they participated in a clinical trial involving administration of drugs within the last four weeks or if they belong to a vulnerable group (e.g. children, adults with physical or mental disabilities).

### **Design/setting**

This study has a longitudinal component aimed at deciphering within-host dynamics and a cross-sectional component, aimed at understanding the diversity of HPV infections in young adults in the area of Montpellier, France. The general structure of the study is shown in Fig 1.

If a woman fits the main inclusion criteria, she can go through an inclusion visit ( $V_1$ ) with a physician (gynaecologist or midwife) at the CeGIDD. During this visit, she presents the study and checks all inclusion criteria before asking the participant to read and sign the informed consent form. Participants then undergo a medical consultation during which a number of samples are collected (see below). They then fill out health and lifestyle questionnaires and are given cotton-flocked swabs for self-sampling at home the next visit, along with instructions on how to fill in weekly questionnaires through an online form (these are performed throughout the study).

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

An appointment is scheduled four weeks later for the Results visit ( $V_2$ ), where the cervical cytology results are communicated. We collect some samples and provide more self-sample swabs for home collection.

The next return visits ( $V_i$ , where  $i > 2$ ) are as follows:

- Participants with a positive DEIA HPV test (see below), i.e. infected by an *Alphapapillomavirus*, at  $V_1$  join the HPV positive (HPV+) arm of the study with return visits scheduled every 2 months.
- Participants with a negative DEIA HPV test at  $V_1$  join the HPV negative (HPV-) arm with return visits scheduled every 4 months.
- HPV- participants infected by an *Alphapapillomavirus* move to the HPV+ arm.

Intervals between visits are based on earlier results showing that HPV infections last from 9 to 18 months on average depending on the HPV type [5, 7–9] and that a follow-up of 4 months yields results that are difficult to analyse [21]. The longer interval in the HPV- arm is based on the estimated incidence for HPV genital infections in young women, which is greater than 30% [34, 35].

Participants in the HPV- arm are followed until month 32 of the study.

Participants in the HPV+ arm are followed until they clear the infection or until they have been infected for 24 months (after which we consider that the infection is persistent). Clearance is defined as being negative at two visits in a row for the first HPV type detected in the follow-up.

In between these visits to the CeGIDD, participants are asked to perform regular (every week for HPV+ and every second week for HPV-) self-samples using vaginal swabs, along with a measure of vaginal pH and filling a short questionnaire. Self-samples are stored in the participants' freezer and brought back at every visit.

The study will end with the last HPV+ participant having cleared the infection or been infected for 24 months.

## Patients and public involvement

Since all participants are healthy, they are referred to as participants rather than patients. As in any longitudinal study, ensuring participant commitment is challenging. To achieve this goal, we have set up a compensation of 40 EUR per visit and an additional 10 EUR in case of a complete follow-up. Furthermore, participants who have answered a sufficient number of questionnaires and brought back a sufficient number of self samples will get a 100 EUR bonus at the end. Overall, a participant performing 12 return visits would gain a total compensation of 650 EUR.

Participants did not play a role in the design of this study.

Results of the study will be disseminated to participants who have left the study and to the general public via an email newsletter in French.

## Visits

The summary of the visit schedule and of the samples collected at each visit is shown in Table 1.

### Inclusion visit (V1)

This visit takes place at the CeGIDD and is scheduled by the Clinical Research Technician (TEC) via phone or email.

Women meet a study investigator, who explains the goals and requirements of the study. The physician also checks that the inclusion criteria are met. If so, after a general discussion, the informed consent forms are signed.

The female physician/midwife performs a general exam and then a gynaecological exam during which the following samples are taken:

- vaginal pH cotton swab (EcoCare™),
- vaginal swab (Copan ESwab™) in 1mL Amies liquid for DNA extraction and microbiota analysis,
- vaginal swab (Copan ESwab™) in 1mL of RNA preservation medium,
- ophthalmic sponge (Weck-cel®) to collect cervical secretions for cytokines analysis,
- cervical smear in 20mL of Thinprep® (Preservcyt® liquid) for HPV and HSV assays, and cytology evaluation.

Following the gynaecological consultation, the participant meets with a nurse to measure body temperature, blood pressure and draw 20mL of blood (a 5mL tube for SNPs sequencing, a 10mL tube for immunophenotyping and a 5mL tube for HPV antibody titration). For the longitudinal study, the nurse provides the participant with 3 self-sampling kits, 3 pH strips, a freezer box to bring back to the next visit, as well as instructions on how to perform the home sampling and store the samples in her personal freezer until the next visit.

If the participant has not been tested for a STI in the last 3 months, the nurse draws an additional blood tube of 5mL to test for STIs (HIV, HCV, HBV) and collects vaginal self-samples for chlamydiae and gonorrhoea detection. Syphilis testing is prescribed to participants who meet the STI clinic's guidelines.

Finally, the participant meets with the TEC to fill in questionnaires #1 (inclusion visit) and #3 (home). The TEC answers any remaining questions, explains how to fill the home questionnaires (#3) and sets an appointment for the Results visit.

### **Results visit (V2)**

During this visit, the participants are given the result of cervical lesion screening using the liquid cytology (normal, ASCUS, LSIL or HSIL). Participants with a high-grade lesion (HSIL) exit the study and are referred to the gynaecology service of the CHU of Montpellier.

During this visit, the physician/midwife collects additional samples: 2 vaginal swabs for DNA and RNA analysis, and a cervical smear in 10mL of PBS (to confirm HPV status and perform flow

cytometry analyses).

1  
2  
3 The participant fills in questionnaires #2 (for return visits) and #3 (home). An appointment for  
4  
5 the next visit is set and swabs for home self-sampling are given.  
6  
7

#### 8 9 **Return visits ( $V_i$ )**

10  
11 These visits only occur in the longitudinal study.  
12  
13

14  
15 **HPV- arm** Participants uninfected by HPV visit the clinic every 4 months until month 26. During  
16  
17 these visits, the same samples as in the inclusion visit ( $V_1$ ) are collected by the  
18  
19 physician/midwife except for the cervical smear, which is put in PBS instead of Thinprep.  
20  
21

22  
23 The nurse only draws blood if a screening test for STIs other than HPV is required. The  
24  
25 participant then fills in questionnaires #2 and #3 and an appointment is set for the next visit in  
26  
27 16 weeks.  
28  
29

30  
31 If an HPV infection is detected in the cervical smear collected during this visit, the participant  
32  
33 moves to the HPV+ arm and the TEC contacts the participant to move her appointment forward.  
34  
35

36  
37 **HPV+ arm** Participants infected by HPV visit the clinic every 2 months. They cannot switch arm  
38  
39 and will remain in the HPV+ arm until clearance or the end of the study. During the visits, the  
40  
41 same samples as in the inclusion visit ( $V_0$ ) are collected by the physician/midwife except for the  
42  
43 cervical smear, which is put in PBS instead of Thinprep.  
44  
45

46  
47 The nurse then draws 5mL of blood for HPV antibody titration. If this is the first HPV+ visit  
48  
49 following an HPV- visit, the nurse also draws 10mL of blood for immunophenotyping. Finally, if a  
50  
51 test for additional STIs is needed, the nurse draws 5mL of blood and collects vaginal self-  
52  
53 samples for STI detection.  
54  
55

56  
57 Importantly, if the participant has been infected by a HR-HPV for more than 12 months and  
58  
59 cytology has not been performed within the last 12 months, the cervical smear is put in  
60  
Thinprep<sup>®</sup> fixation medium, instead of PBS, for cytological analysis (cervical lesion screening).



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Finally, the participant fills in questionnaires #2 and #3, receives self-samples for home collection and an appointment is set for the next visit in 8 weeks.

## Endpoints

The primary endpoint for the study is the inclusion and follow-up of HPV-infected women in order to describe the kinetics of HPV virus load, and the associated immune response.

Secondary endpoints are the characterisation of the interactions between the course of the infection (e.g. duration), the HPV type(s), the abundance and taxonomic diversity of bacteria, fungi and viruses in the vaginal microbiota, human genetics (SNPs) and basal immunological status.

## Technical procedures

### DNA extraction

DNA extraction from cervical smears will be performed using Nuclisens EasyMAG from Biomerieux or an equivalent protocol. For the microbiota analyses, special kits involving physical (via beads) and/or enzymatic breaking of the cellular barrier will be favoured following standard protocols to study the vaginal microbiome [36], e.g. the MagAttract<sup>®</sup> PowerMicrobiome<sup>®</sup> DNA/RNA kit from Qiagen.

### HPV detection, typing and quantification

The participants' infection status (HPV+ or HPV-) will be assessed using the DEIA test, which is based on a PCR of the short SPF10 amplicon [37] and detects all *Alphapapillomaviruses* with great sensitivity.

If the DEIA test is positive, HPVs will be typed using the LiPA<sub>25</sub> kit, which is based on the same SPF10-PCR, and has a lower detection threshold compared to other hybridisation-based typing methods [38].

The reason for basing the detection on the DEIA rather than the LiPA<sub>25</sub> is that some

*Alphapapillomavirus* may be detected by DEIA but not genotyped by LiPA and also that the

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

DEIA is more sensitive than the LiPA. If the DEIA is positive and the LiPA<sub>25</sub> is negative, typing will be performed by sequencing the product of a PGMY09/11 PCR [39], which targets another region of the HPV genome than the SPF10 PCR.

The quantification of HPV DNA genome copy number in the samples will be performed using the protocol set up by Micalessi et al. [40].

### **Cytokine titration**

Cytokines can be used as markers of immune activation or immunosuppression and can also inform us on which components of the immune system are involved. Cervical sponges are centrifuged after the addition of 175 $\mu$ L of PBS. Cervical secretions are analysed for a set of 5 to 6 cytokines levels using the Meso Scale Discovery (MSD) Multiplex ELISA platform, which has a low detection threshold and a slowly saturating dose-response curve. Based on earlier results [41, 42], we will first investigate a large panel of 20 cytokines (IFN- $\alpha$ 2a, IFN- $\gamma$ , IL-1 $\alpha$ , IL-5, IL-6, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-23, IL-25, IP-10, MCP-1, MIP-1 $\alpha$ , MIP-3 $\alpha$ , MIP-3 $\beta$ , TNF- $\alpha$ , TNF- $\beta$ ) to choose the most relevant ones for a longitudinal follow-up.

### **Flow cytometry**

Analysing immune cells via flow cytometry is extremely challenging on cells as fragile as the ones from cervical smears. However, several studies suggest that this is feasible [41–43]. Here, we follow the protocol described in [44].

Stainings are performed using a Duraclone custom mix targeting CD45, CD3, CD4, CD8, CD16, CD56, CD69, CD161 and TCR $\gamma\delta$ . The last marker, Live&Dead tests for cellular viability.

Samples are acquired using a Navios flow cytometer (Beckman Coulter, three-laser configuration).

### **Sequencing**

Sequencing will be performed for microbiota profiling. It involves PCR amplification of the V3-V4 region of 16S RNA for bacteria [45] and ITS1 for fungi [46]. We anticipate that the bacteria should belong to the operational taxonomic units (OTU) described in the five community state

types found in vaginal communities [47, 48]. The virome will also be explored using shotgun sequencing and rolling circle PCR amplification [49]. Human genetics are explored using chip sequencing for SNPs.

## Statistical analyses

### Times series analyses

The core results of the study will come from the longitudinal follow-up of infected women, which will generate time series, i.e. a set of values collected from the same individual over time (Figure 2). There will be several time series per individual (virus load, number of immune cells, cytokine and antibody levels). These time series will be used to fit mathematical viral kinetics models that describe the interaction between viruses, host target cells (here, in the case of HPV, keratinocytes) and the immune response. These models are commonly developed for viral infections [13, 50–52], including those caused by HPVs [53]. We anticipate our follow-up to yield adequate data for such a fit based on the estimated duration of HPV infections (9 to 18 months [5, 7–9]). Furthermore, the weekly self-samples allow us to increase the resolution if necessary.

We will use non-linear mixed effect models [54] to jointly analyse time series from all participants. More precisely, we will rely on *R* packages such as nlme [55] or lme4 [55]. Note that, in addition to estimating model parameters (e.g. life-expectancy of infected cells or virion production rate of infected cells), this approach also allows us to compare biological models using statistical tools based on model likelihood such as Akaike Information Criterion. For an example of such analysis in the case of HIV, see [51].

### Microbiota dynamics

The composition of the vaginal microbiota has already been described and shown to exhibit considerably less diversity than the gut microbiota [47]. The dynamics of this microbiota has also been studied and shown to closely follow menstrual cycles [48].

Using the time series of OTU abundances (measured via 16S RNA sequencing and qPCR) we

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

will infer interaction parameters by assuming an underlying Lotka-Volterra competition model [56]. This work will include time series analysis techniques (e.g. auto-correlation or local similarity analysis) and statistical inference methods in order to infer community structure and interactions from the next-generation sequencing (NGS) datasets [57]. Finally, statistical methods from ecology will also be used to study community diversity (e.g diversity indices) and community assembly, such as cluster and ordination analyses [58].

### **Genome Wide Association Studies**

We will use human single nucleotide polymorphisms (SNPs) inferred by chip sequencing to look for genetic determinants of key traits (e.g. microbiota composition or HPV infection duration). This is classically done by performing a Genome Wide Association Study (GWAS), which is a complex regression method designed for situations where there are many explanatory variables (here millions of SNPs) for a single trait of interest. GWAS will be performed using classical methods [59]. Earlier GWAS studies have been applied to HPV infections for instance to test for determinants to the ability to seroconvert following infection [60] and cervical cancer (see [61] for a review). Here, our expected sample ( $N = 300$  women) is limited but SNPs with large effects have been detected by studies with comparable sizes [62].

### **Additional analyses**

For all collected variables, descriptive statistics will be calculated according to the level of measurement. For metric variables these statistics can be mean and standard deviation as well as quantiles and more robust statistics [63]. In case of categorical variables group proportions and contingency tables are prepared.

Univariate inferential statistics follow a descriptive analysis. Generally, parametric testing procedures are preferred to non-parametric tests, as the former have higher power. That is why, for metric variables, we will first check whether the data can be assumed to be normally distributed. For normally distributed variables, ANOVA statistics are done to detect differences between groups. In case of significance, post-hoc analysis (Tukey test) are planned to reveal pairwise differences. If the data are not normally distributed or ordinally scaled, non-parametric

analyses will be used. These contain the Kruskal-Wallis test and the Wilcoxon test as a post-hoc test with an appropriate correction of the significance level. Since the cell counts are expected to be small, Fisher's exact test will be performed for contingency tables instead of the asymptotic  $\chi^2$  test for categorical variables.

### Sample size calculation

The study will enrol a total of  $N = 300$  women, with  $N = 150$  in a longitudinal study and  $N = 150$  in a cross-sectional study. The goal of the longitudinal study is to follow 75 women longitudinally, preferentially before they are infected (see above). For the following calculations, we assumed a high percentage of lost during follow-up (30%).

With 150 enrolments and considering that the prevalence of HPV infection in young women is  $\approx 60\%$  (based on our preliminary data) and 30% of lost to follow-up, we expect to detect (and successfully follow) 63 infections at inclusion [CI95: 51 – 75, assuming a binomial distribution to calculate the 95% confidence interval].

Among women who are uninfected at the first visit and considering the yearly incidence being close to 30% [64], we expect 12 [CI95: 6 – 20] to be infected during the first year of follow-up (still assuming 30% of lost to follow-up).

In the end, with 150 enrolments and assuming a high percentage of lost to follow-up (30%), we expect to successfully follow 75 [CI95: 56 – 95] women infected at different stages of HPV infection: beginning, during and end.

This will be made possible by the probability of transmission of HPV, which is estimated to be  $\approx 90\%$  without condom use and still high with condom use ( $\approx 40\%$ ) [34].

Finally, regarding potential interference with the HPV vaccines, we do not anticipate any

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52

significant problem for two reasons. First, as mentioned above, the vaccine coverage is low in France [32]. Second, and more importantly, the vaccines only target few HPV types, thus leaving open the possibility of infection by dozens of types. Furthermore, studying the kinetics of a non-vaccine HPV type in a vaccinated woman will be extremely informative, e.g. to detect any potential cross-reactivity [65].

To run cross-sectional analyses (especially on the microbiota and human genetics), we will enrol  $N = 150$  additional women who will only perform the inclusion and the results visits. This sample size was chosen to reach that of earlier GWAS studies [61, 62].

## 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

### Trial governance

#### Sponsor

This study is sponsored by the Centre Hospitalier Universitaire (CHU) of Montpellier. The CHU is involved in the implementation of the trial, legal/ethical submissions (see below for details on Ethics approval) and implementing the clinical database (eCRF), which is hosted by Ennov-Clinical (ClinSight). The CHU is not involved in the analysis or interpretation of the data. The CHU of Montpellier performs regular quality control assessments. A clinical research assistant will visit the CeGIDD every 4 months to ensure that implementation is in accordance with the protocol. The CHU has taken out insurance from the Société hospitalière d'assurances mutuelles, 18, rue Edouard Rochet-6 9372 Lyon cedex 08 (contract number 138983) through the full research period, covering its own civil liability and that of any agent (clinical or research staff), in accordance with article L.1121-10 of the French Public Health Code.

#### Scientific committee

The scientific committee comprises the study investigators, clinicians, scientific experts and representatives of the sponsor. The committee meets yearly and is responsible for following research progress, monitoring compliance with good clinical practices and patient safety. It can also decide relevant modification of the protocol. Requests from third parties to access data

collected during the study will be evaluated by the committee.

### **Monitoring**

Monitoring is performed during the whole study at CeGIDD according to the sponsor specific SOP. Routine monitoring visits are made by the monitors designated by the sponsor to check compliance with the protocol, the completeness, accuracy and consistency of the data, and adherence to GCP. The principal investigator ensures that eCRFs are completed in a timely manner and must allow periodical access to eCRFs, patient records, drug logs, and all other study-related documents and materials. The frequency of monitoring visits is determined by factors such as study design and the site enrolment requirements but visits will normally occur at least once every 4 months.

### **Trial registration**

The trial has been registered to ClinicalTrials.gov on 27 Oct 2016 with ID number NCT02946346.

## **Discussion**

### **Expected results**

Acute infections by HPVs are important to study because vaccination is most effective when performed before the first infection. However, we currently know very little about the early stages of HPV infections. This clinical study will give us an unprecedented level of detail into the natural history of HPV infections in young women. Variations in virus load over time have been studied but in the context of cervical cancer in older women [66]. In addition, we will also describe the nature and the dynamics of the immune response (local immune cells and cytokines, circulating anti-HPV antibodies) and of the vaginal microbiota. Beyond these kinetics, we will also have access to data such as infection clearance or not in 24 months, presence of more than one HPV type or coinfection by other STIs.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

These data will be analysed in the light of numerous cofactors. One of the most important will be human genetics, with the sequencing of millions of SNPs. Others will be related to the sexual behaviour (number of partners, contraception methods, sexual practices) and general lifestyle. We, therefore, expect broader insights regarding sexual health in young women.

### **Practical and operational issues**

One of the main practical challenges resides in the analysis of cervical smears by flow cytometry. Indeed, the tissues are known to be fragile, adhesive and auto-fluorescent. Even though standard protocols now exist [44], they require processing fresh samples in less than 2 hours.

Another potential issue has to do with contamination by HPV DNA between samples, which are frequent in the HPV field due to the robustness of the virions and the sensitivity of the tests. To certify our ability to control for these, we have entered the 2017 GLOBAL HPV DNA Proficiency Panel from the WHO HPV LabNet [67].

Regarding the enrolment of the participants, we do not expect issues with enrolling 150 women in 28 months for the longitudinal study and 150 for the cross-sectional study. This is due to the number of visitors of the centre who fit the inclusion criteria (more than 3,000 per year) and because of earlier high participation rates in the same population ([33] enrolled 1381 participants in 5 months for their study).

Concerning the follow-up, the high incidence rate of HPV can also lead to transient carriage, i.e. women who are positive for a type only at a single visit. This has been observed for instance in longitudinal studies with a tight follow-up interval [21]. To control for this, we will run the HPV detection test on the cells from the cervical smear after washing with RPMI.



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**Table 1: Summary of the visit schedules and samples take.** The cross-sectional study only includes the first two columns (V1 and V2). The  $\alpha$  indicate samples taken at visits. + participants infected by a HR-HPV for 12 month will have one PBS smear replaced by a Thinprep® smear to perform a cytology and check for lesions.  $\triangleleft$  this sample is only taken at the first HPV+ visit of a formerly HPV- participant. \* STI detection will be performed at inclusion unless the participant has been tested within the last 3 months and during the study every 6 months if a new partner has been reported or upon request.

|                                          | Inclusion<br>(V1) | Results<br>(V2) | Return<br>(Vi, with i > 2) |            |
|------------------------------------------|-------------------|-----------------|----------------------------|------------|
| Participants                             | all               | all             | HPV+                       | HPV-       |
| Time                                     | day 0             | + 4 weeks       | + 8 weeks                  | + 16 weeks |
| Eligibility                              | ☐                 |                 |                            |            |
| Consent                                  | ☐                 |                 |                            |            |
| Gynecological consult                    | ☐                 | ☐               | ☐                          | ☐          |
| Vaginal pH cotton swab                   | ☐                 | ☐               | ☐                          | ☐          |
| 2 vaginal swab samples (Copan<br>ESwab™) | ☐                 | ☐               | ☐                          | ☐          |
| 1 ophthalmological sponge sample         | ☐                 |                 | ☐                          | ☐          |
| 1 cervical smear in Thinprep® (cytology) | ☐                 |                 | +                          |            |
| 1 cervical smear in PBS                  |                   | ☐               | +                          | ☐          |
| Blood sampling (HPV antibodies)          | ☐                 |                 | ☐                          |            |
| Blood sampling (sequencing)              | ☐                 |                 |                            |            |
| Blood sampling (immunophenotyping)       | ☐                 |                 | △                          |            |
| Other STI detection                      | *                 | *               | *                          | *          |
| Questionnaire #1 (inclusion)             | ☐                 |                 |                            |            |
| Questionnaire #2 (visit)                 |                   | ☐               | ☐                          | ☐          |
| Questionnaire #3 (home)                  | ☐                 | ☐               | ☐                          | ☐          |
| Returning self-sampling samples          |                   | ☐               | ☐                          | ☐          |
| Serious Adverse Event collection         |                   | ☐               | ☐                          | ☐          |

## Abbreviations

1  
2  
3 ANOVA: Analysis of variance,

4  
5 ASC-US: Atypical squamous cells of undetermined significance,

6  
7 CD: Cluster of differentiation,

8  
9 CI95: 95% Confidence interval,

10  
11 CeGIDD: Centre Gratuit d'Information de Dépistage et de Diagnostic,

12  
13  
14 CHU: Centre Hospitalier Universitaire,

15  
16 CIN: Cervical intraepithelial Neoplasia,

17  
18 ELISA: enzyme-linked immunosorbent assay,

19  
20  
21 GWAS: Genome Wide Association Study,

22  
23 HIV: Human Immunodeficiency Virus,

24  
25 HPV: Human Papillomavirus,

26  
27 HR: high-risk,

28  
29 ITS: Internal Transcribed Spacer,

30  
31  
32 HSIL: High grade Squamous Intraepithelial Lesion,

33  
34 LR: low-risk,

35  
36  
37 LSIL: Low grade Squamous Intraepithelial Lesion,

38  
39 NGS: Next Generation Sequencing,

40  
41 OTU: Operational Taxonomic Unit,

42  
43  
44 PBMC: Peripheral Blood Mononuclear Cell,

45  
46 PBS: Phosphate Buffered Saline,

47  
48 RPMI: Roswell Park Memorial Institute medium,

49  
50  
51 SNP: Single Nucleotide Polymorphism,

52  
53 TCR: T-cell receptor,

54  
55  
56 WHO: World Health Organisation.

## Trial status

The study began on Oct 1, 2016 and the first inclusion was on Nov 3, 2016. On Jun 23, 2018, 89 participants have been included in the longitudinal study. Inclusions in the longitudinal study will continue until March 2019 and the study is expected to last until Aug 2021.

## Conflicts of interests

The authors have read and understood BMJ policy on declaration of interests and declare that they have no competing interests.

## Funding

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement No 648963). The authors acknowledge further support from the Centre National de la Recherche Scientifique (CNRS), the Institute de Recherche pour le Développement (IRD) and the Centre Hospitalier Universitaire (CHU) of Montpellier.

## Data statement

All personal and identifying information collected from participants are kept in a secure place at the CeGIDD during the duration of the trial and will be destroyed at the end of the study. The final raw dataset will be accessible only to the sponsor (CHU) and the chief scientist's (SA) team. Anonymous data will be available to external parties upon approval of both the sponsor and the scientific committee. All publications will be made green or gold open access and the corresponding data will be provided as supplementary material or via a public repository (e.g. Zenodo), provided that there is no conflict with ethical guidelines.

## Author contributions

Samuel Alizon, Carmen Lia Murall and Massical Rahmoun were the major contributors in the conception of the protocol. Samuel Alizon wrote the initial version of the manuscript. Christian

Selinger, Monique Baldellou, Claire Bernat, Marine Bonneau, Vanina Boué, Mathilde Buisson, Guillaume Christophe, Giuseppe D'Auria, Florence De Taroni, Vincent Foulongne, Rémy Froissart, Christelle Graf, Sophie Grasset, Soraya Groc, Christophe Hirtz, Audrey Jaussent, Frédérique Lorcy, Eric Picot, Marie-Christine Picot, Jacques Ravel, Jacques Reynes, Thérèse Rousset, Aziza Seddiki, Martine Teirlinck, Vincent Tribout, Édouard Tuailon, Tim Waterboer, Nathalie Jacobs, Ignacio G Bravo, Michel Segondy and Natalie Boule were involved in the conception of the protocol, in the implementation of the study and read and approved the final manuscript.

## **Ethics approval and consent to participate**

The PAPCLEAR trial obtained favourable opinions from the Comité de Protection des Personnes (CPP) Sud Méditerranée I on May 11, 2016 (CPP number 16 42, reference number ID RCB 2016-A00712-49); from the Comité Consultatif sur le Traitement de l'Information en matière de Recherche dans le domaine de la Santé (CCTIRS) on July 12, 2016 (reference number 16.504); and from the Commission Nationale Informatique et Libertés (CNIL) on Dec 16, 2016 (reference number MMS/ABD/AR1612278, decision number DR-2016-488). This trial was authorised by the Agence Nationale de Sécurité du Médicament et des Produits de Santé (ANSM) on July 20, 2016 (reference 20160072000007).

The protocol has been modified since its initial version and the latest modification was accepted by the CPP on Dec 12, 2018.

All participants in the study will sign an informed consent form prior to participation.

## **Acknowledgements**

We thank all the study participants and the CeGIDD staff for their commitment to the study. We also thank reviewers and, in particular, Dr. Andrew Brouwer for his meticulous reading of the manuscript.

## References

1. Tota, J.E., Chevarie-Davis, M., Richardson, L.A., Devries, M., Franco, E.L.: Epidemiology and burden of HPV infection and related diseases: implications for prevention strategies. *Prev Med* **53 Suppl 1**, 12–21 (2011). doi:[10.1016/j.ypmed.2011.08.017](https://doi.org/10.1016/j.ypmed.2011.08.017)
2. Monsonego, J., Zerat, L., Syrjänen, K., Zerat, J.C., Smith, J.S., Halfon, P.: Prevalence of genotype-specific HPV infection among women in France: implications for screening and vaccination. *Gynecol Obstet Fertil* **41**(5), 305–313 (2013). doi:[10.1016/j.gyobfe.2013.03.003](https://doi.org/10.1016/j.gyobfe.2013.03.003)
3. Brun-Micaleff, E., Coffy, A., Rey, V., Didelot, M.-N., Combecal, J., Doutre, S., Daurès, J.-P., Segondy, M., Boulle, N.: Cervical cancer screening by cytology and human papillomavirus testing during pregnancy in french women with poor adhesion to regular cervical screening. *J Med Virol* **86**(3), 536–45 (2014). doi:[10.1002/jmv.23764](https://doi.org/10.1002/jmv.23764)
4. Bruni, L., Diaz, M., Castellsagué, X., Ferrer, E., Bosch, F.X., de Sanjosé, S.: Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. *J Infect Dis* **202**(12), 1789–99 (2010). doi:[10.1086/657321](https://doi.org/10.1086/657321)
5. Insinga, R.P., Dasbach, E.J., Elbasha, E.H., Liaw, K.-L., Barr, E.: Incidence and duration of cervical human papillomavirus 6, 11, 16, and 18 infections in young women: an evaluation from multiple analytic perspectives. *Cancer Epidemiol Biomarkers Prev* **16**(4), 709–15 (2007). doi:[10.1158/1055-9965.EPI-06-0846](https://doi.org/10.1158/1055-9965.EPI-06-0846)
6. Woodman, C.B.J., Collins, S.I., Young, L.S.: The natural history of cervical HPV infection: unresolved issues. *Nat Rev Cancer* **7**(1), 11–22 (2007). doi:[10.1038/nrc2050](https://doi.org/10.1038/nrc2050)
7. Rodríguez, A.C., Schiffman, M., Herrero, R., Wacholder, S., Hildesheim, A., Castle, P.E., Solomon, D., Burk, R., Proyecto Epidemiológico Guanacaste Group: Rapid clearance of human papillomavirus and implications for clinical focus on persistent infections. *J Natl Cancer Inst* **100**(7), 513–7 (2008). doi:[10.1093/jnci/djn044](https://doi.org/10.1093/jnci/djn044)

- 1  
2  
3  
4  
5  
6  
7  
8  
9
8. Trottier, H., Mahmud, S., Prado, J.C.M., Sobrinho, J.S., Costa, M.C., Rohan, T.E., Villa, L.L., Franco, E.L.: Type-Specific Duration of Human Papillomavirus Infection: Implications for Human Papillomavirus Screening and Vaccination. *J Infect Dis* **197**(10), 1436–1447 (2008). doi:[10.1086/587698](https://doi.org/10.1086/587698)
- 10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24
9. Ramanakumar, A.V., Naud, P., Roteli-Martins, C.M., de Carvalho, N.S., de Borja, P.C., Teixeira, J.C., Blatter, M., Moscicki, A.-B., Harper, D.M., Romanowski, B., Tying, S.K., Ramjattan, B., Schuind, A., Dubin, G., Franco, E.L.: Incidence and duration of type-specific human papillomavirus infection in high-risk HPV-naïve women: results from the control arm of a phase II HPV-16/18 vaccine trial. *BMJ Open* **6**(8), 011371 (2016). doi:[10.1136/bmjopen-2016-011371](https://doi.org/10.1136/bmjopen-2016-011371)
- 25  
26  
27  
28  
29  
30  
31  
32  
33  
34
10. Houlihan, C.F., Baisley, K., Bravo, I.G., Kapiga, S., de Sanjosé, S., Changalucha, J., Ross, D.A., Hayes, R.J., Watson-Jones, D.: Rapid acquisition of HPV around the time of sexual debut in adolescent girls in Tanzania. *Int J Epidemiol* **45**(3), 762–773 (2016). doi:[10.1093/ije/dyv367](https://doi.org/10.1093/ije/dyv367)
- 35  
36  
37  
38  
39  
40
11. Alizon, S., Murall, C.L., Bravo, I.G.: Why Human Papillomavirus Acute Infections Matter. *Viruses* **9**(10), 293 (2017). doi:[10.3390/v9100293](https://doi.org/10.3390/v9100293)
- 41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54
12. Herrero, R., Wacholder, S., Rodríguez, A.C., Solomon, D., González, P., Kreimer, A.R., Porras, C., Schussler, J., Jiménez, S., Sherman, M.E., Quint, W., Schiller, J.T., Lowy, D.R., Schiffman, M., Hildesheim, A., Costa Rica Vaccine Trial Group: Prevention of persistent human papillomavirus infection by an HPV16/18 vaccine: a community-based randomized clinical trial in Guanacaste, Costa Rica. *Cancer Discov* **1**(5), 408–19 (2011). doi:[10.1158/2159-8290.CD-11-0131](https://doi.org/10.1158/2159-8290.CD-11-0131)
- 55  
56  
57  
58  
59  
60
13. Canini, L., Perelson, A.S.: Viral kinetic modeling: state of the art. *J Pharmacokinet Pharmacodyn* **41**(5), 431–443 (2014). doi:[10.1007/s10928-014-9363-3](https://doi.org/10.1007/s10928-014-9363-3)
14. Stanley, M.: Immune responses to human papillomavirus. *Vaccine* **24**(S1), 16–22 (2006).

doi:[10.1016/j.vaccine.2005.09.002](https://doi.org/10.1016/j.vaccine.2005.09.002)

15. Ferenczy, A., Franco, E.: Persistent human papillomavirus infection and cervical neoplasia. *Lancet Oncol* **3**(1), 11–6 (2002)
16. zur Hausen, H.: Review: Papillomaviruses — to Vaccination and Beyond. *Biochemistry* **73**(5), 498–503 (2008). doi:[10.1134/S0006297908050027](https://doi.org/10.1134/S0006297908050027)
17. Einstein, M.H., Schiller, J.T., Viscidi, R.P., Strickler, H.D., Coursaget, P., Tan, T., Halsey, N., Jenkins, D.: Clinician's guide to human papillomavirus immunology: knowns and unknowns. *Lancet Infect Dis* **9**(6), 347–56 (2009). doi:[10.1016/S1473-3099\(09\)70108-2](https://doi.org/10.1016/S1473-3099(09)70108-2)
18. Van Hede, D., Langers, I., Delvenne, P., Jacobs, N.: Origin and immunoescape of uterine cervical cancer. *Presse Med* **43**(12P2), 413–421 (2014). doi:[10.1016/j.lpm.2014.09.005](https://doi.org/10.1016/j.lpm.2014.09.005)
19. Stanley, M.: Immunology of HPV infection. *Curr Obstet Gynecol Rep* **4**(4), 195–200 (2015). doi:[10.1007/s13669-015-0134-y](https://doi.org/10.1007/s13669-015-0134-y). Accessed 2017-03-20
20. Gao, W., Weng, J., Gao, Y., Chen, X.: Comparison of the vaginal microbiota diversity of women with and without human papillomavirus infection: a cross-sectional study. *BMC Infect Dis* **13**, 271 (2013). doi:[10.1186/1471-2334-13-271](https://doi.org/10.1186/1471-2334-13-271)
21. Brotman, R.M., Shardell, M.D., Gajer, P., Tracy, J.K., Zenilman, J.M., Ravel, J., Gravitt, P.E.: Interplay between the temporal dynamics of the vaginal microbiota and human papillomavirus detection. *J Infect Dis* **210**(11), 1723–33 (2014). doi:[10.1093/infdis/jiu330](https://doi.org/10.1093/infdis/jiu330)
22. Koutsky, L.A., Ault, K.A., Wheeler, C.M., Brown, D.R., Barr, E., Alvarez, F.B., Chiacchierini, L.M., Jansen, K.U., Proof of Principle Study Investigators: A controlled trial of a human papillomavirus type 16 vaccine. *N Engl J Med* **347**(21), 1645–51 (2002). doi:[10.1056/NEJMoa020586](https://doi.org/10.1056/NEJMoa020586)
23. Riethmuller, D., Jacquard, A.-C., Lacau St Guily, J., Aubin, F., Carcopino, X., Pradat, P., Dahlab, A., Pr  tet, J.-L.: Potential impact of a nonavalent hpv vaccine on the occurrence of hpv-



related diseases in france. BMC Public Health **15**, 453 (2015). doi:[10.1186/s12889-015-1779-1](https://doi.org/10.1186/s12889-015-1779-1)

- 1  
2  
3 24. Joura, E.A., Giuliano, A.R., Iversen, O.-E., Bouchard, C., Mao, C., Mehlsen, J., Moreira,  
4 E.D. Jr, Ngan, Y., Petersen, L.K., Lazcano-Ponce, E., Pitisuttithum, P., Restrepo, J.A., Stuart,  
5 G., Woelber, L., Yang, Y.C., Cuzick, J., Garland, S.M., Huh, W., Kjaer, S.K., Bautista, O.M.,  
6 Chan, I.S.F., Chen, J., Gesser, R., Moeller, E., Ritter, M., Vuocolo, S., Luxembourg, A., Broad  
7 Spectrum HPV Vaccine Study: A 9-valent HPV vaccine against infection and intraepithelial  
8 neoplasia in women. N Engl J Med **372**(8), 711–23 (2015). doi:[10.1056/NEJMoa1405044](https://doi.org/10.1056/NEJMoa1405044)  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18 25. Murall, C.L., Bauch, C.T., Day, T.: Could the human papillomavirus vaccines drive  
19 virulence evolution? Proc Biol Sci **282**, 20141069 (2015). doi:[10.1098/rspb.2014.1069](https://doi.org/10.1098/rspb.2014.1069)  
20  
21  
22  
23  
24 26. Alizon, S., Méthot, P.-O.: Reconciling Pasteur and Darwin to control infectious diseases.  
25 PLoS Biol **16**(1), 2003815 (2018). doi:[10.1371/journal.pbio.2003815](https://doi.org/10.1371/journal.pbio.2003815)  
26  
27  
28  
29  
30 27. Moscicki, A.-B., Ma, Y., Wibbelsman, C., Darragh, T.M., Powers, A., Farhat, S., Shiboski,  
31 S.: Rate of and Risks for Regression of CIN-2 in adolescents and young women. Obstet  
32 Gynecol **116**(6), 1373–1380 (2010). doi:[10.1097/AOG.0b013e3181fe777f](https://doi.org/10.1097/AOG.0b013e3181fe777f)  
33  
34  
35  
36  
37  
38 28. Buck Jr., H.W.: Warts (genital). BMJ Clin Evid **2015**, 1602 (2015)  
39  
40  
41  
42 29. Herrero, R., González, P., Markowitz, L.E.: Present status of human papillomavirus  
43 vaccine development and implementation. Lancet Oncol **16**(5), 206–16 (2015).  
44 doi:[10.1016/S1470-2045\(14\)70481-4](https://doi.org/10.1016/S1470-2045(14)70481-4)  
45  
46  
47  
48  
49 30. Maver, P.J., Poljak, M.: Progress in prophylactic human papillomavirus (HPV) vaccination  
50 in 2016: A literature review. Vaccine (2018). doi:[10.1016/j.vaccine.2017.07.113](https://doi.org/10.1016/j.vaccine.2017.07.113)  
51  
52  
53  
54  
55 31. Fagot, J.-P., Boutrelle, A., Ricordeau, P., Weill, A., Allemand, H.: HPV vaccination in  
56 France: uptake, costs and issues for the National Health Insurance. Vaccine **29**(19), 3610–6  
57 (2011). doi:[10.1016/j.vaccine.2011.02.064](https://doi.org/10.1016/j.vaccine.2011.02.064)  
58  
59  
60

- 1  
2  
3 32. Ben Hadj Yahia, M.-B., Dervaux, B., Duport, N., Floret, D., Gaillot, J., Heard, I., Jacquet,  
4 A., Le Goaster, C., Lévy-Bruhl, D., Morer, I., Parent du Chatelet, I., Peigue-Lafeuille, H.,  
5 Rumeau-Pichon, C.: Vaccination contre les infections à papilloamvirus. Technical report, Haut  
6 Conseil de la Santé Publique, Paris, France (2014).  
7  
8 <https://www.hcsp.fr/Explore.cgi/avisrapportsdomaine?clefr=454>  
9  
10  
11  
12  
13  
14  
15 33. Clarivet, B., Picot, E., Marchandin, H., Tribout, V., Rachedi, N., Schwartzentruber, E.,  
16 Ledésert, B., Dereure, O., Guillot, B., Picot, M.-C.: Prevalence of Chlamydia trachomatis,  
17 Neisseria gonorrhoeae and Mycoplasma genitalium in asymptomatic patients under 30 years of  
18 age screened in a French sexually transmitted infections clinic. *Eur J Dermatol* **24**(5), 611–6  
19 (2014). doi:[10.1684/ejd.2014.2413](https://doi.org/10.1684/ejd.2014.2413)  
20  
21  
22  
23  
24  
25  
26  
27  
28 34. Winer, R.L., Hughes, J.P., Feng, Q., O'Reilly, S., Kiviat, N.B., Holmes, K.K., Koutsky, L.A.:  
29 Condom use and the risk of genital human papillomavirus infection in young women. *N Engl J*  
30 *Med* **354**(25), 2645–54 (2006). doi:[10.1056/NEJMoa053284](https://doi.org/10.1056/NEJMoa053284)  
31  
32  
33  
34  
35  
36 35. Winer, R.L., Hughes, J.P., Feng, Q., Stern, J.E., Xi, L.F., Koutsky, L.A.: Incident Detection  
37 of High-Risk Human Papillomavirus Infections in a Cohort of High-Risk Women Aged 25-65  
38 Years. *J Infect Dis* **214**(5), 665–75 (2016). doi:[10.1093/infdis/jiw074](https://doi.org/10.1093/infdis/jiw074)  
39  
40  
41  
42  
43  
44 36. Ravel, J., Brotman, R.M., Gajer, P., Ma, B., Nandy, M., Fadrosh, D.W., Sakamoto, J.,  
45 Koenig, S.S., Fu, L., Zhou, X., Hickey, R.J., Schwebke, J.R., Forney, L.J.: Daily temporal  
46 dynamics of vaginal microbiota before, during and after episodes of bacterial vaginosis.  
47 *Microbiome* **1**(1), 29 (2013). doi:[10.1186/2049-2618-1-29](https://doi.org/10.1186/2049-2618-1-29)  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60 37. Kleter, B., van Doorn, L.-J., ter Schegget, J., Schrauwen, L., van Krimpen, K., Burger, M.,

ter Harmsel, B., Quint, W.: Novel Short-Fragment PCR Assay for Highly Sensitive Broad-Spectrum Detection of Anogenital Human Papillomaviruses. *Am J Pathol* **153**(6), 1731–1739 (1998). doi:[10.1016/S0002-9440\(10\)65688-X](https://doi.org/10.1016/S0002-9440(10)65688-X)

38. Geraets, D.T., Struijk, L., Kleter, B., Molijn, A., van Doorn, L.-J., Quint, W.G.V., Colau, B.: The original SPF10 LiPA25 algorithm is more sensitive and suitable for epidemiologic HPV research than the SPF10 INNO-LiPA Extra. *J Virol Meth* **215-216**, 22–29 (2015). doi:[10.1016/j.jviromet.2015.01.001](https://doi.org/10.1016/j.jviromet.2015.01.001)

39. Gravitt, P.E., Peyton, C.L., Alessi, T.Q., Wheeler, C.M., Coutlée, F., Hildesheim, A., Schiffman, M.H., Scott, D.R., Apple, R.J.: Improved amplification of genital Human Papillomaviruses. *J Clin Microbiol* **38**(1), 357–361 (2000)

40. Micalessi, I.M., Boulet, G.A.V., Bogers, J.J., Benoy, I.H., Depuydt, C.E.: High-throughput detection, genotyping and quantification of the human papillomavirus using real-time PCR. *Clin Chem Lab Med* **50**(4), 655–61 (2012). doi:[10.1515/cclm.2011.835](https://doi.org/10.1515/cclm.2011.835)

41. Hunter, P.J., Sheikh, S., David, A.L., Peebles, D.M., Klein, N.: Cervical leukocytes and spontaneous preterm birth. *Journal of Reproductive Immunology* **113**, 42–49 (2016). doi:[10.1016/j.jri.2015.11.002](https://doi.org/10.1016/j.jri.2015.11.002)

42. Shannon, B., Yi, T.J., Perusini, S., Gajer, P., Ma, B., Humphrys, M.S., Thomas-Pavanel, J., Chieza, L., Janakiram, P., Saunders, M., Tharao, W., Huibner, S., Shahabi, K., Ravel, J., Rebbapragada, A., Kaul, R.: Association of HPV infection and clearance with cervicovaginal immunology and the vaginal microbiota. *Mucosal Immunology* **10**(5), 1310–1319 (2017). doi:[10.1038/mi.2016.129](https://doi.org/10.1038/mi.2016.129)

43. Lajoie, J., Juno, J., Burgener, A., Rahman, S., Mogk, K., Wachihi, C., Mwanjewe, J., Plummer, F.A., Kimani, J., Ball, T.B., Fowke, K.R.: A distinct cytokine and chemokine profile at the genital mucosa is associated with HIV-1 protection among HIV-exposed seronegative commercial sex workers. *Mucosal Immunol* **5**(3), 277–287 (2012). doi:[10.1038/mi.2012.7](https://doi.org/10.1038/mi.2012.7)

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
44. Juno, J.A., Boily-Larouche, G., Lajoie, J., Fowke, K.R.: Collection, isolation, and flow cytometric analysis of human endocervical samples. *J Vis Exp* **89**, 51906 (2014).  
doi:[10.3791/51906](https://doi.org/10.3791/51906)
45. Frank, J.A., Reich, C.I., Sharma, S., Weisbaum, J.S., Wilson, B.A., Olsen, G.J.: Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Appl Environ Microbiol* **74**(8), 2461 (2008). doi:[10.1128/AEM.02272-07](https://doi.org/10.1128/AEM.02272-07)
46. Findley, K., Oh, J., Yang, J., Conlan, S., Deming, C., Meyer, J.A., Schoenfeld, D., Nomicos, E., Park, M., NIH Intramural Sequencing Center Comparative Sequencing Program, Kong, H.H., Segre, J.A.: Topographic diversity of fungal and bacterial communities in human skin. *Nature* **498**(7454), 367–370 (2013). doi:[10.1038/nature12171](https://doi.org/10.1038/nature12171). Accessed 2017-09-13
47. Ravel, J., Gajer, P., Abdo, Z., Schneider, G.M., Koenig, S.S.K., McCulle, S.L., Karlebach, S., Gorle, R., Russell, J., Tacket, C.O., Brotman, R.M., Davis, C.C., Ault, K., Peralta, L., Forney, L.J.: Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci USA* **108**, 4680–7 (2011). doi:[10.1073/pnas.1002611107](https://doi.org/10.1073/pnas.1002611107)
48. Gajer, P., Brotman, R.M., Bai, G., Sakamoto, J., Schütte, U.M.E., Zhong, X., Koenig, S.S.K., Fu, L., Ma, Z.S., Zhou, X., Abdo, Z., Forney, L.J., Ravel, J.: Temporal dynamics of the human vaginal microbiota. *Sci Transl Med* **4**(132), 132–52 (2012).  
doi:[10.1126/scitranslmed.3003605](https://doi.org/10.1126/scitranslmed.3003605)
49. Johne, R., Müller, H., Rector, A., van Ranst, M., Stevens, H.: Rolling-circle amplification of viral DNA genomes using phi29 polymerase. *Trends Microbiol* **17**(5), 205–211 (2009).  
doi:[10.1016/j.tim.2009.02.004](https://doi.org/10.1016/j.tim.2009.02.004)
50. Nowak, M.A., May, R.M.: *Virus Dynamics: Mathematical Principles of Immunology and Virology*. Oxford University Press, Oxford, USA (2000)
51. Stafford, M.A., Corey, L., Cao, Y., Daar, E.S., Ho, D.D., Perelson, A.S.: Modeling plasma virus concentration during primary HIV infection. *J. theor. Biol.* **203**(3), 285–301 (2000).

doi:[10.1006/jtbi.2000.1076](https://doi.org/10.1006/jtbi.2000.1076)

1  
2  
3 52. Perelson, A.S.: Modelling viral and immune system dynamics. *Nat. Rev. Immunol.* **2**(1),  
4 28–36 (2002). doi:[10.1038/nri700](https://doi.org/10.1038/nri700)

5  
6  
7  
8 53. Murall, C.L., Jackson, R., Zehbe, I., Boulle, N., Segondy, M., Alizon, S.: Epithelial  
9 stratification shapes infection dynamics. *PLoS Comput Biol* **15**(1), 1006646 (2019).

10  
11  
12  
13 doi:[10.1371/journal.pcbi.1006646](https://doi.org/10.1371/journal.pcbi.1006646)

14  
15  
16  
17 54. Steimer, J.L., Vozech, S., Racine Poon, A., Holford, N., O'Neil, R.: The population  
18 approach: rationale, methods and applications in clinical pharmacology and drug development.  
19 In: Balant, P.G.W..L. (ed.) *Handbook of Experimental Pharmacology*, vol. 110, pp. 405–451.

20  
21  
22  
23 Springer, Berlin (1994)

24  
25  
26  
27 55. Bates, D., Mächler, M., Bolker, B., Walker, S.: Fitting linear mixed-effects models using  
28 lme4. *Journal of Statistical Software* **67**(1) (2015). doi:[10.18637/jss.v067.i01](https://doi.org/10.18637/jss.v067.i01)

29  
30  
31  
32 56. Bucci, V., Tzen, B., Li, N., Simmons, M., Tanoue, T., Bogart, E., Deng, L., Yeliseyev, V.,  
33 Delaney, M.L., Liu, Q., Olle, B., Stein, R.R., Honda, K., Bry, L., Gerber, G.K.: MDSINE:  
34 Microbial dynamical systems INference engine for microbiome time-series analyses. *Genome*  
35 *Biology* **17**, 121 (2016). doi:[10.1186/s13059-016-0980-6](https://doi.org/10.1186/s13059-016-0980-6). Accessed 2017-03-09

36  
37  
38  
39 57. Faust, K., Lahti, L., Gonze, D., de Vos, W.M., Raes, J.: Metagenomics meets time series  
40 analysis: unraveling microbial community dynamics. *Curr Opin Microbiol* **25**, 56–66 (2015).

41  
42  
43  
44  
45  
46  
47  
48 doi:[10.1016/j.mib.2015.04.004](https://doi.org/10.1016/j.mib.2015.04.004)

49  
50  
51 58. Fox, G.A., Negrete-Yankelevich, S., Sosa, V.J.: *Ecological Statistics: Contemporary*  
52 *Theory and Application*. Oxford University Press, Oxford, USA (2015)

53  
54  
55  
56  
57 59. Shi, Y., Li, L., Hu, Z., Li, S., Wang, S., Liu, J., Wu, C., He, L., Zhou, J., Li, Z., Hu, T., Chen,  
58 Y., Jia, Y., Wang, S., Wu, L., Cheng, X., Yang, Z., Yang, R., Li, X., Huang, K., Zhang, Q., Zhou,  
59 H., Tang, F., Chen, Z., Shen, J., Jiang, J., Ding, H., Xing, H., Zhang, S., Qu, P., Song, X., Lin,

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Z., Deng, D., Xi, L., Lv, W., Han, X., Tao, G., Yan, L., Han, Z., Li, Z., Miao, X., Pan, S., Shen, Y., Wang, H., Liu, D., Gong, E., Li, Z., Zhou, L., Luan, X., Wang, C., Song, Q., Wu, S., Xu, H., Shen, J., Qiang, F., Ma, G., Liu, L., Chen, X., Liu, J., Wu, J., Shen, Y., Wen, Y., Chu, M., Yu, J., Hu, X., Fan, Y., He, H., Jiang, Y., Lei, Z., Liu, C., Chen, J., Zhang, Y., Yi, C., Chen, S., Li, W., Wang, D., Wang, Z., Di, W., Shen, K., Lin, D., Shen, H., Feng, Y., Xie, X., Ma, D.: A genome-wide association study identifies two new cervical cancer susceptibility loci at 4q12 and 17q12. *Nat Genet* **45**(8), 918–22 (2013). doi:[10.1038/ng.2687](https://doi.org/10.1038/ng.2687)

60. Chen, D., Gaborieau, V., Zhao, Y., Chabrier, A., Wang, H., Waterboer, T., Zaridze, D., Lissowska, J., Rudnai, P., Fabianova, E., Bencko, V., Janout, V., Foretova, L., Mates, I.N., Szeszenia-Dabrowska, N., Boffetta, P., Pawlita, M., Lathrop, M., Gyllensten, U., Brennan, P., McKay, J.D.: A systematic investigation of the contribution of genetic variation within the MHC region to HPV seropositivity. *Hum Mol Genet* **24**(9), 2681–2688 (2015). doi:[10.1093/hmg/ddv015](https://doi.org/10.1093/hmg/ddv015)

61. Chen, D., Gyllensten, U.: Lessons and implications from association studies and post-GWAS analyses of cervical cancer. *Trends Genet* **31**(1), 41–54 (2015). doi:[10.1016/j.tig.2014.10.005](https://doi.org/10.1016/j.tig.2014.10.005)

62. Fellay, J., Shianna, K.V., Ge, D., Colombo, S., Ledergerber, B., Weale, M., Zhang, K., Gumbs, C., Castagna, A., Cossarizza, A., Cozzi-Lepri, A., De Luca, A., Easterbrook, P., Francioli, P., Mallal, S., Martinez-Picado, J., Miro, J.M., Obel, N., Smith, J.P., Wyniger, J., Descombes, P., Antonarakis, S.E., Letvin, N.L., McMichael, A.J., Haynes, B.F., Telenti, A., Goldstein, D.B.: A whole-genome association study of major determinants for host control of HIV-1. *Science* **317**(5840), 944–947 (2007). doi:[10.1126/science.1143767](https://doi.org/10.1126/science.1143767)

63. Huber, P.J.: The 1972 Wald Lecture Robust Statistics: A Review. *Ann Math Stat* **43**(4), 1041–1067 (1972). doi:[10.1214/aoms/1177692459](https://doi.org/10.1214/aoms/1177692459)

64. Winer, R.L., Feng, Q., Hughes, J.P., O'Reilly, S., Kiviat, N.B., Koutsky, L.A.: Risk of female human papillomavirus acquisition associated with first male sex partner. *J Infect Dis*

**197**(2), 279–82 (2008). doi:[10.1086/524875](https://doi.org/10.1086/524875)

1  
2  
3 65. Herrero, R.: Human Papillomavirus (HPV) Vaccines: Limited Cross-Protection against  
4 Additional HPV Types. *J Infect Dis* **199**(7), 919–922 (2009). doi:[10.1086/597308](https://doi.org/10.1086/597308)  
5  
6  
7

8 66. Depuydt, C.E., Verstraete, L., Berth, M., Beert, J., Bogers, J.-P., Salembier, G.,  
9 Vereecken, A.J., Bosmans, E.: Human papillomavirus positivity in women undergoing  
10 intrauterine insemination has a negative effect on pregnancy rates. *Gynecol Obstet Invest*  
11 **81**(1), 41–6 (2016). doi:[10.1159/000434749](https://doi.org/10.1159/000434749)  
12  
13  
14  
15  
16  
17

18 67. WHO HPV LabNet. World Health Organization.  
19

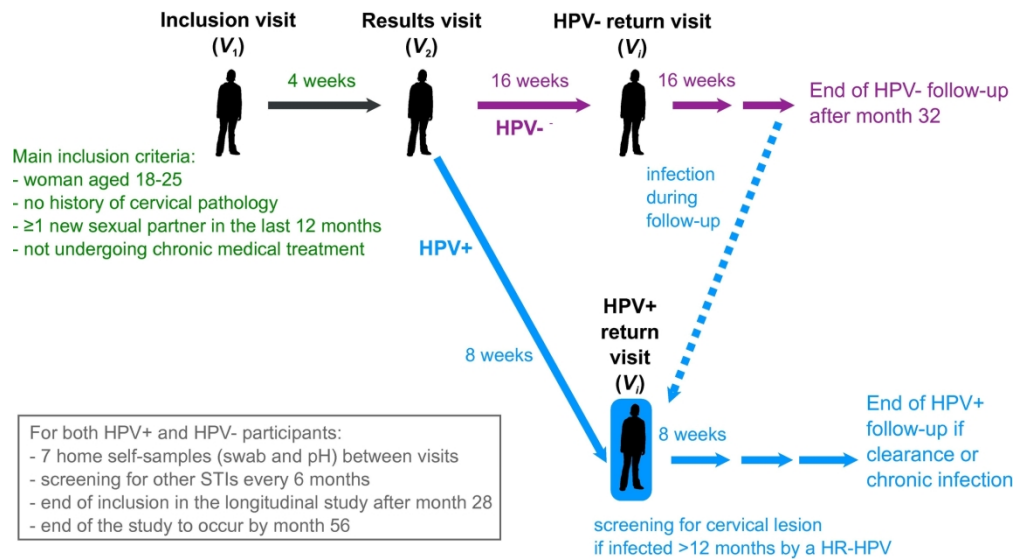
20 [http://www.who.int/biologicals/areas/human\\_papillomavirus/WHO\\_HP\\_V\\_LabNet/en/](http://www.who.int/biologicals/areas/human_papillomavirus/WHO_HP_V_LabNet/en/)  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## Figure captions

1  
2  
3 **Figure 1: General structure of the PAPCLEAR study.** For the longitudinal study, participants  
4 have an inclusion visit ( $V_1$ ), a results visit ( $V_2$ ) and then return visits ( $V_i$  with  $i > 2$ ). For the cross-  
5  
6 sectional study, participants only have  $V_1$  and  $V_2$ .  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21

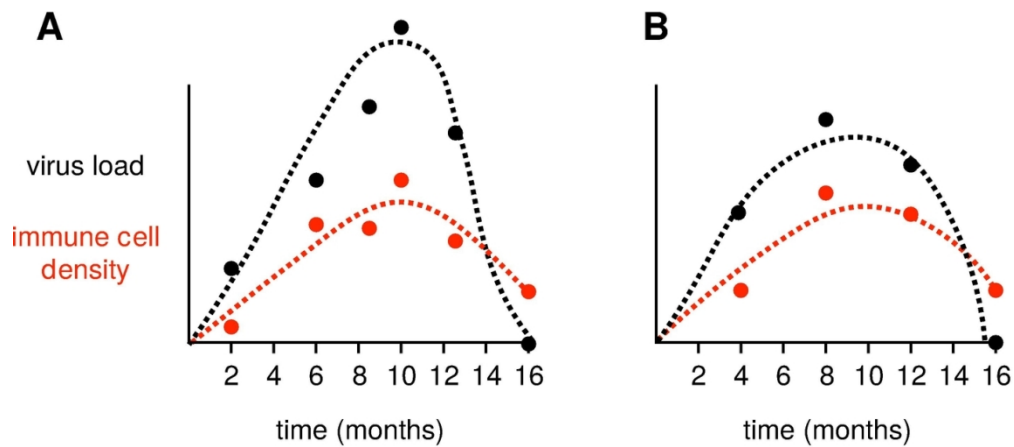
22 **Figure 2: Fitting viral kinetics models to within-host times series.** Dashed lines indicate a  
23 model fitted using virus load (in black) or immune cells (in red) time series. In panel A, the  
24 follow-up is bi-monthly with 2 missing visits and several delayed visits, whereas in panel B the  
25 follow-up is every 4 months without any missing or delayed visits. In spite of missing data this,  
26 the situation shown in panel A is clearly the best for inferring parameter values and for fitting the  
27 underlying dynamics.  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60





General structure of the PAPCLEAR study. For the longitudinal study, participants have an inclusion visit (V<sub>1</sub>), a results visit (V<sub>2</sub>) and then return visits (V<sub>i</sub> with  $i > 2$ ). For the cross-sectional study, participants only have V<sub>1</sub> and V<sub>2</sub>.

190x104mm (300 x 300 DPI)



Fitting kinetics dynamical models to within-host times series. Dashed lines indicate a model fitted using virus load (in black) or immune cells (in red) time series. In panel A, the follow-up is bi-monthly with 2 missing visits and several delayed visits, whereas in panel B the follow-up is every 4 months without any missing or delayed visits. In spite of missing data this, the situation shown in panel A is clearly the best for inferring parameter values and for fitting the underlying dynamics.

120x52mm (300 x 300 DPI)

# BMJ Open

## The natural history, dynamics, and ecology of Human papillomaviruses in genital infections of young women: protocol of the PAPCLEAR cohort study

|                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|-------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Journal:                      | <i>BMJ Open</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Manuscript ID                 | bmjopen-2018-025129.R2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Article Type:                 | Protocol                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Date Submitted by the Author: | 09-Apr-2019                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| Complete List of Authors:     | <p>Murall, Carmen Lia; Centre National de la Recherche Scientifique, MIVEGEC</p> <p>Rahmoun, Massilva; Centre National de la Recherche Scientifique, MIVEGEC</p> <p>Selinger, Christian; Centre National de la Recherche Scientifique, MIVEGEC</p> <p>Baldellou, Monique; Centre Hospitalier Regional Universitaire de Montpellier, CeGIDD</p> <p>Bernat, Claire; Centre National de la Recherche Scientifique, MIVEGEC</p> <p>Bonneau, Marine; Centre Hospitalier Regional Universitaire de Montpellier, Department of Obstetrics and Gynaecology</p> <p>Boué, Vanina; Centre National de la Recherche Scientifique, MIVEGEC</p> <p>Buisson, Mathilde; Centre Hospitalier Regional Universitaire de Montpellier, Department of Research and Innovation</p> <p>Christophe, Guillaume; Centre Hospitalier Regional Universitaire de Montpellier, CeGIDD</p> <p>D'Auria, Giuseppe; Fundacio per al Foment de la Investigacio Sanitaria i Biomedica, Sequencing and Bioinformatics Service; Centro de Investigacion Biomedica en Red de Epidemiologia y Salud Publica De Taroni, Florence; Centre Hospitalier Regional Universitaire de Montpellier, CeGIDD</p> <p>Foulongne, Vincent; Centre Hospitalier Regional Universitaire de Montpellier, Department of Bacteriology and Virology</p> <p>Froissart, Rémy; Centre National de la Recherche Scientifique, MIVEGEC</p> <p>Graf, Christelle; Centre Hospitalier Regional Universitaire de Montpellier, Department of Obstetrics and Gynaecology</p> <p>Grasset, Sophie; Centre Hospitalier Regional Universitaire de Montpellier, Department of Virology; Centre National de la Recherche Scientifique, MIVEGEC</p> <p>Groc, Soraya; Centre Hospitalier Regional Universitaire de Montpellier, Department of Virology; Centre National de la Recherche Scientifique, MIVEGEC</p> <p>Hirtz, Christophe; Centre Hospitalier Regional Universitaire de Montpellier, LBPC/PPC, IRMB</p> <p>Jaussent, Audrey; Centre Hospitalier Regional Universitaire de Montpellier, Department of Medical Information</p> <p>Lajoie, Julie; University of Manitoba College of Medicine, Department of Medical microbiology</p> <p>Lorcy, Frédérique; Centre Hospitalier Regional Universitaire de</p> |

|                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|---------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                 | <p>Montpellier, Laboratoire d'anatomie et cytologie pathologiques<br/> Picot, Eric; Centre Hospitalier Regional Universitaire de Montpellier, CeGIDD<br/> PICOT, Marie-Christine; Centre Hospitalier Regional Universitaire de Montpellier, Department of Medical Information<br/> Ravel, Jacques; University of Maryland School of Medicine, Institute for Genome Sciences<br/> Reynes, Jacques; Centre Hospitalier Regional Universitaire de Montpellier, Department of Infectious and Tropical Diseases<br/> Rousset, Thérèse; Centre Hospitalier Regional Universitaire de Montpellier, Department of pathology and oncobiology<br/> Seddiki, Aziza; Centre Hospitalier Regional Universitaire de Montpellier, Department of Research and Innovation<br/> Teirlinck, Martine; Centre Hospitalier Regional Universitaire de Montpellier, CeGIDD<br/> Tribout, Vincent; Centre Hospitalier Regional Universitaire de Montpellier, CeGIDD<br/> Tuaille, Edouard; Centre Hospitalier Regional Universitaire de Montpellier, Department of bacteriology and virology<br/> Waterboer, Tim; Deutsches Krebsforschungszentrum, Infections and Cancer Epidemiology<br/> Jacobs, Nathalie; Universite de Liege Faculte des Sciences, GIGA-Research, Cellular and molecular immunology<br/> Bravo, Ignacio; Centre National de la Recherche Scientifique, MIVEGEC<br/> Segondy, Michel; Centre Hospitalier Regional Universitaire de Montpellier, Department of Bacteriology and Virology<br/> Bouille, Nathalie; Centre Hospitalier Regional Universitaire de Montpellier, Department of pathology and oncobiology<br/> Alizon, Samuel; Centre National de la Recherche Scientifique, MIVEGEC</p> |
| <b>Primary Subject Heading</b>: | Infectious diseases                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| Secondary Subject Heading:      | Epidemiology, Immunology (including allergy), Genetics and genomics                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| Keywords:                       | VIROLOGY, IMMUNOLOGY, Epidemiology < INFECTIOUS DISEASES, MICROBIOLOGY, GENETICS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |

SCHOLARONE™  
Manuscripts

## STUDY PROTOCOL

# The natural history, dynamics, and ecology of Human papillomaviruses in genital infections of young women: protocol of the PAPCLEAR cohort study

Carmen Lía Murall<sup>1</sup>, Massilva Rahmoun<sup>1</sup>, Christian Selinger<sup>1</sup>, Monique Baldellou<sup>2</sup>, Claire Bernat<sup>1</sup>, Marine Bonneau<sup>3</sup>, Vanina Boué<sup>1</sup>, Mathilde Buisson<sup>4</sup>, Guillaume Christophe<sup>2</sup>, Giuseppe D'Auria<sup>5,6</sup>, Florence De Taroni<sup>2</sup>, Vincent Foulongne<sup>7,8</sup>, Rémy Froissart<sup>1</sup>, Christelle Graf<sup>3</sup>, Sophie Grasset<sup>1,2</sup>, Soraya Groc<sup>1,7</sup>, Christophe Hirtz<sup>9</sup>, Audrey Jausse<sup>10</sup>, Julie Lajoie<sup>11</sup>, Frédérique Lorcy<sup>12</sup>, Eric Picot<sup>2</sup>, Marie-Christine Picot<sup>10</sup>, Jacques Ravel<sup>13</sup>, Jacques Reynes<sup>11</sup>, Thérèse Rousset<sup>12</sup>, Aziza Seddiki<sup>4</sup>, Martine Teirlinck<sup>2</sup>, Vincent Tribut<sup>2</sup>, Édouard Tuillon<sup>8</sup>, Tim Waterboer<sup>15</sup>, Nathalie Jacobs<sup>16</sup>, Ignacio G Bravo<sup>1</sup>, Michel Segondy<sup>7,8</sup>, Nathalie Boule<sup>8,12</sup> and Samuel Alizon<sup>1,\*</sup>

Word count: 5265 words, excluding title page, abstract, references, figures and tables.

1 Laboratoire MIVEGEC (UMR 5290 CNRS, IRD, UM), 911, avenue Agropolis, BP 64501, 34394 Montpellier, France

2 Center for Free Information, Screening and Diagnosis (CeGIDD), Centre Hospitalier Universitaire de Montpellier, Montpellier, France

3 Department of Obstetrics and Gynaecology, Centre Hospitalier Universitaire de Montpellier, Montpellier, France.

4 Department of Research and Innovation (DRI), Centre Hospitalier Universitaire de Montpellier, Montpellier, France

5 Sequencing and Bioinformatics Service, Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunidad Valenciana (FISABIO-Salud Pública), 46020 Valencia, Spain

6 CIBER en Epidemiología y Salud Pública (CIBEResp), Madrid, Spain

7 Department of Virology, Centre Hospitalier Universitaire de Montpellier, Montpellier, France

8 Pathogenesis and Control of Chronic Infections, INSERM, CHU, University of Montpellier, Montpellier, France

9 University of Montpellier, LBPC/PPC- IRMB, CHU de Montpellier, 80 rue Augustin Fliche, Montpellier, France

10 Department of Medical Information (DIM), Centre Hospitalier Universitaire de Montpellier, Montpellier, France

11 Department of Medical microbiology, University of Manitoba, 745 Bannatyne, Winnipeg, Canada

12 Department of pathology and oncobiology, Centre Hospitalier Universitaire de Montpellier, Montpellier, France

13 Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, USA

14 Department of Infectious and Tropical Diseases, Centre Hospitalier Universitaire de Montpellier, Montpellier, France

15 German Cancer Research Center (DKFZ), Infections and Cancer Epidemiology, Im Neuenheimer Feld 280, Heidelberg, Germany

16 GIGA-Research, Cellular and molecular immunology, University of Liège, 3 Avenue de l'Hôpital, 4000 Liège, Belgium

\* Author for correspondence: [samuel.alizon@cnrs.fr](mailto:samuel.alizon@cnrs.fr)

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only

## Abstract

### Introduction

Human papillomaviruses (HPVs) are responsible for one third of all cancers caused by infections. Most HPV studies focus on chronic infections and cancers, and thus, we know little about the early stages of viral infection. In particular, the effects of the dynamic interactions between the immune system, the microbiota, and the viral and host genetics on infection clearance or persistence remains poorly understood.

### Methods and Analysis

We follow 150 women, aged 18-25 years, longitudinally to monitor immune response features (cytokines and immune cells in the genital tract, circulating anti-HPV antibodies), virus load of HPVs, and vaginal microbiota composition. This is complemented by the assessment of viral and human genetics and behavioural data. To increase the statistical power of the epidemiological arm of the study, an additional 150 women are screened cross-sectionally.

### Ethics and Dissemination

This study has been approved by the Comité de Protection des Personnes Sud Méditerranée I (reference number 2016-A00712-49); by the Comité Consultatif sur le Traitement de l'Information en matière de Recherche dans le domaine de la Santé (reference number 16.504); by the Commission Nationale Informatique et Libertés (reference number MMS/ABD/AR1612278, decision number DR-2016-488) and by the Agence Nationale de Sécurité du Médicament et des Produits de Santé (reference 20160072000007). The results will be published in preprint servers, post-print servers, peer-reviewed journals and disseminated through conferences.

**Trial registration number:** NCT02946346

**Keywords:** HPV; acute infection; persistence; virus load; immunity; microbiota; viral kinetics

## Article summary

### Strengths and limitations of this study

- A dense follow-up with visits every two months for infected women and additional self-sampling every week.
- The combination of virological (virus load), immunological (cytokine concentrations and immune cell percentages) and environmental (vaginal microbiota composition, pH) measurements at each visit.
- A limitation is that the density of the follow-up limits the number of participants (N=150), which can affect the power of epidemiological analyses.
- We complement the longitudinal study with a cross-sectional study of N=150 women to increase statistical power.



## Introduction

### Epidemiology of HPV genital infections in young adults and public health implications

Infections by Human Papillomaviruses (HPVs) are likely the most common sexually transmitted infection (STI) globally. It is often estimated that, worldwide, more than 80% of sexually-active individuals will be infected by an HPV type [1]. In France, a study performed in 2013 in the Paris area estimated the prevalence of HPV genital infections to be 25% in women below 25 years of age [2]. In the area of Montpellier (France), the prevalence of oncogenic HPVs (also referred to as 'high-risk', HR, HPVs) in pregnant women aged 16 to 42 years was close to 20% [3]. These numbers are consistent with worldwide estimates according to which HPVs are most prevalent in women under 25 years of age, with an estimated overall prevalence of 24% [4].

Fortunately, the vast majority of infections by HPVs are asymptomatic and benign. Even for HPV16, which is probably the most oncogenic human virus, only a minority of infections (less than 10%) become persistent [5], and then a minority of these (12%) progress to cancer if untreated [1, 6]. Indeed, it is estimated that approximately 70 to 100% of infections by HPVs are cleared within 12 to 24 months, with strong differences between virus types [5, 7–9]. Recent studies suggest that primo-infections could be shorter in young girls [10] but, in general, there are many unknowns about the biology of non-persisting infections [11].

Our lack of knowledge partly comes from the fact that in vaccine trials, from which most of the data on infection duration originate, participants are followed every six months for several years [5, 7, 9, 12]. This frequency is sufficient to estimate the time to clearance (or persistence) but it is not precise enough to understand the within-host dynamics, often referred to as 'kinetics' [13], of infections that last on average 6 to 24 months. Arbitrarily, after 24 months, an infection is often considered to be persistent [14].

Some factors have been shown to correlate with persistence (e.g. immunosuppression, smoking, and co-infection with other STIs [15]) but we do not know how these affect viral kinetics. Also, some changes in viral-immunity interactions appear to be related to persistence

and disease progression [16–19] but, again, we do not know the underlying interactions between the viruses, the host target cells, and the immune response in acute infections [11]. Finally, it has been argued that the vaginal microbiota may differ between HPV-infected and HPV-uninfected women [20] and that specific microbiota composition may interact with HPV detection [21]. However, it is difficult to disentangle the cause and the consequence. For instance, does the microbiota composition change after the establishment of an HPV infection, or do certain microbiota compositions increase susceptibility to HPV infection?

A better understanding of the within-host infection dynamics and of the determinants of clearance and persistence of viral infection is particularly important in the context of vaccination [22–25]. Indeed, the long-term efficacy of the anti-HPVs vaccines at the population level will largely depend on the within-host viral dynamics because, ultimately, most selective pressures on viral populations occur via the immune response [26]. Furthermore, a better understanding of acute HPV infections can shed new light on issues related to latency, fertility, or immunotherapies [11].

## **Prevention strategies and treatment**

### **Treatment**

Since most infections by HPVs are benign in young adults and clear within six to 24 months, the current standard of care is to avoid over-treatment, even in the presence of cervical lesions [27]. Clinical interventions (colposcopies, biopsies, and surgery) are less often performed with young women (< 25 years) and only for high-grade (pre-cancerous) lesions (cervical intraepithelial neoplasia grade 2, CIN-2, or more). Low-grade lesions (CIN-1) are not systematically treated but rather monitored yearly to detect any progression to high-grade lesions.

Genital warts caused by non-oncogenic HPVs (often referred to as ‘low-risk’, LR, HPVs) can be removed by surgery or treated with bi- and trichloroacetic acid, cryotherapy or other treatments [28].

## HPV vaccination

1  
2 There are currently three licensed vaccines: a bivalent vaccine (Cervarix<sup>®</sup>) targeting HPV16  
3  
4 and HPV18 (together accounting for 70% of cervical cancers [1]), a quadrivalent vaccine  
5  
6 (Gardasil<sup>®</sup>) that additionally targets HPV6 and HPV11 (non-oncogenic, but highly prevalent and  
7  
8 associated to benign proliferative lesions) and, since 2014, a nonavalent vaccine (Gardasil 9<sup>®</sup>)  
9  
10 that targets five more oncogenic types (HPV31, HPV33, HPV45, HPV52, and HPV58, which  
11  
12 altogether account for 20% of cervical cancers [24]). These vaccines succeed in eliciting a  
13  
14 protective immune response against new infections by the targeted viruses, and are used  
15  
16 throughout the world, albeit with wide variation in coverage (for reviews, see e.g. [29, 30]).  
17  
18  
19  
20

21  
22 Vaccination campaigns in France started in 2006 but with limited coverage: it reached 28.5% in  
23  
24 2008 [31] and has been decreasing ever since [32]. The vaccine is recommended for girls from  
25  
26 11 to 14 years of age, currently with a vaccination scheme of two doses with a six months  
27  
28 interval. A catch-up is organised for girls aged 15-19 years, with a three-doses vaccination  
29  
30 scheme. Vaccination is reimbursed by the French Social Security but is not mandatory. It is also  
31  
32 recommended for men who have sex with men (MSM) as well as for immuno-compromised  
33  
34 people [32]. Vaccination is now the primary prevention strategy against cervical cancers.  
35  
36  
37  
38

## Screening

39  
40 In France, the secondary prevention strategy against cervical cancer is routine individual  
41  
42 cytology-based screening for pre-cancerous and cancerous cervical lesions in women between  
43  
44 25 and 65 years old. Cytology can also be performed in younger women if they report risk  
45  
46 factors for cervical cancer (multiple partners, chronic STIs or HIV infection [32]). Detection of  
47  
48 oncogenic HPVs is proposed for triage in case of abnormal cytology (i.e. high-grade or low-  
49  
50 grade squamous intraepithelial lesion, HSIL and LSIL respectively, or Atypical Squamous Cells  
51  
52 of Undetermined Significance, ASCUS).  
53  
54  
55  
56  
57

## Primary objectives

58  
59 The first primary objective of this cohort study is to decipher the kinetics and ecology of cervical  
60  
HPV infections in healthy young women, i.e. follow the population dynamics of the virus, the

target epithelial cells, and the immune effectors.

The second primary objective is to characterise the diversity of genital HPVs in young women in the region of Montpellier in relationship with their lifestyle, vaccination status, vaginal microbiota, and human genetics.

### **Secondary objectives**

A secondary objective is to characterise the acquisition and clearance dynamics of cervical HPV infections as a function of viral diversity, host immunity, vaginal microbiota and human genetics.

A final objective is to investigate variations in genetic diversity of HPVs during cervical infections.

## **Methods and analysis**

### **Participants**

The study population is composed of young women at risk of HPV infection. The age class was chosen because it exhibits high HPV prevalence (24% worldwide [4] and approximately 25% in France [2]). Inclusion of younger women would have raised technical issues because of the requirement for parental consent.

Women are recruited through a social media page, and through posters and leaflets distributed at the Universities in Montpellier and at the Montpellier STI screening centre (*Centre Gratuit d'Information de Dépistage et de Diagnostic, CeGIDD*). The composition of the population visiting the CeGIDD has already been documented in an earlier study [33]. In total, the centre is visited by approximately 3,000 women per year, the majority of which are under 25 years of age (80%). Approximately 40% of the attendants report three or more partners over the last twelve months and approximately 50% report using adequate behaviour for prevention against HIV.

## Inclusion criteria

Participants are women from 18 to 25 years old living in the metropolitan area of Montpellier. They must be sexually active with at least one new partner over the last 12 months. This criteria is fixed to maximise the incidence of new HPV infections. Participants must be able to and willing to give written informed consent: they must sign an informed consent form, understand the requirements for the study, and be affiliated to a French social security scheme (which is a state requirement).

Women cannot be included in the study if they have a history of HPV-associated pathology (genital warts or cervical lesions), if they are pregnant or intending to become pregnant in the coming year, infected by HIV, undergoing (or planning to undergo) intense medical treatment (biotherapy, chemotherapy, immunosuppression), planning on moving outside the Montpellier metropolitan area within the next 18 months, in a dependency or employment with the sponsor or the investigator, if they participated in a clinical trial involving administration of drugs within the last four weeks or if they belong to a vulnerable group (e.g. children, adults with physical or mental disabilities).

## Design/setting

This study has a longitudinal component aimed at deciphering within-host dynamics and a cross-sectional component aimed at understanding the diversity of HPV infections in young adults in the area of Montpellier, France. The general structure of the study is shown in Fig 1.

If a woman fits the main inclusion criteria, she can go through an inclusion visit (V1) with a physician (gynaecologist or midwife) at the CeGIDD. During this visit, the study investigator presents the study and checks all inclusion criteria before asking the participant to read and sign the informed consent form. Participants then undergo a medical consultation during which a number of samples are collected (see below). They then fill out health and lifestyle questionnaires and are given cotton-flocked swabs for self-sampling at home the next visit,

1 along with instructions on how to fill in weekly questionnaires through an online form (these are  
2 performed throughout the study).  
3  
4

5 An appointment is scheduled four weeks later for the Results visit ( $V_2$ ), where the cervical  
6 cytology results are communicated. Additional samples are collected and self-sample swabs for  
7 home collection are provided.  
8  
9  
10

11  
12  
13 The next return visits ( $V_i$ , where  $i > 2$ ) are as follows:  
14

- 15  
16  
17 • Participants with a positive DEIA HPV test (see below), i.e. infected by an  
18 *Alphapapillomavirus*, at  $V_1$  join the HPV positive (HPV+) arm of the study with return  
19 visits scheduled every 2 months.  
20  
21  
22
- 23  
24 • Participants with a negative DEIA HPV test at  $V_1$  join the HPV negative (HPV-) arm with  
25 return visits scheduled every 4 months.  
26  
27
- 28  
29 • HPV- participants infected by an *Alphapapillomavirus* move to the HPV+ arm.  
30  
31

32 Intervals between visits are based on earlier results showing that HPV infections last from 9 to  
33 18 months on average depending on the HPV type [5, 7–9] and that a total follow-up of 4  
34 months yields results that are difficult to analyse [21]. The longer interval in the HPV- arm is  
35 based on the estimated incidence for HPV genital infections in young women, which is greater  
36 than 30% [34, 35].  
37  
38  
39  
40  
41  
42  
43  
44

45 Participants in the HPV- arm are followed until month 32 of the study.  
46  
47

48 Participants in the HPV+ arm are followed until they clear the infection or until they have been  
49 infected for 24 months (after which we consider that the infection is persistent). Clearance is  
50 defined as being negative at two visits in a row for the first HPV type detected in the follow-up.  
51  
52  
53  
54

55  
56 In between these visits to the CeGIDD, participants are asked to perform regular (every week  
57 for HPV+ and every second week for HPV-) self-samples using vaginal swabs, to measure  
58 vaginal pH and to fill in a short questionnaire. Self-samples are stored in the participants'  
59 freezer and brought back at every visit.  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

The study will end with the last HPV+ participant having cleared the infection or been infected for 24 months.

## **Patients and public involvement**

Since all participants are healthy, they are referred to as participants rather than patients. As in any longitudinal study, ensuring participant commitment is challenging. To achieve this goal, we have set up a compensation of 40 EUR per visit and an additional 10 EUR in case of a complete follow-up. Furthermore, participants who have answered a sufficient number of questionnaires and brought back a sufficient number of self-samples get a 100 EUR bonus at the end. Overall, a participant performing 12 return visits would gain a total compensation of 650 EUR.

Participants did not play a role in the design of this study.

Results of the study will be disseminated to participants who have left the study and to the general public via an email newsletter in French.

## **Visits**

The summary of the visit schedule and of the samples collected at each visit is shown in Table 1.

### **Inclusion visit (V1)**

This visit takes place at the CeGIDD and is scheduled by the Clinical Research Technician (CRT) via phone or email.

Women meet a study investigator, who explains the goals and requirements of the study and checks that the inclusion criteria are met. If so, after a general discussion, the informed consent forms are signed.

1 A female physician/midwife performs a general exam and then a gynaecological exam during  
2 which the following samples are taken:  
3  
4

- 5 • vaginal pH cotton swab (EcoCare™),
- 6
- 7 • vaginal swab (Copan ESwab™) in 1mL Amies liquid for DNA extraction and microbiota
- 8 analysis,
- 9
- 10 • vaginal swab (Copan ESwab™) in 1mL of RNA preservation medium,
- 11
- 12 • ophthalmic sponge (Weck-cel®) to collect cervical secretions for cytokines analysis,
- 13
- 14 • cervical smear in 20mL of Thinprep® (Preservcyt® liquid) for HPV and HSV assays, and
- 15 cytology evaluation.
- 16
- 17
- 18
- 19
- 20
- 21
- 22

23 Following the gynaecological consultation, the participant meets with a nurse to measure body  
24 temperature, blood pressure and draw 20mL of blood (a 5mL tube for SNPs sequencing, a  
25 10mL tube for immunophenotyping and a 5mL tube for HPV antibody titration). For the  
26 longitudinal study, the nurse provides the participant with 3 self-sampling kits, 3 pH strips, a  
27 freezer box to bring back to the next visit, as well as instructions on how to perform the home  
28 sampling and store the samples in her personal freezer until the next visit.  
29

30 If the participant has not been tested for a STI in the last 3 months, the nurse draws an  
31 additional blood tube of 5mL to test for STIs (HIV, HCV, HBV) and collects vaginal self-samples  
32 for chlamydiae and gonorrhoea detection. Syphilis testing is prescribed to participants who meet  
33 the STI clinic's guidelines.  
34

35 Finally, the participant meets with the CRT to fill in questionnaires #1 (inclusion visit) and #3  
36 (home). The CRT answers any remaining questions, explains how to fill the home  
37 questionnaires (#3) and sets an appointment for the Results visit.  
38

#### 39 **Results visit (V2)**

40 During this visit, the participants are given the result of cervical lesion screening using the liquid  
41 cytology (normal, ASCUS, LSIL or HSIL). Participants with a high-grade lesion (HSIL) exit the  
42 study and are referred to the gynaecology service of the CHU of Montpellier.  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



1 During this visit, the physician/midwife collects additional samples: 2 vaginal swabs for DNA and  
2 RNA analysis, and a cervical smear in 10mL of PBS (to confirm HPV status and perform flow  
3 cytometry analyses).  
4  
5

6  
7 The participant fills in questionnaires #2 (for return visits) and #3 (home). An appointment for  
8 the next visit is set and swabs for home self-sampling are given.  
9  
10  
11

### 12 **Return visits ( $V_i$ )**

13 These visits only occur in the longitudinal study.  
14  
15

16  
17  
18 **HPV- arm.** Participants uninfected by HPV visit the clinic every 4 months until month 26. During  
19 these visits, the same samples as in the inclusion visit ( $V_1$ ) are collected by the  
20 physician/midwife except for the cervical smear, which is put in PBS instead of Thinprep.  
21  
22  
23

24  
25  
26 The nurse only draws blood if a screening test for STIs other than HPV is required. The  
27 participant then fills in questionnaires #2 and #3 and an appointment is set for the next visit in  
28 16 weeks.  
29  
30  
31  
32

33  
34  
35 If an HPV infection is detected in the cervical smear collected during this visit, the participant  
36 moves to the HPV+ arm and the CRT contacts the participant to move her appointment forward.  
37  
38  
39

40  
41 **HPV+ arm.** Participants infected by HPV visit the clinic every 2 months. They cannot switch arm  
42 and will remain in the HPV+ arm until clearance or the end of the study. During the visits, the  
43 same samples as in the inclusion visit ( $V_0$ ) are collected by the physician/midwife except for the  
44 cervical smear, which is put in PBS instead of Thinprep.  
45  
46  
47  
48

49  
50  
51 The nurse then draws 5mL of blood for HPV antibody titration. If this is the first HPV+ visit  
52 following an HPV- visit, the nurse also draws 10mL of blood for immunophenotyping. Finally, if a  
53 test for additional STIs is needed, the nurse draws 5mL of blood and collects vaginal self-  
54 samples for STI detection.  
55  
56  
57  
58  
59  
60

Importantly, if the participant has been infected by a HR-HPV for more than 12 months and

1 cytology has not been performed within the last 12 months, the cervical smear is put in  
2 Thinprep<sup>®</sup> fixation medium, instead of PBS, for cytological analysis (cervical lesion screening).  
3  
4  
5 Finally, the participant fills in questionnaires #2 and #3, receives self-samples for home  
6  
7 collection and an appointment is set for the next visit in 8 weeks.  
8  
9  
10  
11  
12  
13

## 14 **Endpoints**

15  
16  
17 The primary endpoint for the study is the inclusion and follow-up of HPV-infected women in  
18  
19 order to describe the kinetics of HPV virus load, and the associated immune response.  
20  
21  
22

23 Secondary endpoints are the characterisation of the interactions between the course of the  
24  
25 infection (e.g. duration), the HPV type(s), the abundance and taxonomic diversity of bacteria,  
26  
27 fungi and viruses in the vaginal microbiota, human genetics (SNPs) and basal immunological  
28  
29 status.  
30  
31  
32  
33  
34  
35  
36

## 37 **Technical procedures**

### 38 **DNA extraction**

39  
40  
41 DNA extraction from cervical smears will be performed using Nuclisens EasyMAg from  
42  
43 Biomerieux or an equivalent protocol. For the microbiota analyses, special kits involving  
44  
45 physical (via beads) and/or enzymatic breaking of the cellular barrier will be favoured following  
46  
47 standard protocols to study the vaginal microbiome [36], e.g. the MagAttract<sup>®</sup>  
48  
49 PowerMicrobiome<sup>®</sup> DNA/RNA kit from Qiagen.  
50  
51  
52  
53

### 54 **HPV detection, typing and quantification**

55  
56  
57 The participants' infection status (HPV+ or HPV-) will be assessed using the DEIA test, which is  
58  
59 based on a PCR of the short SPF10 amplicon [37] and detects all *Alphapapillomaviruses* with  
60  
great sensitivity.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

If the DEIA test is positive, HPVs will be typed using the LiPA25 kit, which is based on the same SPF10-PCR, and has a lower detection threshold compared to other hybridisation-based typing methods [38].

The reason for basing the detection on the DEIA rather than the LiPA25 is that some *Alphapapillomavirus* may be detected by DEIA but not genotyped by LiPA and also that the DEIA is more sensitive than the LiPA. If the DEIA is positive and the LiPA25 is negative, typing will be performed by sequencing the product of a PGMY09/11 PCR [39], which targets another region of the HPV genome than the SPF10 PCR.

The quantification of HPV DNA genome copy number in the samples will be performed using the protocol set up by Micalessi et al. [40].

#### **Cytokine titration**

Cytokines can be used as markers of immune activation or immunosuppression and can also inform us on which components of the immune system are involved. Cervical sponges are centrifuged after the addition of 175 $\mu$ L of PBS. Cervical secretions are analysed for a set of 5 to 6 cytokines levels using the Meso Scale Discovery (MSD) Multiplex ELISA platform, which has a low detection threshold and a slowly saturating dose-response curve. Based on earlier results [41, 42], we will first investigate a large panel of 20 cytokines (IFN- $\alpha$ 2a, IFN- $\gamma$ , IL-1 $\alpha$ , IL-5, IL-6, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-23, IL-25, IP-10, MCP-1, MIP-1 $\alpha$ , MIP-3 $\alpha$ , MIP-3 $\beta$ , TNF- $\alpha$ , TNF- $\beta$ ) to choose the most relevant ones for a longitudinal follow-up.

#### **Flow cytometry**

Analysing immune cells via flow cytometry is extremely challenging on cells as fragile as the ones from cervical smears. However, several studies suggest that this is feasible [41–43]. Here, we follow the protocol described in [44].

Staining is performed using a Duraclone custom mix targeting CD45, CD3, CD4, CD8, CD16, CD56, CD69, CD161 and TCR $\gamma\delta$ . The last marker, Live&Dead tests for cellular viability.

Samples are acquired using a Navios flow cytometer (Beckman Coulter, three-laser

configuration).

## Sequencing

Sequencing will be performed for microbiota profiling. It involves PCR amplification of the V3-V4 region of 16S RNA for bacteria [45] and ITS1 for fungi [46]. We anticipate that the bacteria should belong to the operational taxonomic units (OTU) described in the five community state types found in vaginal communities [47, 48]. The virome will also be explored using shotgun sequencing and rolling circle PCR amplification [49]. Human genetics are explored using chip sequencing for SNPs.

## Statistical analyses

### Times series analyses

The core results of the study will come from the longitudinal follow-up of infected women, which will generate time series, i.e. a set of values collected from the same individual over time (Figure 2). There will be several time series per individual (virus load, number of immune cells, cytokine and antibody levels). These time series will be used to fit mathematical viral kinetics models that describe the interaction between viruses, host target cells (here, in the case of HPV, keratinocytes) and the immune response. These models are commonly developed for viral infections [13, 50–52], including those caused by HPVs [53]. We anticipate our follow-up to yield adequate data for such a fit based on the estimated duration of HPV infections (9 to 18 months [5, 7–9]). Furthermore, the weekly self-samples allow us to increase the resolution if necessary.

We will use non-linear mixed effect models [54] to jointly analyse time series from all participants. More precisely, we will rely on *R* packages such as nlme [55] or lme4 [55]. Note that, in addition to estimating model parameters (e.g. life-expectancy of infected cells or virion production rate of infected cells), this approach also allows us to compare biological models using statistical tools based on model likelihood such as Akaike Information Criterion. For an example of such analysis in the case of HIV, see [51].

### Microbiota dynamics

1  
2 The composition of the vaginal microbiota has already been described and shown to exhibit  
3  
4 considerably less diversity than the gut microbiota [47]. The dynamics of this microbiota has  
5  
6 also been studied and shown to closely follow menstrual cycles [48].  
7  
8  
9

10 Using the time series of OTU abundances (measured via 16S RNA sequencing and qPCR) we  
11  
12 will infer interaction parameters by assuming an underlying Lotka-Volterra competition model  
13  
14 [56]. This work will include time series analysis techniques (e.g. auto-correlation or local  
15  
16 similarity analysis) and statistical inference methods in order to infer community structure and  
17  
18 interactions from the next-generation sequencing (NGS) datasets [57]. Finally, statistical  
19  
20 methods from ecology will also be used to study community diversity (e.g diversity indices) and  
21  
22 community assembly, such as cluster and ordination analyses [58].  
23  
24  
25  
26

### Genome Wide Association Studies

27  
28 We will use human single nucleotide polymorphisms (SNPs) inferred by chip sequencing to look  
29  
30 for genetic determinants of key traits (e.g. microbiota composition or HPV infection duration).  
31  
32 This is classically done by performing a Genome Wide Association Study (GWAS), which is a  
33  
34 complex regression method designed for situations where there are many explanatory variables  
35  
36 (here millions of SNPs) for a single trait of interest. GWAS will be performed using classical  
37  
38 methods [59]. Earlier GWAS studies have been applied to HPV infections for instance to test for  
39  
40 determinants to the ability to seroconvert following infection [60] and cervical cancer (see [61]  
41  
42 for a review). Here, our expected sample ( $N = 300$  women) is limited but SNPs with large effects  
43  
44 have been detected by studies with comparable sizes [62].  
45  
46  
47  
48  
49  
50

### Additional analyses

51  
52 For all collected variables, descriptive statistics will be calculated according to the level of  
53  
54 measurement. For metric variables these statistics can be mean and standard deviation as well  
55  
56 as quantiles and more robust statistics [63]. In case of categorical variables group proportions  
57  
58 and contingency tables are prepared.  
59  
60

Univariate inferential statistics follow a descriptive analysis. Generally, parametric testing

procedures are preferred to non-parametric tests, as the former have higher power. That is why, for metric variables, we will first check whether the data can be assumed to be normally distributed. For normally distributed variables, ANOVA statistics are done to detect differences between groups. In case of significance, post-hoc analysis (Tukey test) are planned to reveal pairwise differences. If the data are not normally distributed or ordinally scaled, non-parametric analyses will be used. These contain the Kruskal-Wallis test and the Wilcoxon test as a post-hoc test with an appropriate correction of the significance level. Since the cell counts are expected to be small, Fisher's exact test will be performed for contingency tables instead of the asymptotic  $\chi^2$  test for categorical variables.

### Sample size calculation

The study will enrol a total of  $N = 300$  women, with  $N = 150$  in a longitudinal study and  $N = 150$  in a cross-sectional study. The goal of the longitudinal study is to follow 75 women longitudinally, preferentially before they are infected (see above). For the following calculations, we assumed a high percentage of lost during follow-up (30%).

With 150 enrolments and considering that the prevalence of HPV infection in young women is  $\approx 60\%$  (based on our preliminary data) and 30% of lost to follow-up, we expect to detect (and successfully follow) 63 infections at inclusion [CI95: 51–75, assuming a binomial distribution to calculate the 95% confidence interval].

Among women who are uninfected at the first visit and considering the yearly incidence being close to 30% [64], we expect 12 [CI95: 6–20] to be infected during the first year of follow-up (still assuming 30% of lost to follow-up).

In the end, with 150 enrolments and assuming a high percentage of lost to follow-up (30%), we expect to successfully follow 75 [CI95: 56–95] women infected at different stages of HPV

infection: beginning, during and end.

This will be made possible by the probability of transmission of HPV, which is estimated to be  $\approx$  90% without condom use and still high with condom use ( $\approx$  40%) [34].

Finally, regarding potential interference with the HPV vaccines, we do not anticipate any significant problem for two reasons. First, as mentioned above, the vaccine coverage is low in France [32]. Second, and more importantly, the vaccines only target few HPV types, thus leaving open the possibility of infection by dozens of types. Furthermore, studying the kinetics of a non-vaccine HPV type in a vaccinated woman will be extremely informative, e.g. to detect any potential cross-reactivity [65].

To run cross-sectional analyses (especially on the microbiota and human genetics), we will enrol  $N = 150$  additional women who will only perform the inclusion and the results visits. This sample size was chosen to reach that of earlier GWAS studies [61, 62].

## **Trial governance**

### **Sponsor**

This study is sponsored by the Centre Hospitalier Universitaire (CHU) of Montpellier. The CHU is involved in the implementation of the trial, legal/ethical submissions (see below for details on Ethics approval) and implementing the clinical database (eCRF), which is hosted by Ennov-Clinical (ClinSight). The CHU is not involved in the analysis or interpretation of the data. The CHU of Montpellier performs regular quality control assessments. A clinical research assistant will visit the CeGIDD every 4 months to ensure that implementation is in accordance with the protocol. The CHU has taken out insurance from the Société hospitalière d'assurances mutuelles, 18, rue Edouard Rochet-6 9372 Lyon cedex 08 (contract number 138983) through the full research period, covering its own civil liability and that of any agent (clinical or research staff), in accordance with article L.1121-10 of the French Public Health Code.

### Scientific committee

1  
2 The scientific committee comprises the study investigators, clinicians, scientific experts and  
3  
4 representatives of the sponsor. The committee meets yearly and is responsible for following  
5  
6 research progress, monitoring compliance with good clinical practices and patient safety. It can  
7  
8 also decide relevant modification of the protocol. Requests from third parties to access data  
9  
10 collected during the study will be evaluated by the committee.  
11  
12  
13

### Monitoring

14  
15  
16 Monitoring is performed during the whole study at CeGIDD according to the sponsor specific  
17  
18 SOP. Routine monitoring visits are made by the monitors designated by the sponsor to check  
19  
20 compliance with the protocol, the completeness, accuracy and consistency of the data, and  
21  
22 adherence to GCP. The principal investigator ensures that eCRFs are completed in a timely  
23  
24 manner and must allow periodical access to eCRFs, patient records, drug logs, and all other  
25  
26 study-related documents and materials. The frequency of monitoring visits is determined by  
27  
28 factors such as study design and the site enrolment requirements but visits will normally occur  
29  
30 at least once every 4 months.  
31  
32  
33  
34  
35

### Trial registration

36  
37  
38 The trial has been registered to ClinicalTrials.gov on 27 Oct 2016 with ID number  
39  
40 NCT02946346.  
41  
42  
43  
44  
45  
46  
47

### Ethics and Dissemination

48  
49  
50  
51 The PAPCLEAR trial obtained favourable opinions from the Comité de Protection des  
52  
53 Personnes (CPP) Sud Méditerranée I on May 11, 2016 (CPP number 16 42, reference number  
54  
55 ID RCB 2016-A00712-49); from the Comité Consultatif sur le Traitement de l'Information en  
56  
57 matière de Recherche dans le domaine de la Santé (CCTIRS) on July 12, 2016 (reference  
58  
59 number 16.504); and from the Commission Nationale Informatique et Libertés (CNIL) on Dec  
60  
16, 2016 (reference number MMS/ABD/AR1612278, decision number DR-2016-488). This trial



1  
2 was authorised by the Agence Nationale de Sécurité du Médicament et des Produits de Santé  
3 (ANSM) on July 20, 2016 (reference 20160072000007).  
4

5 The protocol has been modified since its initial version and the latest modification was accepted  
6  
7 by the CPP on Dec 12, 2018.  
8  
9

10 All participants in the study will sign an informed consent form prior to participation.  
11  
12

13 The results will be published on preprint servers (e.g. BioRxiv), peer-reviewed journals, post-  
14  
15 print servers (e.g. HAL) and disseminated through conferences.  
16  
17  
18  
19  
20  
21  
22

## 23 **Discussion**

### 24 **Expected results**

25  
26  
27 Acute infections by HPVs are important to study because vaccination is most effective when  
28  
29 performed before the first infection. However, we currently know very little about the early  
30  
31 stages of HPV infections. This clinical study will give us an unprecedented level of detail into the  
32  
33 natural history of HPV infections in young women. Variations in virus load over time have been  
34  
35 studied but in the context of cervical cancer in older women [66]. In addition, we will also  
36  
37 describe the nature and the dynamics of the immune response (local immune cells and  
38  
39 cytokines, circulating anti-HPV antibodies) and of the vaginal microbiota. Beyond these kinetics,  
40  
41 we will also have access to data such as infection clearance or not in 24 months, presence of  
42  
43 more than one HPV type or coinfection by other STIs.  
44  
45  
46  
47  
48  
49

50  
51 These data will be analysed in the light of numerous cofactors. One of the most important will  
52  
53 be human genetics, with the sequencing of millions of SNPs. Others will be related to the sexual  
54  
55 behaviour (number of partners, contraception methods, sexual practices) and general lifestyle.  
56  
57 We, therefore, expect broader insights regarding sexual health in young women.  
58  
59  
60

## Practical and operational issues

1  
2 One of the main practical challenges resides in the analysis of cervical smears by flow  
3  
4 cytometry. Indeed, the tissues are known to be fragile, adhesive and auto-fluorescent. Even  
5  
6 though standard protocols now exist [44], they require processing fresh samples in less than 2  
7  
8 hours.  
9

10  
11  
12 Another potential issue has to do with contamination by HPV DNA between samples, which are  
13  
14 frequent in the HPV field due to the robustness of the virions and the sensitivity of the tests. To  
15  
16 certify our ability to control for these, we have entered the 2017 GLOBAL HPV DNA Proficiency  
17  
18 Panel from the WHO HPV LabNet [67].  
19  
20  
21

22  
23 Regarding the enrolment of the participants, we do not expect issues with enrolling 150 women  
24  
25 in 28 months for the longitudinal study and 150 for the cross-sectional study. This is due to the  
26  
27 number of visitors of the centre who fit the inclusion criteria (more than 3,000 per year) and  
28  
29 because of earlier high participation rates in the same population ([33] enrolled 1381  
30  
31 participants in 5 months for their study).  
32  
33  
34

35  
36 Concerning the follow-up, the high incidence rate of HPV can also lead to transient carriage,  
37  
38 i.e. women who are positive for a type only at a single visit. This has been observed for instance  
39  
40 in longitudinal studies with a tight follow-up interval [21]. To control for this, we will run the HPV  
41  
42 detection test on the cells from the cervical smear after washing with RPMI.  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 **Table 1: Summary of the visit schedules and samples take.** The cross-sectional study only  
5 includes the first two columns (V1 and V2). The  $\alpha$  indicate samples taken at visits. + participants  
6 infected by a HR-HPV for 12 month will have one PBS smear replaced by a Thinprep<sup>®</sup> smear  
7 to perform a cytology and check for lesions.  $\triangleleft$  this sample is only taken at the first HPV+ visit of  
8 a formerly HPV- participant. \* STI detection will be performed at inclusion unless the participant  
9 has been tested within the last 3 months and during the study every 6 months if a new partner  
10 has been reported or upon request.  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

|                                          | Inclusion<br>(V <sub>1</sub> ) | Results<br>(V <sub>2</sub> ) | Return<br>(V <sub>i</sub> , with $i > 2$ ) |            |
|------------------------------------------|--------------------------------|------------------------------|--------------------------------------------|------------|
| Participants                             | all                            | all                          | HPV+                                       | HPV-       |
| Time                                     | day 0                          | + 4 weeks                    | + 8 weeks                                  | + 16 weeks |
| Eligibility                              | ☐                              |                              |                                            |            |
| Consent                                  | ☐                              |                              |                                            |            |
| Gynecological consult                    | ☐                              | ☐                            | ☐                                          | ☐          |
| Vaginal pH cotton swab                   | ☐                              | ☐                            | ☐                                          | ☐          |
| 2 vaginal swab samples (Copan<br>ESwab™) | ☐                              | ☐                            | ☐                                          | ☐          |
| 1 ophthalmological sponge sample         | ☐                              |                              | ☐                                          | ☐          |
| 1 cervical smear in Thinprep® (cytology) | ☐                              |                              | +                                          |            |
| 1 cervical smear in PBS                  |                                | ☐                            | +                                          | ☐          |
| Blood sampling (HPV antibodies)          | ☐                              |                              | ☐                                          |            |
| Blood sampling (sequencing)              | ☐                              |                              |                                            |            |
| Blood sampling (immunophenotyping)       | ☐                              |                              | △                                          |            |
| Other STI detection                      | *                              | *                            | *                                          | *          |
| Questionnaire #1 (inclusion)             | ☐                              |                              |                                            |            |
| Questionnaire #2 (visit)                 |                                | ☐                            | ☐                                          | ☐          |
| Questionnaire #3 (home)                  | ☐                              | ☐                            | ☐                                          | ☐          |
| Returning self-sampling samples          |                                | ☐                            | ☐                                          | ☐          |
| Serious Adverse Event collection         |                                | ☐                            | ☐                                          | ☐          |

## Abbreviations

1  
2  
3 ANOVA: Analysis of variance,

4  
5 ASC-US: Atypical squamous cells of undetermined significance,

6  
7 CD: Cluster of differentiation,

8  
9 CI95: 95% Confidence interval,

10  
11 CeGIDD: Centre Gratuit d'Information de Dépistage et de Diagnostic,

12  
13  
14 CHU: Centre Hospitalier Universitaire,

15  
16 CIN: Cervical intraepithelial Neoplasia,

17  
18 CRT: Clinical Research Technician,

19  
20 ELISA: enzyme-linked immunosorbent assay,

21  
22 GWAS: Genome Wide Association Study,

23  
24 HIV: Human Immunodeficiency Virus,

25  
26 HPV: Human Papillomavirus,

27  
28 HR: high-risk,

29  
30 ITS: Internal Transcribed Spacer,

31  
32 HSIL: High grade Squamous Intraepithelial Lesion,

33  
34 LR: low-risk,

35  
36 LSIL: Low grade Squamous Intraepithelial Lesion,

37  
38 NGS: Next Generation Sequencing,

39  
40 OTU: Operational Taxonomic Unit,

41  
42 PBMC: Peripheral Blood Mononuclear Cell,

43  
44 PBS: Phosphate Buffered Saline,

45  
46 RPMI: Roswell Park Memorial Institute medium,

47  
48 SNP: Single Nucleotide Polymorphism,

49  
50 TCR: T-cell receptor,

51  
52 WHO: World Health Organisation.

## Trial status

The study began on Oct 1, 2016 and the first inclusion was on Nov 3, 2016. On Jun 23, 2018, 89 participants have been included in the longitudinal study. Inclusions in the longitudinal study will continue until March 2019 and the study is expected to last until Aug 2021.

## Conflicts of interests

The authors have read and understood BMJ policy on declaration of interests and declare that they have no competing interests.

## Funding

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement No 648963). The authors acknowledge further support from the Centre National de la Recherche Scientifique (CNRS), the Institute de Recherche pour le Développement (IRD) and the Centre Hospitalier Universitaire (CHU) of Montpellier.

## Data statement

All personal and identifying information collected from participants are kept in a secure place at the CeGIDD during the duration of the trial and will be destroyed at the end of the study. The final raw dataset will be accessible only to the sponsor (CHU) and the chief scientist's (SA) team. Anonymous data will be available to external parties upon approval of both the sponsor and the scientific committee. All publications will be made green or gold open access and the corresponding data will be provided as supplementary material or via a public repository (e.g. Zenodo), provided that there is no conflict with ethical guidelines.

## Author contributions

Samuel Alizon, Carmen Lia Murall and Massical Rahmoun were the major contributors in the conception of the protocol. Samuel Alizon wrote the initial version of the manuscript. Christian

1 Selinger, Monique Baldellou, Claire Bernat, Marine Bonneau, Vanina Boué, Mathilde Buisson,  
2 Guillaume Christophe, Giuseppe D'Auria, Florence De Taroni, Vincent Foulongne, Rémy  
3  
4 Froissart, Christelle Graf, Sophie Grasset, Soraya Groc, Christophe Hirtz, Audrey Jausset,  
5  
6 Julie Lajoie, Frédérique Lorcy, Eric Picot, Marie-Christine Picot, Jacques Ravel, Jacques  
7  
8 Reynes, Thérèse Rousset, Aziza Seddiki, Martine Teirlinck, Vincent Tribout, Édouard Tuillon,  
9  
10 Tim Waterboer, Nathalie Jacobs, Ignacio G Bravo, Michel Segondy and Natalie Boulle were  
11  
12 involved in the conception of the protocol, in the implementation of the study and read and  
13  
14 approved the final manuscript.  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32

## 33 **Acknowledgements**

34  
35 We thank all the study participants and the CeGIDD staff for their commitment to the study. We  
36  
37 also thank the reviewers and, in particular, Dr. Andrew Brouwer for his meticulous reading of the  
38  
39 manuscript.  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## References

1. Tota, J.E., Chevarie-Davis, M., Richardson, L.A., Devries, M., Franco, E.L.: Epidemiology and burden of HPV infection and related diseases: implications for prevention strategies. *Prev Med* **53 Suppl 1**, 12–21 (2011). doi:[10.1016/j.ypmed.2011.08.017](https://doi.org/10.1016/j.ypmed.2011.08.017)
2. Monsonego, J., Zerat, L., Syrjänen, K., Zerat, J.C., Smith, J.S., Halfon, P.: Prevalence of genotype-specific HPV infection among women in France: implications for screening and vaccination. *Gynecol Obstet Fertil* **41**(5), 305–313 (2013). doi:[10.1016/j.gyobfe.2013.03.003](https://doi.org/10.1016/j.gyobfe.2013.03.003)
3. Brun-Micaleff, E., Coffy, A., Rey, V., Didelot, M.-N., Combecal, J., Doutre, S., Daurès, J.-P., Segondy, M., Boulle, N.: Cervical cancer screening by cytology and human papillomavirus testing during pregnancy in french women with poor adhesion to regular cervical screening. *J Med Virol* **86**(3), 536–45 (2014). doi:[10.1002/jmv.23764](https://doi.org/10.1002/jmv.23764)
4. Bruni, L., Diaz, M., Castellsagué, X., Ferrer, E., Bosch, F.X., de Sanjosé, S.: Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. *J Infect Dis* **202**(12), 1789–99 (2010). doi:[10.1086/657321](https://doi.org/10.1086/657321)
5. Insinga, R.P., Dasbach, E.J., Elbasha, E.H., Liaw, K.-L., Barr, E.: Incidence and duration of cervical human papillomavirus 6, 11, 16, and 18 infections in young women: an evaluation from multiple analytic perspectives. *Cancer Epidemiol Biomarkers Prev* **16**(4), 709–15 (2007). doi:[10.1158/1055-9965.EPI-06-0846](https://doi.org/10.1158/1055-9965.EPI-06-0846)
6. Woodman, C.B.J., Collins, S.I., Young, L.S.: The natural history of cervical HPV infection: unresolved issues. *Nat Rev Cancer* **7**(1), 11–22 (2007). doi:[10.1038/nrc2050](https://doi.org/10.1038/nrc2050)
7. Rodríguez, A.C., Schiffman, M., Herrero, R., Wacholder, S., Hildesheim, A., Castle, P.E., Solomon, D., Burk, R., Proyecto Epidemiológico Guanacaste Group: Rapid clearance of human papillomavirus and implications for clinical focus on persistent infections. *J Natl Cancer Inst* **100**(7), 513–7 (2008). doi:[10.1093/jnci/djn044](https://doi.org/10.1093/jnci/djn044)



- 1  
2  
3  
4  
5  
6  
7  
8  
9
8. Trottier, H., Mahmud, S., Prado, J.C.M., Sobrinho, J.S., Costa, M.C., Rohan, T.E., Villa, L.L., Franco, E.L.: Type-Specific Duration of Human Papillomavirus Infection: Implications for Human Papillomavirus Screening and Vaccination. *J Infect Dis* **197**(10), 1436–1447 (2008). doi:[10.1086/587698](https://doi.org/10.1086/587698)
- 10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24
9. Ramanakumar, A.V., Naud, P., Roteli-Martins, C.M., de Carvalho, N.S., de Borba, P.C., Teixeira, J.C., Blatter, M., Moscicki, A.-B., Harper, D.M., Romanowski, B., Tying, S.K., Ramjattan, B., Schuind, A., Dubin, G., Franco, E.L.: Incidence and duration of type-specific human papillomavirus infection in high-risk HPV-naïve women: results from the control arm of a phase II HPV-16/18 vaccine trial. *BMJ Open* **6**(8), 011371 (2016). doi:[10.1136/bmjopen-2016-011371](https://doi.org/10.1136/bmjopen-2016-011371)
- 25  
26  
27  
28  
29  
30  
31  
32  
33  
34
10. Houlihan, C.F., Baisley, K., Bravo, I.G., Kapiga, S., de Sanjosé, S., Changalucha, J., Ross, D.A., Hayes, R.J., Watson-Jones, D.: Rapid acquisition of HPV around the time of sexual debut in adolescent girls in Tanzania. *Int J Epidemiol* **45**(3), 762–773 (2016). doi:[10.1093/ije/dyv367](https://doi.org/10.1093/ije/dyv367)
- 35  
36  
37  
38  
39  
40
11. Alizon, S., Murall, C.L., Bravo, I.G.: Why Human Papillomavirus Acute Infections Matter. *Viruses* **9**(10), 293 (2017). doi:[10.3390/v9100293](https://doi.org/10.3390/v9100293)
- 41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54
12. Herrero, R., Wacholder, S., Rodríguez, A.C., Solomon, D., González, P., Kreimer, A.R., Porras, C., Schussler, J., Jiménez, S., Sherman, M.E., Quint, W., Schiller, J.T., Lowy, D.R., Schiffman, M., Hildesheim, A., Costa Rica Vaccine Trial Group: Prevention of persistent human papillomavirus infection by an HPV16/18 vaccine: a community-based randomized clinical trial in Guanacaste, Costa Rica. *Cancer Discov* **1**(5), 408–19 (2011). doi:[10.1158/2159-8290.CD-11-0131](https://doi.org/10.1158/2159-8290.CD-11-0131)
- 55  
56  
57  
58  
59  
60
13. Canini, L., Perelson, A.S.: Viral kinetic modeling: state of the art. *J Pharmacokinet Pharmacodyn* **41**(5), 431–443 (2014). doi:[10.1007/s10928-014-9363-3](https://doi.org/10.1007/s10928-014-9363-3)
14. Stanley, M.: Immune responses to human papillomavirus. *Vaccine* **24**(S1), 16–22 (2006).

doi:[10.1016/j.vaccine.2005.09.002](https://doi.org/10.1016/j.vaccine.2005.09.002)

- 1  
2  
3 15. Ferenczy, A., Franco, E.: Persistent human papillomavirus infection and cervical  
4  
5 neoplasia. *Lancet Oncol* **3**(1), 11–6 (2002)  
6  
7
- 8  
9 16. zur Hausen, H.: Review: Papillomaviruses — to Vaccination and Beyond. *Biochemistry*  
10  
11 **73**(5), 498–503 (2008). doi:[10.1134/S0006297908050027](https://doi.org/10.1134/S0006297908050027)  
12  
13
- 14  
15 17. Einstein, M.H., Schiller, J.T., Viscidi, R.P., Strickler, H.D., Coursaget, P., Tan, T., Halsey,  
16  
17 N., Jenkins, D.: Clinician's guide to human papillomavirus immunology: knowns and unknowns.  
18  
19 *Lancet Infect Dis* **9**(6), 347–56 (2009). doi:[10.1016/S1473-3099\(09\)70108-2](https://doi.org/10.1016/S1473-3099(09)70108-2)  
20  
21
- 22  
23 18. Van Hede, D., Langers, I., Delvenne, P., Jacobs, N.: Origin and immunoescape of uterine  
24  
25 cervical cancer. *Presse Med* **43**(12P2), 413–421 (2014). doi:[10.1016/j.lpm.2014.09.005](https://doi.org/10.1016/j.lpm.2014.09.005)  
26  
27
- 28  
29 19. Stanley, M.: Immunology of HPV infection. *Curr Obstet Gynecol Rep* **4**(4), 195–200  
30  
31 (2015). doi:[10.1007/s13669-015-0134-y](https://doi.org/10.1007/s13669-015-0134-y). Accessed 2017-03-20  
32  
33
- 34  
35 20. Gao, W., Weng, J., Gao, Y., Chen, X.: Comparison of the vaginal microbiota diversity of  
36  
37 women with and without human papillomavirus infection: a cross-sectional study. *BMC Infect*  
38  
39 *Dis* **13**, 271 (2013). doi:[10.1186/1471-2334-13-271](https://doi.org/10.1186/1471-2334-13-271)  
40  
41
- 42  
43 21. Brotman, R.M., Shardell, M.D., Gajer, P., Tracy, J.K., Zenilman, J.M., Ravel, J., Gravitt,  
44  
45 P.E.: Interplay between the temporal dynamics of the vaginal microbiota and human  
46  
47 papillomavirus detection. *J Infect Dis* **210**(11), 1723–33 (2014). doi:[10.1093/infdis/jiu330](https://doi.org/10.1093/infdis/jiu330)  
48  
49
- 50  
51 22. Koutsky, L.A., Ault, K.A., Wheeler, C.M., Brown, D.R., Barr, E., Alvarez, F.B.,  
52  
53 Chiacchierini, L.M., Jansen, K.U., Proof of Principle Study Investigators: A controlled trial of a  
54  
55 human papillomavirus type 16 vaccine. *N Engl J Med* **347**(21), 1645–51 (2002).  
56  
57 doi:[10.1056/NEJMoa020586](https://doi.org/10.1056/NEJMoa020586)  
58  
59
- 60  
23. Riethmuller, D., Jacquard, A.-C., Lacau St Guily, J., Aubin, F., Carcopino, X., Pradat, P.,  
Dahlab, A., Pr  tet, J.-L.: Potential impact of a nonavalent hpv vaccine on the occurrence of hpv-

related diseases in france. BMC Public Health **15**, 453 (2015). doi:[10.1186/s12889-015-1779-1](https://doi.org/10.1186/s12889-015-1779-1)

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
24. Joura, E.A., Giuliano, A.R., Iversen, O.-E., Bouchard, C., Mao, C., Mehlsen, J., Moreira, E.D. Jr, Ngan, Y., Petersen, L.K., Lazcano-Ponce, E., Pitisuttithum, P., Restrepo, J.A., Stuart, G., Woelber, L., Yang, Y.C., Cuzick, J., Garland, S.M., Huh, W., Kjaer, S.K., Bautista, O.M., Chan, I.S.F., Chen, J., Gesser, R., Moeller, E., Ritter, M., Vuocolo, S., Luxembourg, A., Broad Spectrum HPV Vaccine Study: A 9-valent HPV vaccine against infection and intraepithelial neoplasia in women. N Engl J Med **372**(8), 711–23 (2015). doi:[10.1056/NEJMoa1405044](https://doi.org/10.1056/NEJMoa1405044)
25. Murall, C.L., Bauch, C.T., Day, T.: Could the human papillomavirus vaccines drive virulence evolution? Proc Biol Sci **282**, 20141069 (2015). doi:[10.1098/rspb.2014.1069](https://doi.org/10.1098/rspb.2014.1069)
26. Alizon, S., Méthot, P.-O.: Reconciling Pasteur and Darwin to control infectious diseases. PLoS Biol **16**(1), 2003815 (2018). doi:[10.1371/journal.pbio.2003815](https://doi.org/10.1371/journal.pbio.2003815)
27. Moscicki, A.-B., Ma, Y., Wibbelsman, C., Darragh, T.M., Powers, A., Farhat, S., Shiboski, S.: Rate of and Risks for Regression of CIN-2 in adolescents and young women. Obstet Gynecol **116**(6), 1373–1380 (2010). doi:[10.1097/AOG.0b013e3181fe777f](https://doi.org/10.1097/AOG.0b013e3181fe777f)
28. Buck Jr., H.W.: Warts (genital). BMJ Clin Evid **2015**, 1602 (2015)
29. Herrero, R., González, P., Markowitz, L.E.: Present status of human papillomavirus vaccine development and implementation. Lancet Oncol **16**(5), 206–16 (2015). doi:[10.1016/S1470-2045\(14\)70481-4](https://doi.org/10.1016/S1470-2045(14)70481-4)
30. Maver, P.J., Poljak, M.: Progress in prophylactic human papillomavirus (HPV) vaccination in 2016: A literature review. Vaccine (2018). doi:[10.1016/j.vaccine.2017.07.113](https://doi.org/10.1016/j.vaccine.2017.07.113)
31. Fagot, J.-P., Boutrelle, A., Ricordeau, P., Weill, A., Allemand, H.: HPV vaccination in France: uptake, costs and issues for the National Health Insurance. Vaccine **29**(19), 3610–6 (2011). doi:[10.1016/j.vaccine.2011.02.064](https://doi.org/10.1016/j.vaccine.2011.02.064)

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

32. Ben Hadj Yahia, M.-B., Dervaux, B., Duport, N., Floret, D., Gaillot, J., Heard, I., Jacquet, A., Le Goaster, C., Lévy-Bruhl, D., Morer, I., Parent du Chatelet, I., Peigue-Lafeuille, H., Rumeau-Pichon, C.: Vaccination contre les infections à papilloamvirus. Technical report, Haut Conseil de la Santé Publique, Paris, France (2014).

<https://www.hcsp.fr/Explore.cgi/avisrapportsdomaine?clefr=454>

33. Clarivet, B., Picot, E., Marchandin, H., Tribout, V., Rachedi, N., Schwartzentruber, E., Ledésert, B., Dereure, O., Guillot, B., Picot, M.-C.: Prevalence of Chlamydia trachomatis, Neisseria gonorrhoeae and Mycoplasma genitalium in asymptomatic patients under 30 years of age screened in a French sexually transmitted infections clinic. *Eur J Dermatol* **24**(5), 611–6 (2014). doi:[10.1684/ejd.2014.2413](https://doi.org/10.1684/ejd.2014.2413)

34. Winer, R.L., Hughes, J.P., Feng, Q., O'Reilly, S., Kiviat, N.B., Holmes, K.K., Koutsky, L.A.: Condom use and the risk of genital human papillomavirus infection in young women. *N Engl J Med* **354**(25), 2645–54 (2006). doi:[10.1056/NEJMoa053284](https://doi.org/10.1056/NEJMoa053284)

35. Winer, R.L., Hughes, J.P., Feng, Q., Stern, J.E., Xi, L.F., Koutsky, L.A.: Incident Detection of High-Risk Human Papillomavirus Infections in a Cohort of High-Risk Women Aged 25-65 Years. *J Infect Dis* **214**(5), 665–75 (2016). doi:[10.1093/infdis/jiw074](https://doi.org/10.1093/infdis/jiw074)

36. Ravel, J., Brotman, R.M., Gajer, P., Ma, B., Nandy, M., Fadrosh, D.W., Sakamoto, J., Koenig, S.S., Fu, L., Zhou, X., Hickey, R.J., Schwebke, J.R., Forney, L.J.: Daily temporal dynamics of vaginal microbiota before, during and after episodes of bacterial vaginosis. *Microbiome* **1**(1), 29 (2013). doi:[10.1186/2049-2618-1-29](https://doi.org/10.1186/2049-2618-1-29)

37. Kleter, B., van Doorn, L.-J., ter Schegget, J., Schrauwen, L., van Krimpen, K., Burger, M.,

- ter Harmsel, B., Quint, W.: Novel Short-Fragment PCR Assay for Highly Sensitive Broad-Spectrum Detection of Anogenital Human Papillomaviruses. *Am J Pathol* **153**(6), 1731–1739 (1998). doi:[10.1016/S0002-9440\(10\)65688-X](https://doi.org/10.1016/S0002-9440(10)65688-X)
38. Geraets, D.T., Struijk, L., Kleter, B., Molijn, A., van Doorn, L.-J., Quint, W.G.V., Colau, B.: The original SPF10 LiPA25 algorithm is more sensitive and suitable for epidemiologic HPV research than the SPF10 INNO-LiPA Extra. *J Virol Meth* **215-216**, 22–29 (2015). doi:[10.1016/j.jviromet.2015.01.001](https://doi.org/10.1016/j.jviromet.2015.01.001)
39. Gravitt, P.E., Peyton, C.L., Alessi, T.Q., Wheeler, C.M., Coutlée, F., Hildesheim, A., Schiffman, M.H., Scott, D.R., Apple, R.J.: Improved amplification of genital Human Papillomaviruses. *J Clin Microbiol* **38**(1), 357–361 (2000)
40. Micalessi, I.M., Boulet, G.A.V., Bogers, J.J., Benoy, I.H., Depuydt, C.E.: High-throughput detection, genotyping and quantification of the human papillomavirus using real-time PCR. *Clin Chem Lab Med* **50**(4), 655–661 (2012). doi:[10.1515/cclm.2011.835](https://doi.org/10.1515/cclm.2011.835)
41. Hunter, P.J., Sheikh, S., David, A.L., Peebles, D.M., Klein, N.: Cervical leukocytes and spontaneous preterm birth. *Journal of Reproductive Immunology* **113**, 42–49 (2016). doi:[10.1016/j.jri.2015.11.002](https://doi.org/10.1016/j.jri.2015.11.002)
42. Shannon, B., Yi, T.J., Perusini, S., Gajer, P., Ma, B., Humphrys, M.S., Thomas-Pavanel, J., Chieza, L., Janakiram, P., Saunders, M., Tharao, W., Huibner, S., Shahabi, K., Ravel, J., Rebbapragada, A., Kaul, R.: Association of HPV infection and clearance with cervicovaginal immunology and the vaginal microbiota. *Mucosal Immunology* **10**(5), 1310–1319 (2017). doi:[10.1038/mi.2016.129](https://doi.org/10.1038/mi.2016.129)
43. Lajoie, J., Juno, J., Burgener, A., Rahman, S., Mogk, K., Wachihi, C., Mwanjewe, J., Plummer, F.A., Kimani, J., Ball, T.B., Fowke, K.R.: A distinct cytokine and chemokine profile at the genital mucosa is associated with HIV-1 protection among HIV-exposed seronegative commercial sex workers. *Mucosal Immunol* **5**(3), 277–287 (2012). doi:[10.1038/mi.2012.7](https://doi.org/10.1038/mi.2012.7)

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
44. Juno, J.A., Boily-Larouche, G., Lajoie, J., Fowke, K.R.: Collection, isolation, and flow cytometric analysis of human endocervical samples. *J Vis Exp* **89**, 51906 (2014).  
doi:[10.3791/51906](https://doi.org/10.3791/51906)
45. Frank, J.A., Reich, C.I., Sharma, S., Weisbaum, J.S., Wilson, B.A., Olsen, G.J.: Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Appl Environ Microbiol* **74**(8), 2461 (2008). doi:[10.1128/AEM.02272-07](https://doi.org/10.1128/AEM.02272-07)
46. Findley, K., Oh, J., Yang, J., Conlan, S., Deming, C., Meyer, J.A., Schoenfeld, D., Nomicos, E., Park, M., NIH Intramural Sequencing Center Comparative Sequencing Program, Kong, H.H., Segre, J.A.: Topographic diversity of fungal and bacterial communities in human skin. *Nature* **498**(7454), 367–370 (2013). doi:[10.1038/nature12171](https://doi.org/10.1038/nature12171). Accessed 2017-09-13
47. Ravel, J., Gajer, P., Abdo, Z., Schneider, G.M., Koenig, S.S.K., McCulle, S.L., Karlebach, S., Gorle, R., Russell, J., Tacket, C.O., Brotman, R.M., Davis, C.C., Ault, K., Peralta, L., Forney, L.J.: Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci USA* **108**, 4680–7 (2011). doi:[10.1073/pnas.1002611107](https://doi.org/10.1073/pnas.1002611107)
48. Gajer, P., Brotman, R.M., Bai, G., Sakamoto, J., Schütte, U.M.E., Zhong, X., Koenig, S.S.K., Fu, L., Ma, Z.S., Zhou, X., Abdo, Z., Forney, L.J., Ravel, J.: Temporal dynamics of the human vaginal microbiota. *Sci Transl Med* **4**(132), 132–52 (2012).  
doi:[10.1126/scitranslmed.3003605](https://doi.org/10.1126/scitranslmed.3003605)
49. Johne, R., Müller, H., Rector, A., van Ranst, M., Stevens, H.: Rolling-circle amplification of viral DNA genomes using phi29 polymerase. *Trends Microbiol* **17**(5), 205–211 (2009).  
doi:[10.1016/j.tim.2009.02.004](https://doi.org/10.1016/j.tim.2009.02.004)
50. Nowak, M.A., May, R.M.: *Virus Dynamics: Mathematical Principles of Immunology and Virology*. Oxford University Press, Oxford, USA (2000)
51. Stafford, M.A., Corey, L., Cao, Y., Daar, E.S., Ho, D.D., Perelson, A.S.: Modeling plasma virus concentration during primary HIV infection. *J. theor. Biol.* **203**(3), 285–301 (2000).

doi:[10.1006/jtbi.2000.1076](https://doi.org/10.1006/jtbi.2000.1076)

1  
2  
3 52. Perelson, A.S.: Modelling viral and immune system dynamics. *Nat. Rev. Immunol.* **2**(1),  
4 28–36 (2002). doi:[10.1038/nri700](https://doi.org/10.1038/nri700)

5  
6  
7  
8  
9 53. Murall, C.L., Jackson, R., Zehbe, I., Boulle, N., Segondy, M., Alizon, S.: Epithelial  
10 stratification shapes infection dynamics. *PLoS Comput Biol* **15**(1), 1006646 (2019).  
11  
12  
13 doi:[10.1371/journal.pcbi.1006646](https://doi.org/10.1371/journal.pcbi.1006646)

14  
15  
16  
17 54. Steimer, J.L., Vozech, S., Racine Poon, A., Holford, N., O'Neil, R.: The population  
18 approach: rationale, methods and applications in clinical pharmacology and drug development.  
19 In: Balant, P.G.W..L. (ed.) *Handbook of Experimental Pharmacology*, vol. 110, pp. 405–451.  
20  
21  
22  
23 Springer, Berlin (1994)

24  
25  
26  
27 55. Bates, D., Mächler, M., Bolker, B., Walker, S.: Fitting linear mixed-effects models using  
28 lme4. *Journal of Statistical Software* **67**(1) (2015). doi:[10.18637/jss.v067.i01](https://doi.org/10.18637/jss.v067.i01)

29  
30  
31  
32  
33 56. Bucci, V., Tzen, B., Li, N., Simmons, M., Tanoue, T., Bogart, E., Deng, L., Yeliseyev, V.,  
34 Delaney, M.L., Liu, Q., Olle, B., Stein, R.R., Honda, K., Bry, L., Gerber, G.K.: MDSINE:  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65  
66  
67  
68  
69  
70  
71  
72  
73  
74  
75  
76  
77  
78  
79  
80  
81  
82  
83  
84  
85  
86  
87  
88  
89  
90  
91  
92  
93  
94  
95  
96  
97  
98  
99  
100  
101  
102  
103  
104  
105  
106  
107  
108  
109  
110  
111  
112  
113  
114  
115  
116  
117  
118  
119  
120  
121  
122  
123  
124  
125  
126  
127  
128  
129  
130  
131  
132  
133  
134  
135  
136  
137  
138  
139  
140  
141  
142  
143  
144  
145  
146  
147  
148  
149  
150  
151  
152  
153  
154  
155  
156  
157  
158  
159  
160  
161  
162  
163  
164  
165  
166  
167  
168  
169  
170  
171  
172  
173  
174  
175  
176  
177  
178  
179  
180  
181  
182  
183  
184  
185  
186  
187  
188  
189  
190  
191  
192  
193  
194  
195  
196  
197  
198  
199  
200  
201  
202  
203  
204  
205  
206  
207  
208  
209  
210  
211  
212  
213  
214  
215  
216  
217  
218  
219  
220  
221  
222  
223  
224  
225  
226  
227  
228  
229  
230  
231  
232  
233  
234  
235  
236  
237  
238  
239  
240  
241  
242  
243  
244  
245  
246  
247  
248  
249  
250  
251  
252  
253  
254  
255  
256  
257  
258  
259  
260  
261  
262  
263  
264  
265  
266  
267  
268  
269  
270  
271  
272  
273  
274  
275  
276  
277  
278  
279  
280  
281  
282  
283  
284  
285  
286  
287  
288  
289  
290  
291  
292  
293  
294  
295  
296  
297  
298  
299  
300  
301  
302  
303  
304  
305  
306  
307  
308  
309  
310  
311  
312  
313  
314  
315  
316  
317  
318  
319  
320  
321  
322  
323  
324  
325  
326  
327  
328  
329  
330  
331  
332  
333  
334  
335  
336  
337  
338  
339  
340  
341  
342  
343  
344  
345  
346  
347  
348  
349  
350  
351  
352  
353  
354  
355  
356  
357  
358  
359  
360  
361  
362  
363  
364  
365  
366  
367  
368  
369  
370  
371  
372  
373  
374  
375  
376  
377  
378  
379  
380  
381  
382  
383  
384  
385  
386  
387  
388  
389  
390  
391  
392  
393  
394  
395  
396  
397  
398  
399  
400  
401  
402  
403  
404  
405  
406  
407  
408  
409  
410  
411  
412  
413  
414  
415  
416  
417  
418  
419  
420  
421  
422  
423  
424  
425  
426  
427  
428  
429  
430  
431  
432  
433  
434  
435  
436  
437  
438  
439  
440  
441  
442  
443  
444  
445  
446  
447  
448  
449  
450  
451  
452  
453  
454  
455  
456  
457  
458  
459  
460  
461  
462  
463  
464  
465  
466  
467  
468  
469  
470  
471  
472  
473  
474  
475  
476  
477  
478  
479  
480  
481  
482  
483  
484  
485  
486  
487  
488  
489  
490  
491  
492  
493  
494  
495  
496  
497  
498  
499  
500  
501  
502  
503  
504  
505  
506  
507  
508  
509  
510  
511  
512  
513  
514  
515  
516  
517  
518  
519  
520  
521  
522  
523  
524  
525  
526  
527  
528  
529  
530  
531  
532  
533  
534  
535  
536  
537  
538  
539  
540  
541  
542  
543  
544  
545  
546  
547  
548  
549  
550  
551  
552  
553  
554  
555  
556  
557  
558  
559  
560  
561  
562  
563  
564  
565  
566  
567  
568  
569  
570  
571  
572  
573  
574  
575  
576  
577  
578  
579  
580  
581  
582  
583  
584  
585  
586  
587  
588  
589  
590  
591  
592  
593  
594  
595  
596  
597  
598  
599  
600  
601  
602  
603  
604  
605  
606  
607  
608  
609  
610  
611  
612  
613  
614  
615  
616  
617  
618  
619  
620  
621  
622  
623  
624  
625  
626  
627  
628  
629  
630  
631  
632  
633  
634  
635  
636  
637  
638  
639  
640  
641  
642  
643  
644  
645  
646  
647  
648  
649  
650  
651  
652  
653  
654  
655  
656  
657  
658  
659  
660  
661  
662  
663  
664  
665  
666  
667  
668  
669  
670  
671  
672  
673  
674  
675  
676  
677  
678  
679  
680  
681  
682  
683  
684  
685  
686  
687  
688  
689  
690  
691  
692  
693  
694  
695  
696  
697  
698  
699  
700  
701  
702  
703  
704  
705  
706  
707  
708  
709  
710  
711  
712  
713  
714  
715  
716  
717  
718  
719  
720  
721  
722  
723  
724  
725  
726  
727  
728  
729  
730  
731  
732  
733  
734  
735  
736  
737  
738  
739  
740  
741  
742  
743  
744  
745  
746  
747  
748  
749  
750  
751  
752  
753  
754  
755  
756  
757  
758  
759  
760  
761  
762  
763  
764  
765  
766  
767  
768  
769  
770  
771  
772  
773  
774  
775  
776  
777  
778  
779  
780  
781  
782  
783  
784  
785  
786  
787  
788  
789  
790  
791  
792  
793  
794  
795  
796  
797  
798  
799  
800  
801  
802  
803  
804  
805  
806  
807  
808  
809  
810  
811  
812  
813  
814  
815  
816  
817  
818  
819  
820  
821  
822  
823  
824  
825  
826  
827  
828  
829  
830  
831  
832  
833  
834  
835  
836  
837  
838  
839  
840  
841  
842  
843  
844  
845  
846  
847  
848  
849  
850  
851  
852  
853  
854  
855  
856  
857  
858  
859  
860  
861  
862  
863  
864  
865  
866  
867  
868  
869  
870  
871  
872  
873  
874  
875  
876  
877  
878  
879  
880  
881  
882  
883  
884  
885  
886  
887  
888  
889  
890  
891  
892  
893  
894  
895  
896  
897  
898  
899  
900  
901  
902  
903  
904  
905  
906  
907  
908  
909  
910  
911  
912  
913  
914  
915  
916  
917  
918  
919  
920  
921  
922  
923  
924  
925  
926  
927  
928  
929  
930  
931  
932  
933  
934  
935  
936  
937  
938  
939  
940  
941  
942  
943  
944  
945  
946  
947  
948  
949  
950  
951  
952  
953  
954  
955  
956  
957  
958  
959  
960  
961  
962  
963  
964  
965  
966  
967  
968  
969  
970  
971  
972  
973  
974  
975  
976  
977  
978  
979  
980  
981  
982  
983  
984  
985  
986  
987  
988  
989  
990  
991  
992  
993  
994  
995  
996  
997  
998  
999  
1000

58. Fox, G.A., Negrete-Yankelevich, S., Sosa, V.J.: *Ecological Statistics: Contemporary Theory and Application*. Oxford University Press, Oxford, USA (2015)

59. Shi, Y., Li, L., Hu, Z., Li, S., Wang, S., Liu, J., Wu, C., He, L., Zhou, J., Li, Z., Hu, T., Chen, Y., Jia, Y., Wang, S., Wu, L., Cheng, X., Yang, Z., Yang, R., Li, X., Huang, K., Zhang, Q., Zhou, H., Tang, F., Chen, Z., Shen, J., Jiang, J., Ding, H., Xing, H., Zhang, S., Qu, P., Song, X., Lin,

1 Z., Deng, D., Xi, L., Lv, W., Han, X., Tao, G., Yan, L., Han, Z., Li, Z., Miao, X., Pan, S., Shen, Y.,  
2 Wang, H., Liu, D., Gong, E., Li, Z., Zhou, L., Luan, X., Wang, C., Song, Q., Wu, S., Xu, H.,  
3 Shen, J., Qiang, F., Ma, G., Liu, L., Chen, X., Liu, J., Wu, J., Shen, Y., Wen, Y., Chu, M., Yu, J.,  
4 Hu, X., Fan, Y., He, H., Jiang, Y., Lei, Z., Liu, C., Chen, J., Zhang, Y., Yi, C., Chen, S., Li, W.,  
5 Wang, D., Wang, Z., Di, W., Shen, K., Lin, D., Shen, H., Feng, Y., Xie, X., Ma, D.: A genome-  
6 wide association study identifies two new cervical cancer susceptibility loci at 4q12 and 17q12.  
7 Nat Genet **45**(8), 918–22 (2013). doi:[10.1038/ng.2687](https://doi.org/10.1038/ng.2687)  
8  
9

10  
11  
12  
13  
14  
15  
16  
17 60. Chen, D., Gaborieau, V., Zhao, Y., Chabrier, A., Wang, H., Waterboer, T., Zaridze, D.,  
18 Lissowska, J., Rudnai, P., Fabianova, E., Bencko, V., Janout, V., Foretova, L., Mates, I.N.,  
19 Szeszenia-Dabrowska, N., Boffetta, P., Pawlita, M., Lathrop, M., Gyllensten, U., Brennan, P.,  
20 McKay, J.D.: A systematic investigation of the contribution of genetic variation within the MHC  
21 region to HPV seropositivity. Hum Mol Genet **24**(9), 2681–2688 (2015).  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

60. Chen, D., Gyllensten, U.: Lessons and implications from association studies and post-  
GWAS analyses of cervical cancer. Trends Genet **31**(1), 41–54 (2015).  
doi:[10.1016/j.tig.2014.10.005](https://doi.org/10.1016/j.tig.2014.10.005)

62. Fellay, J., Shianna, K.V., Ge, D., Colombo, S., Ledergerber, B., Weale, M., Zhang, K.,  
Gumbs, C., Castagna, A., Cossarizza, A., Cozzi-Lepri, A., De Luca, A., Easterbrook, P.,  
Francioli, P., Mallal, S., Martinez-Picado, J., Miro, J.M., Obel, N., Smith, J.P., Wyniger, J.,  
Descombes, P., Antonarakis, S.E., Letvin, N.L., McMichael, A.J., Haynes, B.F., Telenti, A.,  
Goldstein, D.B.: A whole-genome association study of major determinants for host control of  
HIV-1. Science **317**(5840), 944–947 (2007). doi:[10.1126/science.1143767](https://doi.org/10.1126/science.1143767)

63. Huber, P.J.: The 1972 Wald Lecture Robust Statistics: A Review. Ann Math Stat **43**(4),  
1041–1067 (1972). doi:[10.1214/aoms/1177692459](https://doi.org/10.1214/aoms/1177692459)

64. Winer, R.L., Feng, Q., Hughes, J.P., O'Reilly, S., Kiviat, N.B., Koutsky, L.A.: Risk of  
female human papillomavirus acquisition associated with first male sex partner. J Infect Dis



1  
2  
3 **197**(2), 279–82 (2008). doi:[10.1086/524875](https://doi.org/10.1086/524875)  
4

5 65. Herrero, R.: Human Papillomavirus (HPV) Vaccines: Limited Cross-Protection against  
6 Additional HPV Types. *J Infect Dis* **199**(7), 919–922 (2009). doi:[10.1086/597308](https://doi.org/10.1086/597308)  
7

8 66. Depuydt, C.E., Verstraete, L., Berth, M., Beert, J., Bogers, J.-P., Salembier, G.,  
9 Vereecken, A.J., Bosmans, E.: Human papillomavirus positivity in women undergoing  
10 intrauterine insemination has a negative effect on pregnancy rates. *Gynecol Obstet Invest*  
11 **81**(1), 41–6 (2016). doi:[10.1159/000434749](https://doi.org/10.1159/000434749)  
12  
13  
14  
15  
16  
17

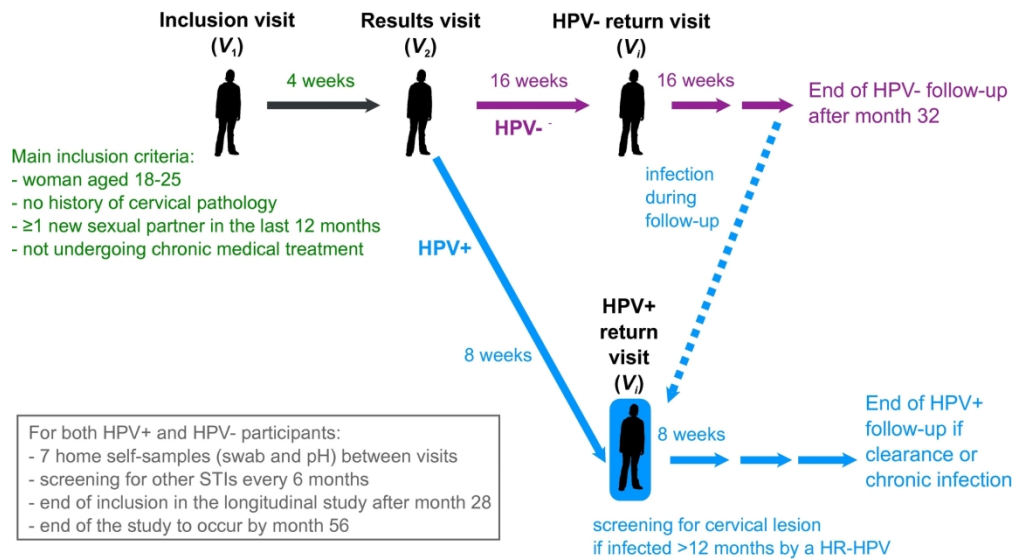
18 67. WHO HPV LabNet. World Health Organization.  
19

20 [http://www.who.int/biologicals/areas/human\\_papillomavirus/WHO\\_HP\\_V\\_LabNet/en/](http://www.who.int/biologicals/areas/human_papillomavirus/WHO_HP_V_LabNet/en/)  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## Figure captions

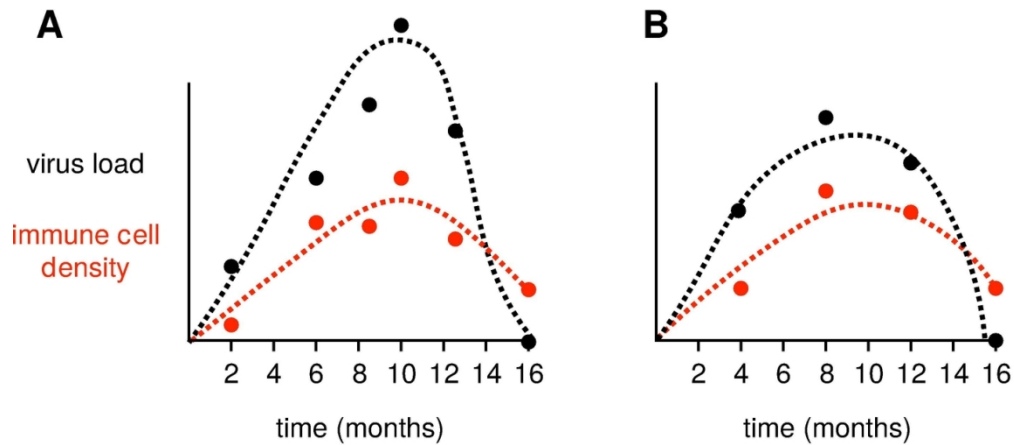
1  
2  
3 **Figure 1: General structure of the PAPCLEAR study.** For the longitudinal study, participants  
4 have an inclusion visit ( $V_1$ ), a results visit ( $V_2$ ) and then return visits ( $V_i$  with  $i > 2$ ). For the cross-  
5  
6 sectional study, participants only have  $V_1$  and  $V_2$ .  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21

22 **Figure 2: Fitting viral kinetics models to within-host times series.** Dashed lines indicate a  
23 model fitted using virus load (in black) or immune cells (in red) time series. In panel A, the  
24 follow-up is bi-monthly with 2 missing visits and several delayed visits, whereas in panel B the  
25 follow-up is every 4 months without any missing or delayed visits. In spite of missing data this,  
26 the situation shown in panel A is clearly the best for inferring parameter values and for fitting the  
27 underlying dynamics.  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



General structure of the PAPCLEAR study. For the longitudinal study, participants have an inclusion visit (V<sub>1</sub>), a results visit (V<sub>2</sub>) and then return visits (V<sub>i</sub> with i > 2). For the cross-sectional study, participants only have V<sub>1</sub> and V<sub>2</sub>.

190x104mm (300 x 300 DPI)



Fitting kinetics dynamical models to within-host times series. Dashed lines indicate a model fitted using virus load (in black) or immune cells (in red) time series. In panel A, the follow-up is bi-monthly with 2 missing visits and several delayed visits, whereas in panel B the follow-up is every 4 months without any missing or delayed visits. In spite of missing data this, the situation shown in panel A is clearly the best for inferring parameter values and for fitting the underlying dynamics.

120x52mm (300 x 300 DPI)

## Note from the Editors: Instructions for reviewers of study protocols

---

Since launching in 2011, BMJ Open has published study protocols for planned or ongoing research studies. If data collection is complete, we will not consider the manuscript.

Publishing study protocols enables researchers and funding bodies to stay up to date in their fields by providing exposure to research activity that may not otherwise be widely publicised. This can help prevent unnecessary duplication of work and will hopefully enable collaboration. Publishing protocols in full also makes available more information than is currently required by trial registries and increases transparency, making it easier for others (editors, reviewers and readers) to see and understand any deviations from the protocol that occur during the conduct of the study.

The scientific integrity and the credibility of the study data depend substantially on the study design and methodology, which is why the study protocol requires a thorough peer-review.

*BMJ Open* will consider for publication protocols for any study design, including observational studies and systematic reviews.

Some things to keep in mind when reviewing the study protocol:

- Protocol papers should report planned or ongoing studies. The dates of the study should be included in the manuscript.
- Unfortunately we are unable to customize the reviewer report form for study protocols. As such, some of the items (i.e., those pertaining to results) on the form should be scored as Not Applicable (N/A).
- While some baseline data can be presented, there should be no results or conclusions present in the study protocol.
- For studies that are ongoing, it is generally the case that very few changes can be made to the methodology. As such, requests for revisions are generally clarifications for the rationale or details relating to the methods. If there is a major flaw in the study that would prevent a sound interpretation of the data, we would expect the study protocol to be rejected.

## STUDY PROTOCOL

# The natural history, dynamics, and ecology of Human papillomaviruses (HPVs) in genital infections of young women: ~~the~~ study protocol of the PAPCLEAR cohort study

Carmen Lía Murall<sup>1</sup>, Massilva Rahmoun<sup>1</sup>, Christian Selinger<sup>1</sup>, Monique Baldellou<sup>2</sup>, Claire Bernat<sup>1</sup>, Marine Bonneau<sup>3</sup>, Vanina Boué<sup>1</sup>, Mathilde Buisson<sup>4</sup>, Guillaume Christophe<sup>2</sup>, Giuseppe D'Auria<sup>5,6</sup>, Florence De Taroni<sup>2</sup>, Vincent Foulongne<sup>7,8</sup>, Rémy Froissart<sup>1</sup>, Christelle Graf<sup>3</sup>, Sophie Grasset<sup>1,2</sup>, Soraya Groc<sup>1,7</sup>, Christophe Hirtz<sup>9</sup>, Audrey Jausse<sup>10</sup>, Julie Lajoie<sup>11</sup>, Frédérique Lorcy<sup>12</sup>, Eric Picot<sup>2</sup>, Marie-Christine Picot<sup>10</sup>, Jacques Ravel<sup>13</sup>, Jacques Reynes<sup>11</sup>, Thérèse Rousset<sup>12</sup>, Aziza Seddiki<sup>4</sup>, Martine Teirlinck<sup>2</sup>, Vincent Tribut<sup>2</sup>, Édouard Tuillon<sup>8</sup>, Tim Waterboer<sup>15</sup>, Nathalie Jacobs<sup>16</sup>, Ignacio G Bravo<sup>1</sup>, Michel Segondy<sup>7,8</sup>, Nathalie Boule<sup>8,12</sup> and Samuel Alizon<sup>1,\*</sup>

Word count: ~~5105~~265 words, excluding title page, abstract, references, figures and tables.

1 Laboratoire MIVEGEC (UMR 5290 CNRS, IRD, UM), 911, avenue Agropolis, BP 64501, 34394 Montpellier, France

2 Center for Free Information, Screening and Diagnosis (CeGIDD), Centre Hospitalier Universitaire de Montpellier, Montpellier, France

3 Department of Obstetrics and Gynaecology, Centre Hospitalier Universitaire de Montpellier, Montpellier, France.

4 Department of Research and Innovation (DRI), Centre Hospitalier Universitaire de Montpellier, Montpellier, France

5 Sequencing and Bioinformatics Service, Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunidad Valenciana (FISABIO-Salud Pública), 46020 Valencia, Spain

6 CIBER en Epidemiología y Salud Pública (CIBEResp), Madrid, Spain

7 Department of Virology, Centre Hospitalier Universitaire de Montpellier, Montpellier, France

8 Pathogenesis and Control of Chronic Infections, INSERM, CHU, University of Montpellier, Montpellier, France

9 University of Montpellier, LBPC/PPC- IRMB, CHU de Montpellier, 80 rue Augustin Fliche, Montpellier, France

10 Department of Medical Information (DIM), Centre Hospitalier Universitaire de Montpellier, Montpellier, France

11 Department of Medical microbiology, University of Manitoba, 745 Bannatyne, Winnipeg, Canada

12 Department of pathology and oncobiology, Centre Hospitalier Universitaire de Montpellier, Montpellier, France

13 Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, USA

14 Department of Infectious and Tropical Diseases, Centre Hospitalier Universitaire de Montpellier, Montpellier, France

15 German Cancer Research Center (DKFZ), Infections and Cancer Epidemiology, Im Neuenheimer Feld 280, Heidelberg, Germany

16 GIGA-Research, Cellular and molecular immunology, University of Liège, 3 Avenue de l'Hôpital, 4000 Liège, Belgium

\* Author for correspondence: [samuel.alizon@cnrs.fr](mailto:samuel.alizon@cnrs.fr)

For peer review only - <http://bmjopen.bmj.com/site/about/guidelines.xhtml>

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only

## Abstract

### Introduction

Human papillomaviruses (HPVs) are responsible for one third of all cancers caused by infections. Most HPV studies focus on chronic infections and cancers, and thus, we know little about the early stages of viral infection. In particular, the effects of the dynamic interactions between the immune system, the microbiota, and the viral and host genetics on infection clearance or persistence remains poorly understood.

### Methods and Analysis

We follow 150 women, aged 18-25 years, longitudinally to monitor immune response features (cytokines and immune cells in the genital tract, circulating anti-HPV antibodies), virus load of HPVs, and vaginal microbiota composition. This is complemented by the assessment of viral and human genetics and behavioural data. To increase the statistical power of the epidemiological arm of the study, an additional 150 women are screened cross-sectionally. ~~This study will provide one of the most detailed follow-up studies of acute HPV infections and their interactions with the host and the vaginal microbiota. It will also allow us to investigate related issues regarding HPV intra-host evolution and diversity, vaginal microbiota dynamics, and sexually transmitted infections.~~

### Ethics and Dissemination

This study has been approved by the Comité de Protection des Personnes Sud Méditerranée I (reference number 2016-A00712-49); by the Comité Consultatif sur le Traitement de l'Information en matière de Recherche dans le domaine de la Santé (reference number 16.504); by the Commission Nationale Informatique et Libertés (reference number MMS/ABD/AR1612278, decision number DR-2016-488) and by the Agence Nationale de Sécurité du Médicament et des Produits de Santé (reference 20160072000007). The results will be published in preprint servers, post-print servers, peer-reviewed journals and disseminated through conferences.

**Trial registration number:** NCT02946346

**Keywords:** HPV; acute infection; persistence; virus load; immunity; microbiota; viral kinetics



## Article summary

### Strengths and limitations of this study

- A ~~D~~dense follow-up with (visits every two months for infected women ~~with~~and additional self-sampling every week) ~~for N=150 women~~.
- ~~The C~~combination of virological (virus load), immunological (cytokine concentrations and immune cell percentages) and environmental (vaginal microbiota composition, pH) measurements s at each visit.
- A limitation is that the density of the follow-up limits the number of participants (N=150), which can affect the power of epidemiological analyses ~~at the epidemiological level~~.
- We complement the longitudinal study with a cross-sectional study of N=150 women to allow for increase ~~the statistical power~~ of epidemiological analyses.

## Introduction

### Epidemiology of HPV genital infections in young adults and public health implications

Infections by Human Papillomaviruses (HPVs) are likely the most common sexually transmitted infection (STI) globally. It is often estimated that, worldwide, more than 7580% of ~~thesexually-~~ active individuals will be infected ~~at some point in their life~~ by an HPV type [1]. In France, a study performed in 2013 in the Paris area estimated the prevalence of HPV genital infections ~~by HPVs at~~ to be 25% ~~of~~ in women below 25 years of age [2]. In the area of Montpellier (France), the prevalence of oncogenic HPVs (~~often also~~ referred to as 'high-risk', HR, HPVs) in pregnant women aged 16 to 42 years was close to 20% [3]. These numbers are consistent with worldwide estimates according to which HPVs are most prevalent in women under 25 years of age, with an estimated overall prevalence of 24% [4].

Fortunately, the vast majority of infections by HPVs are asymptomatic and benign. Even for HPV16, which is probably the most oncogenic ~~biologic agent to humans~~ human virus, only a minority of infections (less than 10%) become persistent [5], and then a minority of these (12%) progress to cancer if untreated [1, 6]. Indeed, it is estimated that approximately 70 to 100% of infections by HPVs are cleared within 12 to 24 months, with strong differences between virus types [5, 7–9]. Recent studies suggest that primo-infections could be shorter in young girls [10] but, in general, there are many unknowns about the biology of non-persisting infections [11].

Our lack of knowledge partly comes from the fact that in vaccine trials, from which most of the data on infection duration originate, participants are followed every six months for several years [5, 7, 9, 12]. This frequency is sufficient to estimate the time to clearance (or ~~to~~ persistence) but it is not precise enough to understand the within-host dynamics, often referred to as 'kinetics' [13], of infections that last on average 6 to 24 months. Arbitrarily, after 24 months ~~of infection~~, an infection is often considered ~~as being~~ to be persistent [14].

Some factors have been shown to correlate with persistence (e.g. immunosuppression,

1 smoking, and co-infection with other STIs [15]) but we do not know how these affect viral  
2 kinetics. Also, some changes in viral-immunity interactions appear to be related to persistence  
3 and disease progression [16–19] but, again, we do not know the underlying interactions  
4 between the viruses, the host target cells, and the immune response in acute infections [11].  
5  
6 Finally, it has been argued that the vaginal microbiota may differ between HPV-infected and  
7 HPV-uninfected women [20] and that specific microbiota composition may interact with HPV  
8 detection [21]. However, it is difficult to disentangle the cause and the consequence. For  
9 instance, does the microbiota composition change after the establishment of an HPV infection,  
10 or do certain microbiota compositions increase susceptibility to HPV infection?  
11  
12

13  
14  
15  
16  
17  
18  
19  
20  
21 A better understanding of the within-host infection dynamics and of the determinants of  
22 clearance and persistence of viral infection is particularly important in the context of vaccination  
23 [22–25]. Indeed, the long-term efficacy of the anti-HPVs vaccines at the population level will  
24 largely depend on the within-host viral dynamics because, ultimately, most selective pressures  
25 on viral populations occur via the immune response [26]. Furthermore, a better understanding of  
26 acute HPV infections can shed a new light on issues related to latency, fertility, or  
27 immunotherapies [11].  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37

## 38 **Prevention strategies and treatment**

### 39 **Treatment**

40  
41 Since most infections by HPVs are benign in young adults and clear within six to 24 months, the  
42 current standard of care is to avoid over-treatment, even in the presence of cervical lesions [27].  
43 Clinical interventions (colposcopies, biopsies, and surgery) are less often performed with young  
44 women (< 25 years) and only for high-grade (pre-cancerous) lesions (cervical intraepithelial  
45 neoplasia grade 2, CIN-2, or more). Low-grade lesions (CIN-1) are not systematically treated  
46 but rather monitored yearly to detect any progression to high-grade lesions.  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57

58 Genital warts caused by non-oncogenic HPVs (often referred to as 'low-risk', LR, HPVs) can be  
59 removed by surgery or treated with bi- and trichloroacetic acid, cryotherapy or other treatments  
60 [28].

## HPV vaccination

1  
2 There are currently three licensed vaccines: a bivalent vaccine (Cervarix<sup>®</sup>) targeting HPV16  
3  
4 and HPV18 (together accounting for 70% of cervical cancers [1]), a quadrivalent vaccine  
5  
6 (Gardasil<sup>®</sup>) that additionally targets HPV6 and HPV11 (non-oncogenic, but highly prevalent and  
7  
8 associated to benign proliferative lesions) and, since 2014, a nonavalent vaccine (Gardasil 9<sup>®</sup>)  
9  
10 that targets five more oncogenic types (HPV31, HPV33, HPV45, HPV52, and HPV58, which  
11  
12 altogether account for 20% of cervical cancers [24]). These vaccines succeed in eliciting a  
13  
14 protective immune response against new infections by the targeted viruses, and are used  
15  
16 throughout the world, albeit with wide variation in coverage (for reviews, see e.g. [29, 30]).  
17  
18  
19  
20

21  
22 Vaccination campaigns in France started in 2006 but with limited coverage: it reached 28.5% in  
23  
24 2008 [31] and has been decreasing ever since [32]. The vaccine is recommended for girls from  
25  
26 11 to 14 years of age, currently with a vaccination scheme of two doses with a six months  
27  
28 interval. A catch-up is organised for girls aged 15-19 years, with a three-doses vaccination  
29  
30 scheme. Vaccination is reimbursed by the French Social Security but is not mandatory. It is also  
31  
32 recommended for men who have sex with men (MSM) as well as for immuno-compromised  
33  
34 people [32]. Vaccination is now the primary prevention strategy against cervical cancers.  
35  
36  
37  
38

## Screening

39  
40 In France, the secondary prevention strategy against cervical cancer is routine individual  
41  
42 cytology-based screening for pre-cancerous and cancerous cervical lesions in women between  
43  
44 25 and 65 years old. Cytology can also be performed in younger women if they report risk  
45  
46 factors for cervical cancer (multiple partners, chronic STIs or HIV infection [32]). Detection of  
47  
48 oncogenic HPVs is proposed for triage in case of abnormal cytology (i.e. high-grade or low-  
49  
50 grade squamous intraepithelial lesion, HSIL and LSIL respectively, or Atypical Squamous Cells  
51  
52 of Undetermined Significance, ASCUS).  
53  
54  
55  
56  
57

## Primary objectives

58  
59 The first primary objective of this cohort study is to decipher the kinetics and ecology of  
60  
cervical HPV infections in healthy young women, i.e. follow the population dynamics of the

virus, the target epithelial cells, and the immune effectors.

The second primary objective is to characterise the diversity of genital HPVs in young women in the region of Montpellier in relationship with their lifestyle, vaccination status, vaginal microbiota, and human genetics.

## Secondary objectives

A secondary objective is to characterise the acquisition and clearance dynamics of cervical HPV infections as a function of viral diversity, host immunity, vaginal microbiota and human genetics.

A final objective is to investigate variations in genetic diversity of HPVs during cervical infections.

## Methods and analysis

### Participants

The study population is composed of young women at risk of HPV infection. The age class was chosen because ~~it exhibits the high HPV prevalence of HPV is the highest~~ (24% worldwide [4] and ~~is~~ approximately 25% in France [2]). Inclusion of younger women would have raised technical issues because of the requirement for parental consent.

Women are recruited through a social media page, and through posters and leaflets distributed at the Universities in Montpellier and at the Montpellier STI screening centre (*Centre Gratuit d'Information de Dépistage et de Diagnostic, CeGIDD*). The composition of the population visiting the CeGIDD has already been documented in an earlier study [33]. In total, the centre is visited by approximately 3,000 women per year, the majority of which are under 25 years of age (80%). Approximately 40% of the attendants report three or more partners over the last twelve months and approximately 50% report using adequate behaviour for prevention against HIV.

## Inclusion criteria

Participants are women from 18 to 25 years old living in the metropole metropolitan area of Montpellier. They must be sexually active with at least one new partner over the last 12 months.

This criteria is fixed to maximise the incidence of new HPV infections. ~~As in any clinical study,~~

Participants must be able to and willing to give written informed consent: they must sign an informed consent form, understand the requirements for the study, and be affiliated to a French social security scheme (which is a state requirement).

Women cannot be included in the study if they have a history of HPV-associated pathology (genital warts or cervical lesions), if they are pregnant or intending to become pregnant in the coming year, infected by HIV, undergoing (or planning to undergo) intense medical treatment (biotherapy, chemotherapy, immunosuppression), planning on moving outside the Montpellier metropolitan area within the next 18 months, in a dependency or employment with the sponsor or the investigator, if they participated in a clinical trial involving administration of drugs within the last four weeks or if they belong to a vulnerable group (e.g. children, adults with physical or mental disabilities).

## Design/setting

This study has a longitudinal component aimed at deciphering within-host dynamics and a cross-sectional component, aimed at understanding the diversity of HPV infections in young adults in the area of Montpellier, France. The general structure of the study is shown in Fig 1.

If a woman fits the main inclusion criteria, she can go through an inclusion visit (V1) with a physician (gynaecologist or midwife) at the CeGIDD. During this visit, ~~she~~ the study investigator presents the study and checks all inclusion criteria before asking the participant to read and sign the informed consent form. Participants then undergo a medical consultation during which a number of samples are collected (see below). They then fill out health and lifestyle questionnaires and are given cotton-flocked swabs for self-sampling at home the next visit,

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

along with instructions on how to fill in weekly questionnaires through an online form (these are performed throughout the study).

An appointment is scheduled four weeks later for the Results visit ( $V_2$ ), where the cervical cytology results are communicated. ~~We collect some~~ Additional samples are collected and ~~provide more~~ self-sample swabs for home collection are provided.

The next return visits ( $V_i$ , where  $i > 2$ ) are as follows:

- Participants with a positive DEIA HPV test (see below), i.e. infected by an *Alphapapillomavirus*, at  $V_1$  join the HPV positive (HPV+) arm of the study with return visits scheduled every 2 months.
- Participants with a negative DEIA HPV test at  $V_1$  join the HPV negative (HPV-) arm with return visits scheduled every 4 months.
- HPV- participants infected by an *Alphapapillomavirus* move to the HPV+ arm.

Intervals between visits are based on earlier results showing that HPV infections last from 9 to 18 months on average depending on the HPV type [5, 7–9] and that a total follow-up of 4 months yields results that are difficult to analyse [21]. The longer interval in the HPV- arm is based on the estimated incidence for HPV genital infections in young women, which is greater than 30% [34, 35].

Participants in the HPV- arm are followed until month 32 of the study.

Participants in the HPV+ arm are followed until they clear the infection or until they have been infected for 24 months (after which we consider that the infection is persistent). Clearance is defined as being negative at two visits in a row for the first HPV type detected in the follow-up.

In between these visits to the CeGIDD, participants are asked to perform regular (every week for HPV+ and every second week for HPV-) self-samples using vaginal swabs, ~~along with a~~ to measure ~~of~~ vaginal pH and to fill in a short questionnaire. Self-samples are stored in the participants' freezer and brought back at every visit.

The study will end with the last HPV+ participant having cleared the infection or been infected for 24 months.

## Patients and public involvement

Since all participants are healthy, they are referred to as participants rather than patients. As in any longitudinal study, ensuring participant commitment is challenging. To achieve this goal, we have set up a compensation of 40 EUR per visit and an additional 10 EUR in case of a complete follow-up. Furthermore, participants who have answered a sufficient number of questionnaires and brought back a sufficient number of ~~self-samples~~self-samples will get a 100 EUR bonus at the end. Overall, a participant performing 12 return visits would gain a total compensation of 650 EUR.

Participants did not play a role in the design of this study.

Results of the study will be disseminated to participants who have left the study and to the general public via an email newsletter in French.

## Visits

The summary of the visit schedule and of the samples collected at each visit is shown in Table 1.

### Inclusion visit (V1)

This visit takes place at the CeGIDD and is scheduled by the Clinical Research Technician (~~TEGCRT~~) via phone or email.

Women meet a study investigator, who explains the goals and requirements of the study. ~~The physician also and~~ checks that the inclusion criteria are met. If so, after a general discussion, the informed consent forms are signed.



The female physician/midwife performs a general exam and then a gynaecological exam during which the following samples are taken:

- vaginal pH cotton swab (EcoCare™),
- vaginal swab (Copan ESwab™) in 1mL Amies liquid for DNA extraction and microbiota analysis,
- vaginal swab (Copan ESwab™) in 1mL of RNA preservation medium,
- ophthalmic sponge (Weck-cel®) to collect cervical secretions for cytokines analysis,
- cervical smear in 20mL of Thinprep® (Preservcyt® liquid) for HPV and HSV assays, and cytology evaluation.

Following the gynaecological consultation, the participant meets with a nurse to measure body temperature, blood pressure and draw 20mL of blood (a 5mL tube for SNPs sequencing, a 10mL tube for immunophenotyping and a 5mL tube for HPV antibody titration). For the longitudinal study, the nurse provides the participant with 3 self-sampling kits, 3 pH strips, a freezer box to bring back to the next visit, as well as instructions on how to perform the home sampling and store the samples in her personal freezer until the next visit.

If the participant has not been tested for a STI in the last 3 months, the nurse draws an additional blood tube of 5mL to test for STIs (HIV, HCV, HBV) and collects vaginal self-samples for chlamydiae and gonorrhoea detection. Syphilis testing is prescribed to participants who meet the STI clinic's guidelines.

Finally, the participant meets with the **TECCRT** to fill in questionnaires #1 (inclusion visit) and #3 (home). The **TECCRT** answers any remaining questions, explains how to fill the home questionnaires (#3) and sets an appointment for the Results visit.

### **Results visit (V2)**

During this visit, the participants are given the result of cervical lesion screening using the liquid cytology (normal, ASCUS, LSIL or HSIL). Participants with a high-grade lesion (HSIL) exit the study and are referred to the gynaecology service of the CHU of Montpellier.

1 During this visit, the physician/midwife collects additional samples: 2 vaginal swabs for DNA and  
2 RNA analysis, and a cervical smear in 10mL of PBS (to confirm HPV status and perform flow  
3 cytometry analyses).  
4  
5

6  
7 The participant fills in questionnaires #2 (for return visits) and #3 (home). An appointment for  
8 the next visit is set and swabs for home self-sampling are given.  
9  
10  
11

### 12 **Return visits ( $V_i$ )**

13 These visits only occur in the longitudinal study.  
14  
15

16  
17  
18  
19 **HPV- arm.** Participants uninfected by HPV visit the clinic every 4 months until month 26. During  
20 these visits, the same samples as in the inclusion visit ( $V_1$ ) are collected by the  
21 physician/midwife except for the cervical smear, which is put in PBS instead of Thinprep.  
22  
23  
24

25  
26  
27 The nurse only draws blood if a screening test for STIs other than HPV is required. The  
28 participant then fills in questionnaires #2 and #3 and an appointment is set for the next visit in  
29 16 weeks.  
30  
31  
32

33  
34  
35 If an HPV infection is detected in the cervical smear collected during this visit, the participant  
36 moves to the HPV+ arm and the **TECCRT** contacts the participant to move her appointment  
37 forward.  
38  
39  
40

41  
42  
43 **HPV+ arm.** Participants infected by HPV visit the clinic every 2 months. They cannot switch arm  
44 and will remain in the HPV+ arm until clearance or the end of the study. During the visits, the  
45 same samples as in the inclusion visit ( $V_0$ ) are collected by the physician/midwife except for the  
46 cervical smear, which is put in PBS instead of Thinprep.  
47  
48  
49

50  
51  
52 The nurse then draws 5mL of blood for HPV antibody titration. If this is the first HPV+ visit  
53 following an HPV- visit, the nurse also draws 10mL of blood for immunophenotyping. Finally, if a  
54 test for additional STIs is needed, the nurse draws 5mL of blood and collects vaginal self-  
55 samples for STI detection.  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Importantly, if the participant has been infected by a HR-HPV for more than 12 months and cytology has not been performed within the last 12 months, the cervical smear is put in Thinprep<sup>®</sup> fixation medium, instead of PBS, for cytological analysis (cervical lesion screening).

Finally, the participant fills in questionnaires #2 and #3, receives self-samples for home collection and an appointment is set for the next visit in 8 weeks.

## Endpoints

The primary endpoint for the study is the inclusion and follow-up of HPV-infected women in order to describe the kinetics of HPV virus load, and the associated immune response.

Secondary endpoints are the characterisation of the interactions between the course of the infection (e.g. duration), the HPV type(s), the abundance and taxonomic diversity of bacteria, fungi and viruses in the vaginal microbiota, human genetics (SNPs) and basal immunological status.

## Technical procedures

### DNA extraction

DNA extraction from cervical smears will be performed using Nuclisens EasyMAG from Biomerieux or an equivalent protocol. For the microbiota analyses, special kits involving physical (via beads) and/or enzymatic breaking of the cellular barrier will be favoured following standard protocols to study the vaginal microbiome [36], e.g. the MagAttract<sup>®</sup> PowerMicrobiome<sup>®</sup> DNA/RNA kit from Qiagen.

### HPV detection, typing and quantification

The participants' infection status (HPV+ or HPV-) will be assessed using the DEIA test, which is based on a PCR of the short SPF10 amplicon [37] and detects all *Alphapapillomaviruses* with great sensitivity

1 If the DEIA test is positive, HPVs will be typed using the LiPA25 kit, which is based on the same  
2 SPF10-PCR, and has a lower detection threshold compared to other hybridisation-based typing  
3 methods [38].  
4  
5

6  
7 The reason for basing the detection on the DEIA rather than the LiPA25 is that some  
8 *Alphapapillomavirus* may be detected by DEIA but not genotyped by LiPA and also that the  
9 DEIA is more sensitive than the LiPA. If the DEIA is positive and the LiPA25 is negative, typing  
10 will be performed by sequencing the product of a PGMY09/11 PCR [39], which targets another  
11 region of the HPV genome than the SPF10 PCR.  
12  
13  
14  
15  
16  
17  
18  
19

20 The quantification of HPV DNA genome copy number in the samples will be performed using  
21 the protocol set up by Micalessi et al. [40].  
22  
23  
24  
25

### 26 **Cytokine titration**

27 Cytokines can be used as markers of immune activation or immunosuppression and can also  
28 inform us on which components of the immune system are involved. Cervical sponges are  
29 centrifuged after the addition of 175 $\mu$ L of PBS. Cervical secretions are analysed for a set of 5 to  
30 6 cytokines levels using the Meso Scale Discovery (MSD) Multiplex ELISA platform, which has  
31 a low detection threshold and a slowly saturating dose-response curve. Based on earlier results  
32 [41, 42], we will first investigate a large panel of 20 cytokines (IFN- $\alpha$ 2a, IFN- $\gamma$ , IL-1 $\alpha$ , IL-5, IL-6,  
33 IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-23, IL-25, IP-10, MCP-1, MIP-1 $\alpha$ , MIP-3 $\alpha$ , MIP-3 $\beta$ , TNF-  
34  $\alpha$ , TNF- $\beta$ ) to choose the most relevant ones for a longitudinal follow-up.  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47

### 48 **Flow cytometry**

49 Analysing immune cells via flow cytometry is extremely challenging on cells as fragile as the  
50 ones from cervical smears. However, several studies suggest that this is feasible [41–43]. Here,  
51 we follow the protocol described in [44].  
52  
53  
54  
55  
56  
57

58 Stainings ~~are is~~ performed using a Duraclone custom mix targeting CD45, CD3, CD4, CD8,  
59 CD16, CD56, CD69, CD161 and TCR $\gamma\delta$ . The last marker, Live&Dead tests for cellular viability.  
60

Samples are acquired using a Navios flow cytometer (Beckman Coulter, three-laser

configuration).

## Sequencing

Sequencing will be performed for microbiota profiling. It involves PCR amplification of the V3-V4 region of 16S RNA for bacteria [45] and ITS1 for fungi [46]. We anticipate that the bacteria should belong to the operational taxonomic units (OTU) described in the five community state types found in vaginal communities [47, 48]. The virome will also be explored using shotgun sequencing and rolling circle PCR amplification [49]. Human genetics are explored using chip sequencing for SNPs.

## Statistical analyses

### Times series analyses

The core results of the study will come from the longitudinal follow-up of infected women, which will generate time series, i.e. a set of values collected from the same individual over time (Figure 2). There will be several time series per individual (virus load, number of immune cells, cytokine and antibody levels). These time series will be used to fit mathematical viral kinetics models that describe the interaction between viruses, host target cells (here, in the case of HPV, keratinocytes) and the immune response. These models are commonly developed for viral infections [13, 50–52], including those caused by HPVs [53]. We anticipate our follow-up to yield adequate data for such a fit based on the estimated duration of HPV infections (9 to 18 months [5, 7–9]). Furthermore, the weekly self-samples allow us to increase the resolution if necessary.

We will use non-linear mixed effect models [54] to jointly analyse time series from all participants. More precisely, we will rely on *R* packages such as nlme [55] or lme4 [55]. Note that, in addition to estimating model parameters (e.g. life-expectancy of infected cells or virion production rate of infected cells), this approach also allows us to compare biological models using statistical tools based on model likelihood such as Akaike Information Criterion. For an example of such analysis in the case of HIV, see [51].

### **Microbiota dynamics**

1  
2 The composition of the vaginal microbiota has already been described and shown to exhibit  
3  
4 considerably less diversity than the gut microbiota [47]. The dynamics of this microbiota has  
5  
6 also been studied and shown to closely follow menstrual cycles [48].  
7  
8  
9

10 Using the time series of OTU abundances (measured via 16S RNA sequencing and qPCR) we  
11  
12 will infer interaction parameters by assuming an underlying Lotka-Volterra competition model  
13  
14 [56]. This work will include time series analysis techniques (e.g. auto-correlation or local  
15  
16 similarity analysis) and statistical inference methods in order to infer community structure and  
17  
18 interactions from the next-generation sequencing (NGS) datasets [57]. Finally, statistical  
19  
20 methods from ecology will also be used to study community diversity (e.g diversity indices) and  
21  
22 community assembly, such as cluster and ordination analyses [58].  
23  
24  
25  
26

### **Genome Wide Association Studies**

27  
28  
29 We will use human single nucleotide polymorphisms (SNPs) inferred by chip sequencing to look  
30  
31 for genetic determinants of key traits (e.g. microbiota composition or HPV infection duration).  
32  
33 This is classically done by performing a Genome Wide Association Study (GWAS), which is a  
34  
35 complex regression method designed for situations where there are many explanatory variables  
36  
37 (here millions of SNPs) for a single trait of interest. GWAS will be performed using classical  
38  
39 methods [59]. Earlier GWAS studies have been applied to HPV infections for instance to test for  
40  
41 determinants to the ability to seroconvert following infection [60] and cervical cancer (see [61]  
42  
43 for a review). Here, our expected sample ( $N = 300$  women) is limited but SNPs with large effects  
44  
45 have been detected by studies with comparable sizes [62].  
46  
47  
48  
49  
50

### **Additional analyses**

51  
52  
53 For all collected variables, descriptive statistics will be calculated according to the level of  
54  
55 measurement. For metric variables these statistics can be mean and standard deviation as well  
56  
57 as quantiles and more robust statistics [63]. In case of categorical variables group proportions  
58  
59 and contingency tables are prepared.  
60

Univariate inferential statistics follow a descriptive analysis. Generally, parametric testing

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

procedures are preferred to non-parametric tests, as the former have higher power. That is why, for metric variables, we will first check whether the data can be assumed to be normally distributed. For normally distributed variables, ANOVA statistics are done to detect differences between groups. In case of significance, post-hoc analysis (Tukey test) are planned to reveal pairwise differences. If the data are not normally distributed or ordinally scaled, non-parametric analyses will be used. These contain the Kruskal-Wallis test and the Wilcoxon test as a post-hoc test with an appropriate correction of the significance level. Since the cell counts are expected to be small, Fisher's exact test will be performed for contingency tables instead of the asymptotic  $\chi^2$  test for categorical variables.

### Sample size calculation

The study will enrol a total of  $N = 300$  women, with  $N = 150$  in a longitudinal study and  $N = 150$  in a cross-sectional study. The goal of the longitudinal study is to follow 75 women longitudinally, preferentially before they are infected (see above). For the following calculations, we assumed a high percentage of lost during follow-up (30%).

With 150 enrolments and considering that the prevalence of HPV infection in young women is  $\approx 60\%$  (based on our preliminary data) and 30% of lost to follow-up, we expect to detect (and successfully follow) 63 infections at inclusion [CI95: 51--75, assuming a binomial distribution to calculate the 95% confidence interval].

Among women who are uninfected at the first visit and considering the yearly incidence being close to 30% [64], we expect 12 [CI95: 6--20] to be infected during the first year of follow-up (still assuming 30% of lost to follow-up).

In the end, with 150 enrolments and assuming a high percentage of lost to follow-up (30%), we expect to successfully follow 75 [CI95: 56--95] women infected at different stages of HPV

infection: beginning, during and end.

This will be made possible by the probability of transmission of HPV, which is estimated to be  $\approx$  90% without condom use and still high with condom use ( $\approx$  40%) [34].

Finally, regarding potential interference with the HPV vaccines, we do not anticipate any significant problem for two reasons. First, as mentioned above, the vaccine coverage is low in France [32]. Second, and more importantly, the vaccines only target few HPV types, thus leaving open the possibility of infection by dozens of types. Furthermore, studying the kinetics of a non-vaccine HPV type in a vaccinated woman will be extremely informative, e.g. to detect any potential cross-reactivity [65].

To run cross-sectional analyses (especially on the microbiota and human genetics), we will enrol  $N = 150$  additional women who will only perform the inclusion and the results visits. This sample size was chosen to reach that of earlier GWAS studies [61, 62].

## **Trial governance**

### **Sponsor**

This study is sponsored by the Centre Hospitalier Universitaire (CHU) of Montpellier. The CHU is involved in the implementation of the trial, legal/ethical submissions (see below for details on Ethics approval) and implementing the clinical database (eCRF), which is hosted by Ennov-Clinical (ClinSight). The CHU is not involved in the analysis or interpretation of the data. The CHU of Montpellier performs regular quality control assessments. A clinical research assistant will visit the CeGIDD every 4 months to ensure that implementation is in accordance with the protocol. The CHU has taken out insurance from the Société hospitalière d'assurances mutuelles, 18, rue Edouard Rochet-6 9372 Lyon cedex 08 (contract number 138983) through the full research period, covering its own civil liability and that of any agent (clinical or research staff), in accordance with article L.1121-10 of the French Public Health Code.



### Scientific committee

1  
2 The scientific committee comprises the study investigators, clinicians, scientific experts and  
3  
4 representatives of the sponsor. The committee meets yearly and is responsible for following  
5  
6 research progress, monitoring compliance with good clinical practices and patient safety. It can  
7  
8 also decide relevant modification of the protocol. Requests from third parties to access data  
9  
10 collected during the study will be evaluated by the committee.  
11  
12

### Monitoring

13  
14  
15  
16 Monitoring is performed during the whole study at CeGIDD according to the sponsor specific  
17  
18 SOP. Routine monitoring visits are made by the monitors designated by the sponsor to check  
19  
20 compliance with the protocol, the completeness, accuracy and consistency of the data, and  
21  
22 adherence to GCP. The principal investigator ensures that eCRFs are completed in a timely  
23  
24 manner and must allow periodical access to eCRFs, patient records, drug logs, and all other  
25  
26 study-related documents and materials. The frequency of monitoring visits is determined by  
27  
28 factors such as study design and the site enrolment requirements but visits will normally occur  
29  
30 at least once every 4 months.  
31  
32  
33  
34  
35

### Trial registration

36  
37  
38 The trial has been registered to ClinicalTrials.gov on 27 Oct 2016 with ID number  
39  
40 NCT02946346.  
41  
42  
43  
44  
45  
46  
47

## **Ethics and Dissemination**

48  
49  
50  
51 The PAPCLEAR trial obtained favourable opinions from the Comité de Protection des  
52  
53 Personnes (CPP) Sud Méditerranée I on May 11, 2016 (CPP number 16 42, reference number  
54  
55 ID RCB 2016-A00712-49); from the Comité Consultatif sur le Traitement de l'Information en  
56  
57 matière de Recherche dans le domaine de la Santé (CCTIRS) on July 12, 2016 (reference  
58  
59 number 16.504); and from the Commission Nationale Informatique et Libertés (CNIL) on Dec  
60  
16, 2016 (reference number MMS/ABD/AR1612278, decision number DR-2016-488). This trial

was authorised by the Agence Nationale de Sécurité du Médicament et des Produits de Santé (ANSM) on July 20, 2016 (reference 20160072000007).

The protocol has been modified since its initial version and the latest modification was accepted by the CPP on Dec 12, 2018.

All participants in the study will sign an informed consent form prior to participation.

The results will be published on preprint servers (e.g. BioRxiv), peer-reviewed journals, post-print servers (e.g. HAL) and disseminated through conferences.

## Discussion

### Expected results

Acute infections by HPVs are important to study because vaccination is most effective when performed before the first infection. However, we currently know very little about the early stages of HPV infections. This clinical study will give us an unprecedented level of detail into the natural history of HPV infections in young women. Variations in virus load over time have been studied but in the context of cervical cancer in older women [66]. In addition, we will also describe the nature and the dynamics of the immune response (local immune cells and cytokines, circulating anti-HPV antibodies) and of the vaginal microbiota. Beyond these kinetics, we will also have access to data such as infection clearance or not in 24 months, presence of more than one HPV type or coinfection by other STIs.

These data will be analysed in the light of numerous cofactors. One of the most important will be human genetics, with the sequencing of millions of SNPs. Others will be related to the sexual behaviour (number of partners, contraception methods, sexual practices) and general lifestyle. We, therefore, expect broader insights regarding sexual health in young women.

## Practical and operational issues

1  
2 One of the main practical challenges resides in the analysis of cervical smears by flow  
3  
4 cytometry. Indeed, the tissues are known to be fragile, adhesive and auto-fluorescent. Even  
5  
6 though standard protocols now exist [44], they require processing fresh samples in less than 2  
7  
8 hours.  
9

10  
11  
12 Another potential issue has to do with contamination by HPV DNA between samples, which are  
13  
14 frequent in the HPV field due to the robustness of the virions and the sensitivity of the tests. To  
15  
16 certify our ability to control for these, we have entered the 2017 GLOBAL HPV DNA Proficiency  
17  
18 Panel from the WHO HPV LabNet [67].  
19  
20  
21

22  
23 Regarding the enrolment of the participants, we do not expect issues with enrolling 150 women  
24  
25 in 28 months for the longitudinal study and 150 for the cross-sectional study. This is due to the  
26  
27 number of visitors of the centre who fit the inclusion criteria (more than 3,000 per year) and  
28  
29 because of earlier high participation rates in the same population ([33] enrolled 1381  
30  
31 participants in 5 months for their study).  
32  
33  
34

35  
36 Concerning the follow-up, the high incidence rate of HPV can also lead to transient carriage,  
37  
38 i.e. women who are positive for a type only at a single visit. This has been observed for instance  
39  
40 in longitudinal studies with a tight follow-up interval [21]. To control for this, we will run the HPV  
41  
42 detection test on the cells from the cervical smear after washing with RPMI.  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**Table 1: Summary of the visit schedules and samples take.** The cross-sectional study only includes the first two columns (V1 and V2). The ∅ indicate samples taken at visits. + participants infected by a HR-HPV for 12 month will have one PBS smear replaced by a Thinprep® smear to perform a cytology and check for lesions. ◁ this sample is only taken at the first HPV+ visit of a formerly HPV- participant. \* STI detection will be performed at inclusion unless the participant has been tested within the last 3 months and during the study every 6 months if a new partner has been reported or upon request.

|                                                      | Inclusion<br>(V <sub>1</sub> ) | Results<br>(V <sub>2</sub> ) | Return<br>(V <sub>i</sub> , with $i > 2$ ) |            |
|------------------------------------------------------|--------------------------------|------------------------------|--------------------------------------------|------------|
| Participants                                         | all                            | all                          | HPV+                                       | HPV-       |
| Time                                                 | day 0                          | + 4 weeks                    | + 8 weeks                                  | + 16 weeks |
| Eligibility                                          | ☐                              |                              |                                            |            |
| Consent                                              | ☐                              |                              |                                            |            |
| Gynecological consult                                | ☐                              | ☐                            | ☐                                          | ☐          |
| Vaginal pH cotton swab                               | ☐                              | ☐                            | ☐                                          | ☐          |
| 2 vaginal swab samples (Copan<br>ESwab™)             | ☐                              | ☐                            | ☐                                          | ☐          |
| 1 ophthalmological sponge sample                     | ☐                              |                              | ☐                                          | ☐          |
| 1 cervical smear in Thinprep <sup>®</sup> (cytology) | ☐                              |                              | +                                          |            |
| 1 cervical smear in PBS                              |                                | ☐                            | +                                          | ☐          |
| Blood sampling (HPV antibodies)                      | ☐                              |                              | ☐                                          |            |
| Blood sampling (sequencing)                          | ☐                              |                              |                                            |            |
| Blood sampling (immunophenotyping)                   | ☐                              |                              | △                                          |            |
| Other STI detection                                  | *                              | *                            | *                                          | *          |
| Questionnaire #1 (inclusion)                         | ☐                              |                              |                                            |            |
| Questionnaire #2 (visit)                             |                                | ☐                            | ☐                                          | ☐          |
| Questionnaire #3 (home)                              | ☐                              | ☐                            | ☐                                          | ☐          |
| Returning self-sampling samples                      |                                | ☐                            | ☐                                          | ☐          |
| Serious Adverse Event collection                     |                                | ☐                            | ☐                                          | ☐          |

## Abbreviations

1  
2  
3 ANOVA: Analysis of variance,

4  
5 ASC-US: Atypical squamous cells of undetermined significance,

6  
7 CD: Cluster of differentiation,

8  
9 CI95: 95% Confidence interval,

10  
11 CeGIDD: Centre Gratuit d'Information de Dépistage et de Diagnostic,

12  
13  
14 CHU: Centre Hospitalier Universitaire,

15  
16  
17 CIN: Cervical intraepithelial Neoplasia,

18  
19 CRT: Clinical Research Technician,

20  
21 ELISA: enzyme-linked immunosorbent assay,

22  
23 GWAS: Genome Wide Association Study,

24  
25 HIV: Human Immunodeficiency Virus,

26  
27 HPV: Human Papillomavirus,

28  
29 HR: high-risk,

30  
31 ITS: Internal Transcribed Spacer,

32  
33 HSIL: High grade Squamous Intraepithelial Lesion,

34  
35 LR: low-risk,

36  
37 LSIL: Low grade Squamous Intraepithelial Lesion,

38  
39 NGS: Next Generation Sequencing,

40  
41 OTU: Operational Taxonomic Unit,

42  
43 PBMC: Peripheral Blood Mononuclear Cell,

44  
45 PBS: Phosphate Buffered Saline,

46  
47 RPMI: Roswell Park Memorial Institute medium,

48  
49 SNP: Single Nucleotide Polymorphism,

50  
51 TCR: T-cell receptor,

52  
53  
54  
55  
56  
57  
58 WHO: World Health Organisation.  
59  
60

## Trial status

The study began on Oct 1, 2016 and the first inclusion was on Nov 3, 2016. On Jun 23, 2018, 89 participants have been included in the longitudinal study. Inclusions in the longitudinal study will continue until March 2019 and the study is expected to last until Aug 2021.

## Conflicts of interests

The authors have read and understood BMJ policy on declaration of interests and declare that they have no competing interests.

## Funding

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement No 648963). The authors acknowledge further support from the Centre National de la Recherche Scientifique (CNRS), the Institute de Recherche pour le Développement (IRD) and the Centre Hospitalier Universitaire (CHU) of Montpellier.

## Data statement

All personal and identifying information collected from participants are kept in a secure place at the CeGIDD during the duration of the trial and will be destroyed at the end of the study. The final raw dataset will be accessible only to the sponsor (CHU) and the chief scientist's (SA) team. Anonymous data will be available to external parties upon approval of both the sponsor and the scientific committee. All publications will be made green or gold open access and the corresponding data will be provided as supplementary material or via a public repository (e.g. Zenodo), provided that there is no conflict with ethical guidelines.

## Author contributions

Samuel Alizon, Carmen Lia Murall and Massical Rahmoun were the major contributors in the conception of the protocol. Samuel Alizon wrote the initial version of the manuscript. Christian

Selinger, Monique Baldellou, Claire Bernat, Marine Bonneau, Vanina Boué, Mathilde Buisson, Guillaume Christophe, Giuseppe D'Auria, Florence De Taroni, Vincent Foulongne, Rémy Froissart, Christelle Graf, Sophie Grasset, Soraya Groc, Christophe Hirtz, Audrey Jaussent, [Julie Lajoie](#), Frédérique Lorcy, Eric Picot, Marie-Christine Picot, Jacques Ravel, Jacques Reynes, Thérèse Rousset, Aziza Seddiki, Martine Teirlinck, Vincent Tribout, Édouard Tuailon, Tim Waterboer, Nathalie Jacobs, Ignacio G Bravo, Michel Segondy and Natalie Boule were involved in the conception of the protocol, in the implementation of the study and read and approved the final manuscript.

### **Ethics approval and consent to participate**

~~The PAPCLEAR trial obtained favourable opinions from the Comité de Protection des Personnes (CPP) Sud Méditerranée I on May 11, 2016 (CPP number 16-42, reference number ID-RCB-2016-A00712-49); from the Comité Consultatif sur le Traitement de l'Information en matière de Recherche dans le domaine de la Santé (CCTIRS) on July 12, 2016 (reference number 16-504); and from the Commission Nationale Informatique et Libertés (CNIL) on Dec 16, 2016 (reference number MMS/ABD/AR1612278, decision number DR-2016-488). This trial was authorised by the Agence Nationale de Sécurité du Médicament et des Produits de Santé (ANSM) on July 20, 2016 (reference 20160072000007).~~

~~The protocol has been modified since its initial version and the latest modification was accepted by the CPP on Dec 12, 2018.~~

~~**All participants in the study will sign an informed consent form prior to participation.**~~

### **Acknowledgements**

We thank all the study participants and the CeGIDD staff for their commitment to the study. We also thank [the](#) reviewers and, in particular, Dr. Andrew Brouwer for his meticulous reading of the manuscript.



## References

1. Tota, J.E., Chevarie-Davis, M., Richardson, L.A., Devries, M., Franco, E.L.: Epidemiology and burden of HPV infection and related diseases: implications for prevention strategies. *Prev Med* **53 Suppl 1**, 12–21 (2011). doi:[10.1016/j.ypmed.2011.08.017](https://doi.org/10.1016/j.ypmed.2011.08.017)
2. Monsonego, J., Zerat, L., Syrjänen, K., Zerat, J.C., Smith, J.S., Halfon, P.: Prevalence of genotype-specific HPV infection among women in France: implications for screening and vaccination. *Gynecol Obstet Fertil* **41**(5), 305–313 (2013). doi:[10.1016/j.gyobfe.2013.03.003](https://doi.org/10.1016/j.gyobfe.2013.03.003)
3. Brun-Micaleff, E., Coffy, A., Rey, V., Didelot, M.-N., Combecal, J., Doutre, S., Daurès, J.-P., Segondy, M., Boulle, N.: Cervical cancer screening by cytology and human papillomavirus testing during pregnancy in french women with poor adhesion to regular cervical screening. *J Med Virol* **86**(3), 536–45 (2014). doi:[10.1002/jmv.23764](https://doi.org/10.1002/jmv.23764)
4. Bruni, L., Diaz, M., Castellsagué, X., Ferrer, E., Bosch, F.X., de Sanjosé, S.: Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. *J Infect Dis* **202**(12), 1789–99 (2010). doi:[10.1086/657321](https://doi.org/10.1086/657321)
5. Insinga, R.P., Dasbach, E.J., Elbasha, E.H., Liaw, K.-L., Barr, E.: Incidence and duration of cervical human papillomavirus 6, 11, 16, and 18 infections in young women: an evaluation from multiple analytic perspectives. *Cancer Epidemiol Biomarkers Prev* **16**(4), 709–15 (2007). doi:[10.1158/1055-9965.EPI-06-0846](https://doi.org/10.1158/1055-9965.EPI-06-0846)
6. Woodman, C.B.J., Collins, S.I., Young, L.S.: The natural history of cervical HPV infection: unresolved issues. *Nat Rev Cancer* **7**(1), 11–22 (2007). doi:[10.1038/nrc2050](https://doi.org/10.1038/nrc2050)
7. Rodríguez, A.C., Schiffman, M., Herrero, R., Wacholder, S., Hildesheim, A., Castle, P.E., Solomon, D., Burk, R., Proyecto Epidemiológico Guanacaste Group: Rapid clearance of human papillomavirus and implications for clinical focus on persistent infections. *J Natl Cancer Inst* **100**(7), 513–7 (2008). doi:[10.1093/jnci/djn044](https://doi.org/10.1093/jnci/djn044)

- 1  
2  
3  
4  
5  
6  
7  
8  
9
8. Trottier, H., Mahmud, S., Prado, J.C.M., Sobrinho, J.S., Costa, M.C., Rohan, T.E., Villa, L.L., Franco, E.L.: Type-Specific Duration of Human Papillomavirus Infection: Implications for Human Papillomavirus Screening and Vaccination. *J Infect Dis* **197**(10), 1436–1447 (2008). doi:[10.1086/587698](https://doi.org/10.1086/587698)
- 10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24
9. Ramanakumar, A.V., Naud, P., Roteli-Martins, C.M., de Carvalho, N.S., de Borja, P.C., Teixeira, J.C., Blatter, M., Moscicki, A.-B., Harper, D.M., Romanowski, B., Tying, S.K., Ramjattan, B., Schuind, A., Dubin, G., Franco, E.L.: Incidence and duration of type-specific human papillomavirus infection in high-risk HPV-naïve women: results from the control arm of a phase II HPV-16/18 vaccine trial. *BMJ Open* **6**(8), 011371 (2016). doi:[10.1136/bmjopen-2016-011371](https://doi.org/10.1136/bmjopen-2016-011371)
- 25  
26  
27  
28  
29  
30  
31  
32  
33  
34
10. Houlihan, C.F., Baisley, K., Bravo, I.G., Kapiga, S., de Sanjosé, S., Changalucha, J., Ross, D.A., Hayes, R.J., Watson-Jones, D.: Rapid acquisition of HPV around the time of sexual debut in adolescent girls in Tanzania. *Int J Epidemiol* **45**(3), 762–773 (2016). doi:[10.1093/ije/dyv367](https://doi.org/10.1093/ije/dyv367)
- 35  
36  
37  
38  
39  
40
11. Alizon, S., Murall, C.L., Bravo, I.G.: Why Human Papillomavirus Acute Infections Matter. *Viruses* **9**(10), 293 (2017). doi:[10.3390/v9100293](https://doi.org/10.3390/v9100293)
- 41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54
12. Herrero, R., Wacholder, S., Rodríguez, A.C., Solomon, D., González, P., Kreimer, A.R., Porras, C., Schussler, J., Jiménez, S., Sherman, M.E., Quint, W., Schiller, J.T., Lowy, D.R., Schiffman, M., Hildesheim, A., Costa Rica Vaccine Trial Group: Prevention of persistent human papillomavirus infection by an HPV16/18 vaccine: a community-based randomized clinical trial in Guanacaste, Costa Rica. *Cancer Discov* **1**(5), 408–19 (2011). doi:[10.1158/2159-8290.CD-11-0131](https://doi.org/10.1158/2159-8290.CD-11-0131)
- 55  
56  
57  
58  
59  
60
13. Canini, L., Perelson, A.S.: Viral kinetic modeling: state of the art. *J Pharmacokinet Pharmacodyn* **41**(5), 431–443 (2014). doi:[10.1007/s10928-014-9363-3](https://doi.org/10.1007/s10928-014-9363-3)
14. Stanley, M.: Immune responses to human papillomavirus. *Vaccine* **24**(S1), 16–22 (2006).

doi:[10.1016/j.vaccine.2005.09.002](https://doi.org/10.1016/j.vaccine.2005.09.002)

15. Ferenczy, A., Franco, E.: Persistent human papillomavirus infection and cervical neoplasia. *Lancet Oncol* **3**(1), 11–6 (2002)
16. zur Hausen, H.: Review: Papillomaviruses — to Vaccination and Beyond. *Biochemistry* **73**(5), 498–503 (2008). doi:[10.1134/S0006297908050027](https://doi.org/10.1134/S0006297908050027)
17. Einstein, M.H., Schiller, J.T., Viscidi, R.P., Strickler, H.D., Coursaget, P., Tan, T., Halsey, N., Jenkins, D.: Clinician's guide to human papillomavirus immunology: knowns and unknowns. *Lancet Infect Dis* **9**(6), 347–56 (2009). doi:[10.1016/S1473-3099\(09\)70108-2](https://doi.org/10.1016/S1473-3099(09)70108-2)
18. Van Hede, D., Langers, I., Delvenne, P., Jacobs, N.: Origin and immunoescape of uterine cervical cancer. *Presse Med* **43**(12P2), 413–421 (2014). doi:[10.1016/j.lpm.2014.09.005](https://doi.org/10.1016/j.lpm.2014.09.005)
19. Stanley, M.: Immunology of HPV infection. *Curr Obstet Gynecol Rep* **4**(4), 195–200 (2015). doi:[10.1007/s13669-015-0134-y](https://doi.org/10.1007/s13669-015-0134-y). Accessed 2017-03-20
20. Gao, W., Weng, J., Gao, Y., Chen, X.: Comparison of the vaginal microbiota diversity of women with and without human papillomavirus infection: a cross-sectional study. *BMC Infect Dis* **13**, 271 (2013). doi:[10.1186/1471-2334-13-271](https://doi.org/10.1186/1471-2334-13-271)
21. Brotman, R.M., Shardell, M.D., Gajer, P., Tracy, J.K., Zenilman, J.M., Ravel, J., Gravitt, P.E.: Interplay between the temporal dynamics of the vaginal microbiota and human papillomavirus detection. *J Infect Dis* **210**(11), 1723–33 (2014). doi:[10.1093/infdis/jiu330](https://doi.org/10.1093/infdis/jiu330)
22. Koutsky, L.A., Ault, K.A., Wheeler, C.M., Brown, D.R., Barr, E., Alvarez, F.B., Chiacchierini, L.M., Jansen, K.U., Proof of Principle Study Investigators: A controlled trial of a human papillomavirus type 16 vaccine. *N Engl J Med* **347**(21), 1645–51 (2002). doi:[10.1056/NEJMoa020586](https://doi.org/10.1056/NEJMoa020586)
23. Riethmuller, D., Jacquard, A.-C., Lacau St Guily, J., Aubin, F., Carcopino, X., Pradat, P., Dahlab, A., Pr  tet, J.-L.: Potential impact of a nonavalent hpv vaccine on the occurrence of hpv-

related diseases in france. BMC Public Health **15**, 453 (2015). doi:[10.1186/s12889-015-1779-1](https://doi.org/10.1186/s12889-015-1779-1)

- 1  
2  
3 24. Joura, E.A., Giuliano, A.R., Iversen, O.-E., Bouchard, C., Mao, C., Mehlsen, J., Moreira,  
4 E.D. Jr, Ngan, Y., Petersen, L.K., Lazcano-Ponce, E., Pitisuttithum, P., Restrepo, J.A., Stuart,  
5 G., Woelber, L., Yang, Y.C., Cuzick, J., Garland, S.M., Huh, W., Kjaer, S.K., Bautista, O.M.,  
6 Chan, I.S.F., Chen, J., Gesser, R., Moeller, E., Ritter, M., Vuocolo, S., Luxembourg, A., Broad  
7 Spectrum HPV Vaccine Study: A 9-valent HPV vaccine against infection and intraepithelial  
8 neoplasia in women. N Engl J Med **372**(8), 711–23 (2015). doi:[10.1056/NEJMoa1405044](https://doi.org/10.1056/NEJMoa1405044)  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18 25. Murall, C.L., Bauch, C.T., Day, T.: Could the human papillomavirus vaccines drive  
19 virulence evolution? Proc Biol Sci **282**, 20141069 (2015). doi:[10.1098/rspb.2014.1069](https://doi.org/10.1098/rspb.2014.1069)  
20  
21  
22  
23  
24 26. Alizon, S., Méthot, P.-O.: Reconciling Pasteur and Darwin to control infectious diseases.  
25 PLoS Biol **16**(1), 2003815 (2018). doi:[10.1371/journal.pbio.2003815](https://doi.org/10.1371/journal.pbio.2003815)  
26  
27  
28  
29  
30 27. Moscicki, A.-B., Ma, Y., Wibbelsman, C., Darragh, T.M., Powers, A., Farhat, S., Shiboski,  
31 S.: Rate of and Risks for Regression of CIN-2 in adolescents and young women. Obstet  
32 Gynecol **116**(6), 1373–1380 (2010). doi:[10.1097/AOG.0b013e3181fe777f](https://doi.org/10.1097/AOG.0b013e3181fe777f)  
33  
34  
35  
36  
37  
38 28. Buck Jr., H.W.: Warts (genital). BMJ Clin Evid **2015**, 1602 (2015)  
39  
40  
41  
42 29. Herrero, R., González, P., Markowitz, L.E.: Present status of human papillomavirus  
43 vaccine development and implementation. Lancet Oncol **16**(5), 206–16 (2015).  
44 doi:[10.1016/S1470-2045\(14\)70481-4](https://doi.org/10.1016/S1470-2045(14)70481-4)  
45  
46  
47  
48  
49 30. Maver, P.J., Poljak, M.: Progress in prophylactic human papillomavirus (HPV) vaccination  
50 in 2016: A literature review. Vaccine (2018). doi:[10.1016/j.vaccine.2017.07.113](https://doi.org/10.1016/j.vaccine.2017.07.113)  
51  
52  
53  
54  
55 31. Fagot, J.-P., Boutrelle, A., Ricordeau, P., Weill, A., Allemand, H.: HPV vaccination in  
56 France: uptake, costs and issues for the National Health Insurance. Vaccine **29**(19), 3610–6  
57 (2011). doi:[10.1016/j.vaccine.2011.02.064](https://doi.org/10.1016/j.vaccine.2011.02.064)  
58  
59  
60

- 1  
2  
3 32. Ben Hadj Yahia, M.-B., Dervaux, B., Duport, N., Floret, D., Gaillot, J., Heard, I., Jacquet,  
4 A., Le Goaster, C., Lévy-Bruhl, D., Morer, I., Parent du Chatelet, I., Peigue-Lafeuille, H.,  
5  
6 Rumeau-Pichon, C.: Vaccination contre les infections à papilloamvirus. Technical report, Haut  
7  
8 Conseil de la Santé Publique, Paris, France (2014).  
9  
10 <https://www.hcsp.fr/Explore.cgi/avisrapportsdomaine?clefr=454>  
11  
12  
13  
14  
15 33. Clarivet, B., Picot, E., Marchandin, H., Tribout, V., Rachedi, N., Schwartzentruber, E.,  
16  
17 Ledésert, B., Dereure, O., Guillot, B., Picot, M.-C.: Prevalence of Chlamydia trachomatis,  
18  
19 Neisseria gonorrhoeae and Mycoplasma genitalium in asymptomatic patients under 30 years of  
20  
21 age screened in a French sexually transmitted infections clinic. *Eur J Dermatol* **24**(5), 611–6  
22  
23 (2014). doi:[10.1684/ejd.2014.2413](https://doi.org/10.1684/ejd.2014.2413)  
24  
25  
26  
27 34. Winer, R.L., Hughes, J.P., Feng, Q., O'Reilly, S., Kiviat, N.B., Holmes, K.K., Koutsky, L.A.:  
28  
29 Condom use and the risk of genital human papillomavirus infection in young women. *N Engl J*  
30  
31 *Med* **354**(25), 2645–54 (2006). doi:[10.1056/NEJMoa053284](https://doi.org/10.1056/NEJMoa053284)  
32  
33  
34  
35 35. Winer, R.L., Hughes, J.P., Feng, Q., Stern, J.E., Xi, L.F., Koutsky, L.A.: Incident Detection  
36  
37 of High-Risk Human Papillomavirus Infections in a Cohort of High-Risk Women Aged 25-65  
38  
39 Years. *J Infect Dis* **214**(5), 665–75 (2016). doi:[10.1093/infdis/jiw074](https://doi.org/10.1093/infdis/jiw074)  
40  
41  
42  
43 36. Ravel, J., Brotman, R.M., Gajer, P., Ma, B., Nandy, M., Fadrosh, D.W., Sakamoto, J.,  
44  
45 Koenig, S.S., Fu, L., Zhou, X., Hickey, R.J., Schwebke, J.R., Forney, L.J.: Daily temporal  
46  
47 dynamics of vaginal microbiota before, during and after episodes of bacterial vaginosis.  
48  
49 *Microbiome* **1**(1), 29 (2013). doi:[10.1186/2049-2618-1-29](https://doi.org/10.1186/2049-2618-1-29)  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60 37. Kleter, B., van Doorn, L.-J., ter Schegget, J., Schrauwen, L., van Krimpen, K., Burger, M.,

ter Harmsel, B., Quint, W.: Novel Short-Fragment PCR Assay for Highly Sensitive Broad-Spectrum Detection of Anogenital Human Papillomaviruses. *Am J Pathol* **153**(6), 1731–1739 (1998). doi:[10.1016/S0002-9440\(10\)65688-X](https://doi.org/10.1016/S0002-9440(10)65688-X)

38. Geraets, D.T., Struijk, L., Kleter, B., Molijn, A., van Doorn, L.-J., Quint, W.G.V., Colau, B.: The original SPF10 LiPA25 algorithm is more sensitive and suitable for epidemiologic HPV research than the SPF10 INNO-LiPA Extra. *J Virol Meth* **215-216**, 22–29 (2015). doi:[10.1016/j.jviromet.2015.01.001](https://doi.org/10.1016/j.jviromet.2015.01.001)

39. Gravitt, P.E., Peyton, C.L., Alessi, T.Q., Wheeler, C.M., Coutlée, F., Hildesheim, A., Schiffman, M.H., Scott, D.R., Apple, R.J.: Improved amplification of genital Human Papillomaviruses. *J Clin Microbiol* **38**(1), 357–361 (2000)

40. Micalessi, I.M., Boulet, G.A.V., Bogers, J.J., Benoy, I.H., Depuydt, C.E.: High-throughput detection, genotyping and quantification of the human papillomavirus using real-time PCR. *Clin Chem Lab Med* **50**(4), 655–61 (2012). doi:[10.1515/cclm.2011.835](https://doi.org/10.1515/cclm.2011.835)

41. Hunter, P.J., Sheikh, S., David, A.L., Peebles, D.M., Klein, N.: Cervical leukocytes and spontaneous preterm birth. *Journal of Reproductive Immunology* **113**, 42–49 (2016). doi:[10.1016/j.jri.2015.11.002](https://doi.org/10.1016/j.jri.2015.11.002)

42. Shannon, B., Yi, T.J., Perusini, S., Gajer, P., Ma, B., Humphrys, M.S., Thomas-Pavanel, J., Chieza, L., Janakiram, P., Saunders, M., Tharao, W., Huibner, S., Shahabi, K., Ravel, J., Rebbapragada, A., Kaul, R.: Association of HPV infection and clearance with cervicovaginal immunology and the vaginal microbiota. *Mucosal Immunology* **10**(5), 1310–1319 (2017). doi:[10.1038/mi.2016.129](https://doi.org/10.1038/mi.2016.129)

43. Lajoie, J., Juno, J., Burgener, A., Rahman, S., Mogk, K., Wachihi, C., Mwanjewe, J., Plummer, F.A., Kimani, J., Ball, T.B., Fowke, K.R.: A distinct cytokine and chemokine profile at the genital mucosa is associated with HIV-1 protection among HIV-exposed seronegative commercial sex workers. *Mucosal Immunol* **5**(3), 277–287 (2012). doi:[10.1038/mi.2012.7](https://doi.org/10.1038/mi.2012.7)

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
44. Juno, J.A., Boily-Larouche, G., Lajoie, J., Fowke, K.R.: Collection, isolation, and flow cytometric analysis of human endocervical samples. *J Vis Exp* **89**, 51906 (2014).  
doi:[10.3791/51906](https://doi.org/10.3791/51906)
45. Frank, J.A., Reich, C.I., Sharma, S., Weisbaum, J.S., Wilson, B.A., Olsen, G.J.: Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Appl Environ Microbiol* **74**(8), 2461 (2008). doi:[10.1128/AEM.02272-07](https://doi.org/10.1128/AEM.02272-07)
46. Findley, K., Oh, J., Yang, J., Conlan, S., Deming, C., Meyer, J.A., Schoenfeld, D., Nomicos, E., Park, M., NIH Intramural Sequencing Center Comparative Sequencing Program, Kong, H.H., Segre, J.A.: Topographic diversity of fungal and bacterial communities in human skin. *Nature* **498**(7454), 367–370 (2013). doi:[10.1038/nature12171](https://doi.org/10.1038/nature12171). Accessed 2017-09-13
47. Ravel, J., Gajer, P., Abdo, Z., Schneider, G.M., Koenig, S.S.K., McCulle, S.L., Karlebach, S., Gorle, R., Russell, J., Tacket, C.O., Brotman, R.M., Davis, C.C., Ault, K., Peralta, L., Forney, L.J.: Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci USA* **108**, 4680–7 (2011). doi:[10.1073/pnas.1002611107](https://doi.org/10.1073/pnas.1002611107)
48. Gajer, P., Brotman, R.M., Bai, G., Sakamoto, J., Schütte, U.M.E., Zhong, X., Koenig, S.S.K., Fu, L., Ma, Z.S., Zhou, X., Abdo, Z., Forney, L.J., Ravel, J.: Temporal dynamics of the human vaginal microbiota. *Sci Transl Med* **4**(132), 132–52 (2012).  
doi:[10.1126/scitranslmed.3003605](https://doi.org/10.1126/scitranslmed.3003605)
49. Johne, R., Müller, H., Rector, A., van Ranst, M., Stevens, H.: Rolling-circle amplification of viral DNA genomes using phi29 polymerase. *Trends Microbiol* **17**(5), 205–211 (2009).  
doi:[10.1016/j.tim.2009.02.004](https://doi.org/10.1016/j.tim.2009.02.004)
50. Nowak, M.A., May, R.M.: *Virus Dynamics: Mathematical Principles of Immunology and Virology*. Oxford University Press, Oxford, USA (2000)
51. Stafford, M.A., Corey, L., Cao, Y., Daar, E.S., Ho, D.D., Perelson, A.S.: Modeling plasma virus concentration during primary HIV infection. *J. theor. Biol.* **203**(3), 285–301 (2000).

doi:[10.1006/jtbi.2000.1076](https://doi.org/10.1006/jtbi.2000.1076)

1  
2  
3 52. Perelson, A.S.: Modelling viral and immune system dynamics. *Nat. Rev. Immunol.* **2**(1),  
4 28–36 (2002). doi:[10.1038/nri700](https://doi.org/10.1038/nri700)

5  
6  
7  
8 53. Murall, C.L., Jackson, R., Zehbe, I., Boulle, N., Segondy, M., Alizon, S.: Epithelial  
9 stratification shapes infection dynamics. *PLoS Comput Biol* **15**(1), 1006646 (2019).

10  
11  
12  
13 doi:[10.1371/journal.pcbi.1006646](https://doi.org/10.1371/journal.pcbi.1006646)

14  
15  
16  
17 54. Steimer, J.L., Vozech, S., Racine Poon, A., Holford, N., O'Neil, R.: The population  
18 approach: rationale, methods and applications in clinical pharmacology and drug development.  
19 In: Balant, P.G.W..L. (ed.) *Handbook of Experimental Pharmacology*, vol. 110, pp. 405–451.

20  
21  
22  
23 Springer, Berlin (1994)

24  
25  
26  
27 55. Bates, D., Mächler, M., Bolker, B., Walker, S.: Fitting linear mixed-effects models using  
28 lme4. *Journal of Statistical Software* **67**(1) (2015). doi:[10.18637/jss.v067.i01](https://doi.org/10.18637/jss.v067.i01)

29  
30  
31  
32 56. Bucci, V., Tzen, B., Li, N., Simmons, M., Tanoue, T., Bogart, E., Deng, L., Yeliseyev, V.,  
33 Delaney, M.L., Liu, Q., Olle, B., Stein, R.R., Honda, K., Bry, L., Gerber, G.K.: MDSINE:  
34 Microbial dynamical systems INference engine for microbiome time-series analyses. *Genome*  
35 *Biology* **17**, 121 (2016). doi:[10.1186/s13059-016-0980-6](https://doi.org/10.1186/s13059-016-0980-6). Accessed 2017-03-09

36  
37  
38  
39 57. Faust, K., Lahti, L., Gonze, D., de Vos, W.M., Raes, J.: Metagenomics meets time series  
40 analysis: unraveling microbial community dynamics. *Curr Opin Microbiol* **25**, 56–66 (2015).

41  
42  
43  
44  
45  
46  
47  
48 doi:[10.1016/j.mib.2015.04.004](https://doi.org/10.1016/j.mib.2015.04.004)

49  
50  
51 58. Fox, G.A., Negrete-Yankelevich, S., Sosa, V.J.: *Ecological Statistics: Contemporary*  
52 *Theory and Application*. Oxford University Press, Oxford, USA (2015)

53  
54  
55  
56  
57 59. Shi, Y., Li, L., Hu, Z., Li, S., Wang, S., Liu, J., Wu, C., He, L., Zhou, J., Li, Z., Hu, T., Chen,  
58 Y., Jia, Y., Wang, S., Wu, L., Cheng, X., Yang, Z., Yang, R., Li, X., Huang, K., Zhang, Q., Zhou,  
59 H., Tang, F., Chen, Z., Shen, J., Jiang, J., Ding, H., Xing, H., Zhang, S., Qu, P., Song, X., Lin,



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Z., Deng, D., Xi, L., Lv, W., Han, X., Tao, G., Yan, L., Han, Z., Li, Z., Miao, X., Pan, S., Shen, Y., Wang, H., Liu, D., Gong, E., Li, Z., Zhou, L., Luan, X., Wang, C., Song, Q., Wu, S., Xu, H., Shen, J., Qiang, F., Ma, G., Liu, L., Chen, X., Liu, J., Wu, J., Shen, Y., Wen, Y., Chu, M., Yu, J., Hu, X., Fan, Y., He, H., Jiang, Y., Lei, Z., Liu, C., Chen, J., Zhang, Y., Yi, C., Chen, S., Li, W., Wang, D., Wang, Z., Di, W., Shen, K., Lin, D., Shen, H., Feng, Y., Xie, X., Ma, D.: A genome-wide association study identifies two new cervical cancer susceptibility loci at 4q12 and 17q12. *Nat Genet* **45**(8), 918–22 (2013). doi:[10.1038/ng.2687](https://doi.org/10.1038/ng.2687)

60. Chen, D., Gaborieau, V., Zhao, Y., Chabrier, A., Wang, H., Waterboer, T., Zaridze, D., Lissowska, J., Rudnai, P., Fabianova, E., Bencko, V., Janout, V., Foretova, L., Mates, I.N., Szeszenia-Dabrowska, N., Boffetta, P., Pawlita, M., Lathrop, M., Gyllensten, U., Brennan, P., McKay, J.D.: A systematic investigation of the contribution of genetic variation within the MHC region to HPV seropositivity. *Hum Mol Genet* **24**(9), 2681–2688 (2015).

doi:[10.1093/hmg/ddv015](https://doi.org/10.1093/hmg/ddv015)

61. Chen, D., Gyllensten, U.: Lessons and implications from association studies and post-GWAS analyses of cervical cancer. *Trends Genet* **31**(1), 41–54 (2015).

doi:[10.1016/j.tig.2014.10.005](https://doi.org/10.1016/j.tig.2014.10.005)

62. Fellay, J., Shianna, K.V., Ge, D., Colombo, S., Ledergerber, B., Weale, M., Zhang, K., Gumbs, C., Castagna, A., Cossarizza, A., Cozzi-Lepri, A., De Luca, A., Easterbrook, P., Francioli, P., Mallal, S., Martinez-Picado, J., Miro, J.M., Obel, N., Smith, J.P., Wyniger, J., Descombes, P., Antonarakis, S.E., Letvin, N.L., McMichael, A.J., Haynes, B.F., Telenti, A., Goldstein, D.B.: A whole-genome association study of major determinants for host control of HIV-1. *Science* **317**(5840), 944–947 (2007). doi:[10.1126/science.1143767](https://doi.org/10.1126/science.1143767)

63. Huber, P.J.: The 1972 Wald Lecture Robust Statistics: A Review. *Ann Math Stat* **43**(4), 1041–1067 (1972). doi:[10.1214/aoms/1177692459](https://doi.org/10.1214/aoms/1177692459)

64. Winer, R.L., Feng, Q., Hughes, J.P., O'Reilly, S., Kiviat, N.B., Koutsky, L.A.: Risk of female human papillomavirus acquisition associated with first male sex partner. *J Infect Dis*

**197**(2), 279–82 (2008). doi:[10.1086/524875](https://doi.org/10.1086/524875)

1  
2  
3 65. Herrero, R.: Human Papillomavirus (HPV) Vaccines: Limited Cross-Protection against  
4 Additional HPV Types. *J Infect Dis* **199**(7), 919–922 (2009). doi:[10.1086/597308](https://doi.org/10.1086/597308)  
5  
6  
7

8 66. Depuydt, C.E., Verstraete, L., Berth, M., Beert, J., Bogers, J.-P., Salembier, G.,  
9 Vereecken, A.J., Bosmans, E.: Human papillomavirus positivity in women undergoing  
10 intrauterine insemination has a negative effect on pregnancy rates. *Gynecol Obstet Invest*  
11  
12  
13  
14  
15  
16 **81**(1), 41–6 (2016). doi:[10.1159/000434749](https://doi.org/10.1159/000434749)  
17  
18

19 67. WHO HPV LabNet. World Health Organization.

20  
21 [http://www.who.int/biologicals/areas/human\\_papillomavirus/WHO\\_HP\\_V\\_LabNet/en/](http://www.who.int/biologicals/areas/human_papillomavirus/WHO_HP_V_LabNet/en/)  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## Figure captions

1  
2  
3 **Figure 1: General structure of the PAPCLEAR study.** For the longitudinal study, participants  
4 have an inclusion visit ( $V_1$ ), a results visit ( $V_2$ ) and then return visits ( $V_i$  with  $i > 2$ ). For the cross-  
5  
6 sectional study, participants only have  $V_1$  and  $V_2$ .  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21

22 **Figure 2: Fitting viral kinetics models to within-host times series.** Dashed lines indicate a  
23 model fitted using virus load (in black) or immune cells (in red) time series. In panel A, the  
24 follow-up is bi-monthly with 2 missing visits and several delayed visits, whereas in panel B the  
25 follow-up is every 4 months without any missing or delayed visits. In spite of missing data this,  
26  
27 the situation shown in panel A is clearly the best for inferring parameter values and for fitting the  
28  
29 underlying dynamics.  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

# BMJ Open

## The natural history, dynamics, and ecology of Human papillomaviruses in genital infections of young women: protocol of the PAPCLEAR cohort study

|                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|-------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Journal:                      | <i>BMJ Open</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Manuscript ID                 | bmjopen-2018-025129.R3                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Article Type:                 | Protocol                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Date Submitted by the Author: | 16-Apr-2019                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| Complete List of Authors:     | <p>Murall, Carmen Lia; Centre National de la Recherche Scientifique, MIVEGEC</p> <p>Rahmoun, Massilva; Centre National de la Recherche Scientifique, MIVEGEC</p> <p>Selinger, Christian; Centre National de la Recherche Scientifique, MIVEGEC</p> <p>Baldellou, Monique; Centre Hospitalier Regional Universitaire de Montpellier, CeGIDD</p> <p>Bernat, Claire; Centre National de la Recherche Scientifique, MIVEGEC</p> <p>Bonneau, Marine; Centre Hospitalier Regional Universitaire de Montpellier, Department of Obstetrics and Gynaecology</p> <p>Boué, Vanina; Centre National de la Recherche Scientifique, MIVEGEC</p> <p>Buisson, Mathilde; Centre Hospitalier Regional Universitaire de Montpellier, Department of Research and Innovation</p> <p>Christophe, Guillaume; Centre Hospitalier Regional Universitaire de Montpellier, CeGIDD</p> <p>D'Auria, Giuseppe; Fundacio per al Foment de la Investigacio Sanitaria i Biomedica, Sequencing and Bioinformatics Service; Centro de Investigacion Biomedica en Red de Epidemiologia y Salud Publica De Taroni, Florence; Centre Hospitalier Regional Universitaire de Montpellier, CeGIDD</p> <p>Foulongne, Vincent; Centre Hospitalier Regional Universitaire de Montpellier, Department of Bacteriology and Virology</p> <p>Froissart, Rémy; Centre National de la Recherche Scientifique, MIVEGEC</p> <p>Graf, Christelle; Centre Hospitalier Regional Universitaire de Montpellier, Department of Obstetrics and Gynaecology</p> <p>Grasset, Sophie; Centre Hospitalier Regional Universitaire de Montpellier, Department of Virology; Centre National de la Recherche Scientifique, MIVEGEC</p> <p>Groc, Soraya; Centre Hospitalier Regional Universitaire de Montpellier, Department of Virology; Centre National de la Recherche Scientifique, MIVEGEC</p> <p>Hirtz, Christophe; Centre Hospitalier Regional Universitaire de Montpellier, LBPC/PPC, IRMB</p> <p>Jaussent, Audrey; Centre Hospitalier Regional Universitaire de Montpellier, Department of Medical Information</p> <p>Lajoie, Julie; University of Manitoba College of Medicine, Department of Medical microbiology</p> <p>Lorcy, Frédérique; Centre Hospitalier Regional Universitaire de</p> |

|                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|---------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                 | <p>Montpellier, Laboratoire d'anatomie et cytologie pathologiques<br/> Picot, Eric; Centre Hospitalier Regional Universitaire de Montpellier, CeGIDD<br/> PICOT, Marie-Christine; Centre Hospitalier Regional Universitaire de Montpellier, Department of Medical Information<br/> Ravel, Jacques; University of Maryland School of Medicine, Institute for Genome Sciences<br/> Reynes, Jacques; Centre Hospitalier Regional Universitaire de Montpellier, Department of Infectious and Tropical Diseases<br/> Rousset, Thérèse; Centre Hospitalier Regional Universitaire de Montpellier, Department of pathology and oncobiology<br/> Seddiki, Aziza; Centre Hospitalier Regional Universitaire de Montpellier, Department of Research and Innovation<br/> Teirlinck, Martine; Centre Hospitalier Regional Universitaire de Montpellier, CeGIDD<br/> Tribout, Vincent; Centre Hospitalier Regional Universitaire de Montpellier, CeGIDD<br/> Tuaille, Edouard; Centre Hospitalier Regional Universitaire de Montpellier, Department of bacteriology and virology<br/> Waterboer, Tim; Deutsches Krebsforschungszentrum, Infections and Cancer Epidemiology<br/> Jacobs, Nathalie; Universite de Liege Faculte des Sciences, GIGA-Research, Cellular and molecular immunology<br/> Bravo, Ignacio; Centre National de la Recherche Scientifique, MIVEGEC<br/> Segondy, Michel; Centre Hospitalier Regional Universitaire de Montpellier, Department of Bacteriology and Virology<br/> Boulle, Nathalie; Centre Hospitalier Regional Universitaire de Montpellier, Department of pathology and oncobiology<br/> Alizon, Samuel; Centre National de la Recherche Scientifique, MIVEGEC</p> |
| <b>Primary Subject Heading</b>: | Infectious diseases                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| Secondary Subject Heading:      | Epidemiology, Immunology (including allergy), Genetics and genomics                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| Keywords:                       | VIROLOGY, IMMUNOLOGY, Epidemiology < INFECTIOUS DISEASES, MICROBIOLOGY, GENETICS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |

SCHOLARONE™  
Manuscripts

## STUDY PROTOCOL

# The natural history, dynamics, and ecology of Human papillomaviruses in genital infections of young women: protocol of the PAPCLEAR cohort study

Carmen Lía Murall<sup>1</sup>, Massilva Rahmoun<sup>1</sup>, Christian Selinger<sup>1</sup>, Monique Baldellou<sup>2</sup>, Claire Bernat<sup>1</sup>, Marine Bonneau<sup>3</sup>, Vanina Boué<sup>1</sup>, Mathilde Buisson<sup>4</sup>, Guillaume Christophe<sup>2</sup>, Giuseppe D'Auria<sup>5,6</sup>, Florence De Taroni<sup>2</sup>, Vincent Foulongne<sup>7,8</sup>, Rémy Froissart<sup>1</sup>, Christelle Graf<sup>3</sup>, Sophie Grasset<sup>1,2</sup>, Soraya Groc<sup>1,7</sup>, Christophe Hirtz<sup>9</sup>, Audrey Jausse<sup>10</sup>, Julie Lajoie<sup>11</sup>, Frédérique Lorcy<sup>12</sup>, Eric Picot<sup>2</sup>, Marie-Christine Picot<sup>10</sup>, Jacques Ravel<sup>13</sup>, Jacques Reynes<sup>11</sup>, Thérèse Rousset<sup>12</sup>, Aziza Seddiki<sup>4</sup>, Martine Teirlinck<sup>2</sup>, Vincent Tribut<sup>2</sup>, Édouard Tuillon<sup>8</sup>, Tim Waterboer<sup>15</sup>, Nathalie Jacobs<sup>16</sup>, Ignacio G Bravo<sup>1</sup>, Michel Segondy<sup>7,8</sup>, Nathalie Boule<sup>8,12</sup> and Samuel Alizon<sup>1,\*</sup>

Word count: 5265 words, excluding title page, abstract, references, figures and tables.

1 Laboratoire MIVEGEC (UMR 5290 CNRS, IRD, UM), 911, avenue Agropolis, BP 64501, 34394 Montpellier, France

2 Center for Free Information, Screening and Diagnosis (CeGIDD), Centre Hospitalier Universitaire de Montpellier, Montpellier, France

3 Department of Obstetrics and Gynaecology, Centre Hospitalier Universitaire de Montpellier, Montpellier, France.

4 Department of Research and Innovation (DRI), Centre Hospitalier Universitaire de Montpellier, Montpellier, France

5 Sequencing and Bioinformatics Service, Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunidad Valenciana (FISABIO-Salud Pública), 46020 Valencia, Spain

6 CIBER en Epidemiología y Salud Pública (CIBEResp), Madrid, Spain

7 Department of Virology, Centre Hospitalier Universitaire de Montpellier, Montpellier, France

8 Pathogenesis and Control of Chronic Infections, INSERM, CHU, University of Montpellier, Montpellier, France

9 University of Montpellier, LBPC/PPC- IRMB, CHU de Montpellier, 80 rue Augustin Fliche, Montpellier, France

10 Department of Medical Information (DIM), Centre Hospitalier Universitaire de Montpellier, Montpellier, France

11 Department of Medical microbiology, University of Manitoba, 745 Bannatyne, Winnipeg, Canada

12 Department of pathology and oncobiology, Centre Hospitalier Universitaire de Montpellier, Montpellier, France

13 Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, USA

14 Department of Infectious and Tropical Diseases, Centre Hospitalier Universitaire de Montpellier, Montpellier, France

15 German Cancer Research Center (DKFZ), Infections and Cancer Epidemiology, Im Neuenheimer Feld 280, Heidelberg, Germany

16 GIGA-Research, Cellular and molecular immunology, University of Liège, 3 Avenue de l'Hôpital, 4000 Liège, Belgium

\* Author for correspondence: [samuel.alizon@cnrs.fr](mailto:samuel.alizon@cnrs.fr)

For peer review only - <http://bmjopen.bmj.com/site/about/guidelines.xhtml>

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only

## Abstract

### Introduction

Human papillomaviruses (HPVs) are responsible for one third of all cancers caused by infections. Most HPV studies focus on chronic infections and cancers, and we know little about the early stages of the infection. Our main objective is to better understand the course and natural history of cervical HPV infections in healthy, unvaccinated and vaccinated, young women, by characterising the dynamics of various infection-related populations (virus, epithelial cells, vaginal microbiota, and immune effectors). Another objective is to analyse HPV diversity within hosts and in the study population, in relation to co-factors (lifestyle characteristics, vaccination status, vaginal microbiota, human genetics).

### Methods and Analysis

The PAPCLEAR study is a mono-centric longitudinal study following 150 women, aged 18-25 years, for up to 2 years. Visits occur every 2 or 4 months (depending on HPV status) during which several variables are measured, such as behaviours (via questionnaires), vaginal pH, HPV presence and viral load (via qPCR), local concentrations of cytokines (via MesoScale Discovery technology) and immune cells (via flow cytometry). Additional analyses are outsourced, such as titration of circulating anti-HPV antibodies, vaginal microbiota sequencing (16S and ITS-1 loci) and human genotyping. To increase the statistical power of the epidemiological arm of the study, an additional 150 women are screened cross-sectionally. Finally, to maximise the resolution of the time series, participants are asked to perform weekly self-samples at home. Statistical analyses will involve classical tools in epidemiology, genomics, and virus kinetics, and will be performed or coordinated by the CNRS in Montpellier.

### Ethics and Dissemination

This study has been approved by the CPP Sud Méditerranée I (reference number 2016-A00712-49); by the CCTIRS (reference number 16.504); by the CNIL (reference number MMS/ABD/AR1612278, decision number DR-2016-488) and by the ANSM (reference 20160072000007). Results will be published in preprint servers, peer-reviewed journals and disseminated through conferences.

**Trial registration number:** NCT02946346



**Keywords:** HPV; acute infection; persistence; virus load; immunity; microbiota; viral kinetics

## Article summary

### Strengths and limitations of this study

- Short time interval between the visits (every two months for infected women) and additional self-sampling every week at home.
- The combination of virological (virus load), immunological (cytokine concentrations and immune cell percentages) and environmental (vaginal microbiota composition, pH) measurements at each visit.
- A limitation is that the density of the follow-up limits the number of participants (N=150), which can affect the power of epidemiological analyses.
- We complement the longitudinal study with a cross-sectional study of N=150 women to increase statistical power.

## Introduction

### Epidemiology of HPV genital infections in young adults and public health implications

Infections by Human Papillomaviruses (HPVs) are likely the most common sexually transmitted infection (STI) globally. It is often estimated that, worldwide, more than 80% of sexually-active individuals will be infected by an HPV type [1]. In France, a study performed in 2013 in the Paris area estimated the prevalence of HPV genital infections to be 25% in women below 25 years of age [2]. In the area of Montpellier (France), the prevalence of oncogenic HPVs (also referred to as 'high-risk', HR, HPVs) in pregnant women aged 16 to 42 years was close to 20% [3]. These numbers are consistent with worldwide estimates according to which HPVs are most prevalent in women under 25 years of age, with an estimated overall prevalence of 24% [4].

Fortunately, the vast majority of infections by HPVs are asymptomatic and benign. Even for HPV16, which is probably the most oncogenic human virus, only a minority of infections (less than 10%) become persistent [5], and then a minority of these (12%) progress to cancer if untreated [1, 6]. Indeed, it is estimated that approximately 70 to 100% of infections by HPVs are cleared within 12 to 24 months, with strong differences between virus types [5, 7–9]. Recent studies suggest that primo-infections could be shorter in young girls [10] but, in general, there are many unknowns about the biology of non-persisting infections [11].

Our lack of knowledge partly comes from the fact that in vaccine trials, from which most of the data on infection duration originate, participants are followed every six months for several years [5, 7, 9, 12]. This frequency is sufficient to estimate the time to clearance (or persistence) but it is not precise enough to understand the within-host dynamics, often referred to as 'kinetics' [13], of infections that last on average 6 to 24 months. Arbitrarily, after 24 months, an infection is often considered to be persistent [14].

Some factors have been shown to correlate with persistence (e.g. immunosuppression, smoking, and co-infection with other STIs [15]) but we do not know how these affect viral kinetics. Also, some changes in viral-immunity interactions appear to be related to persistence

and disease progression [16–19] but, again, we do not know the underlying interactions between the viruses, the host target cells, and the immune response in acute infections [11]. Finally, it has been argued that the vaginal microbiota may differ between HPV-infected and HPV-uninfected women [20] and that specific microbiota composition may interact with HPV detection [21]. However, it is difficult to disentangle the cause and the consequence. For instance, does the microbiota composition change after the establishment of an HPV infection, or do certain microbiota compositions increase susceptibility to HPV infection?

A better understanding of the within-host infection dynamics and of the determinants of clearance and persistence of viral infection is particularly important in the context of vaccination [22–25]. Indeed, the long-term efficacy of the anti-HPVs vaccines at the population level will largely depend on the within-host viral dynamics because, ultimately, most selective pressures on viral populations occur via the immune response [26]. Furthermore, a better understanding of acute HPV infections can shed new light on issues related to latency, fertility, or immunotherapies [11].

## **Prevention strategies and treatment**

### **Treatment**

Since most infections by HPVs are benign in young adults and clear within six to 24 months, the current standard of care is to avoid over-treatment, even in the presence of cervical lesions [27]. Clinical interventions (colposcopies, biopsies, and surgery) are less often performed with young women (< 25 years) and only for high-grade (pre-cancerous) lesions (cervical intraepithelial neoplasia grade 2, CIN-2, or more). Low-grade lesions (CIN-1) are not systematically treated but rather monitored yearly to detect any progression to high-grade lesions.

Genital warts caused by non-oncogenic HPVs (often referred to as ‘low-risk’, LR, HPVs) can be removed by surgery or treated with bi- and trichloroacetic acid, cryotherapy or other treatments [28].

## HPV vaccination

1  
2 There are currently three licensed vaccines: a bivalent vaccine (Cervarix<sup>®</sup>) targeting HPV16  
3  
4 and HPV18 (together accounting for 70% of cervical cancers [1]), a quadrivalent vaccine  
5  
6 (Gardasil<sup>®</sup>) that additionally targets HPV6 and HPV11 (non-oncogenic, but highly prevalent and  
7  
8 associated to benign proliferative lesions) and, since 2014, a nonavalent vaccine (Gardasil 9<sup>®</sup>)  
9  
10 that targets five more oncogenic types (HPV31, HPV33, HPV45, HPV52, and HPV58, which  
11  
12 altogether account for 20% of cervical cancers [24]). These vaccines succeed in eliciting a  
13  
14 protective immune response against new infections by the targeted viruses, and are used  
15  
16 throughout the world, albeit with wide variation in coverage (for reviews, see e.g. [29, 30]).  
17  
18  
19  
20

21  
22 Vaccination campaigns in France started in 2006 but with limited coverage: it reached 28.5% in  
23  
24 2008 [31] and has been decreasing ever since [32]. The vaccine is recommended for girls from  
25  
26 11 to 14 years of age, currently with a vaccination scheme of two doses with a six months  
27  
28 interval. A catch-up is organised for girls aged 15-19 years, with a three-doses vaccination  
29  
30 scheme. Vaccination is reimbursed by the French Social Security but is not mandatory. It is also  
31  
32 recommended for men who have sex with men (MSM) as well as for immuno-compromised  
33  
34 people [32]. Vaccination is now the primary prevention strategy against cervical cancers.  
35  
36  
37  
38

## Screening

39  
40 In France, the secondary prevention strategy against cervical cancer is routine individual  
41  
42 cytology-based screening for pre-cancerous and cancerous cervical lesions in women between  
43  
44 25 and 65 years old. Cytology can also be performed in younger women if they report risk  
45  
46 factors for cervical cancer (multiple partners, chronic STIs or HIV infection [32]). Detection of  
47  
48 oncogenic HPVs is proposed for triage in case of abnormal cytology (i.e. high-grade or low-  
49  
50 grade squamous intraepithelial lesion, HSIL and LSIL respectively, or Atypical Squamous Cells  
51  
52 of Undetermined Significance, ASCUS).  
53  
54  
55  
56  
57

## Primary objectives

58  
59 The first primary objective of this cohort study is to decipher the kinetics and ecology of cervical  
60  
HPV infections in healthy young women, i.e. follow the population dynamics of the virus, the

target epithelial cells, and the immune effectors.

The second primary objective is to characterise the diversity of genital HPVs in young women in the region of Montpellier in relationship with their lifestyle, vaccination status, vaginal microbiota, and human genetics.

### Secondary objectives

A secondary objective is to characterise the acquisition and clearance dynamics of cervical HPV infections as a function of viral diversity, host immunity, vaginal microbiota and human genetics.

A final objective is to investigate variations in genetic diversity of HPVs during cervical infections.

## Methods and analysis

### Participants

The study population is composed of young women at risk of HPV infection. The age class was chosen because it exhibits high HPV prevalence (24% worldwide [4] and approximately 25% in France [2]). Inclusion of younger women would have raised technical issues because of the requirement for parental consent.

Women are recruited through a social media page, and through posters and leaflets distributed at the Universities in Montpellier and at the Montpellier STI screening centre (*Centre Gratuit d'Information de Dépistage et de Diagnostic, CeGIDD*). The composition of the population visiting the CeGIDD has already been documented in an earlier study [33]. In total, the centre is visited by approximately 3,000 women per year, the majority of which are under 25 years of age (80%). Approximately 40% of the attendants report three or more partners over the last twelve months and approximately 50% report using adequate behaviour for prevention against HIV.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Since all participants are healthy, they are referred to as participants rather than patients. As in any longitudinal study, ensuring participant commitment is challenging. To achieve this goal, we have set up a compensation of 40 EUR per visit and an additional 10 EUR in case of a complete follow-up. Furthermore, participants who have answered a sufficient number of questionnaires and brought back a sufficient number of self-samples get a 100 EUR bonus at the end. Overall, a participant performing 12 return visits would gain a total compensation of 650 EUR.

### **Inclusion criteria**

Participants are women from 18 to 25 years old living in the metropolitan area of Montpellier. They must be sexually active with at least one new partner over the last 12 months. This criteria is fixed to maximise the incidence of new HPV infections. Participants must be able to and willing to give written informed consent: they must sign an informed consent form, understand the requirements for the study, and be affiliated to a French social security scheme (which is a state requirement).

Women cannot be included in the study if they have a history of HPV-associated pathology (genital warts or cervical lesions), if they are pregnant or intending to become pregnant in the coming year, infected by HIV, undergoing (or planning to undergo) intense medical treatment (biotherapy, chemotherapy, immunosuppression), planning on moving outside the Montpellier metropolitan area within the next 18 months, in a dependency or employment with the sponsor or the investigator, if they participated in a clinical trial involving administration of drugs within the last four weeks or if they belong to a vulnerable group (e.g. children, adults with physical or mental disabilities).

## Design/setting

1  
2 This study has a longitudinal component aimed at deciphering within-host dynamics and a  
3  
4 cross-sectional component aimed at understanding the diversity of HPV infections in young  
5  
6 adults in the area of Montpellier, France. The general structure of the study is shown in Fig 1.  
7  
8  
9

10  
11 If a woman fits the main inclusion criteria, she can go through an inclusion visit ( $V_1$ ) with a  
12  
13 physician (gynaecologist or midwife) at the CeGIDD. During this visit, the study investigator  
14  
15 presents the study and checks all inclusion criteria before asking the participant to read and  
16  
17 sign the informed consent form. Participants then undergo a medical consultation during which  
18  
19 a number of samples are collected (see below). They then fill out health and lifestyle  
20  
21 questionnaires and are given cotton-flocked swabs for self-sampling at home the next visit,  
22  
23 along with instructions on how to fill in weekly questionnaires through an online form (these are  
24  
25 performed throughout the study).  
26  
27  
28

29  
30 An appointment is scheduled four weeks later for the Results visit ( $V_2$ ), where the cervical  
31  
32 cytology results are communicated. Additional samples are collected and self-sample swabs for  
33  
34 home collection are provided.  
35  
36

37  
38 The next return visits ( $V_i$ , where  $i > 2$ ) are as follows:  
39  
40

- 41  
42 • Participants with a positive DEIA HPV test (see below), i.e. infected by an  
43  
44 *Alphapapillomavirus*, at  $V_1$  join the HPV positive (HPV+) arm of the study with return  
45  
46 visits scheduled every 2 months.
- 47  
48 • Participants with a negative DEIA HPV test at  $V_1$  join the HPV negative (HPV-) arm with  
49  
50 return visits scheduled every 4 months.
- 51  
52 • HPV- participants infected by an *Alphapapillomavirus* move to the HPV+ arm.  
53  
54

55  
56 Intervals between visits are based on earlier results showing that HPV infections last from 9 to  
57  
58 18 months on average depending on the HPV type [5, 7–9] and that a total follow-up of 4  
59  
60 months yields results that are difficult to analyse [21]. The longer interval in the HPV- arm is  
based on the estimated incidence for HPV genital infections in young women, which is greater

than 30% [34, 35].

Participants in the HPV- arm are followed until month 32 of the study.

Participants in the HPV+ arm are followed until they clear the infection or until they have been infected for 24 months (after which we consider that the infection is persistent). Clearance is defined as being negative at two visits in a row for the first HPV type detected in the follow-up.

In between these visits to the CeGIDD, participants are asked to perform regular (every week for HPV+ and every second week for HPV-) self-samples using vaginal swabs, to measure vaginal pH and to fill in a short questionnaire. Self-samples are stored in the participants' freezer and brought back at every visit.

The study will end with the last HPV+ participant having cleared the infection or been infected for 24 months.

## Patients and public involvement

Participants did not play a role in the design of this study and the results of the study will be disseminated to participants who have left the study and to the general public via an email newsletter in French.

## Visits

The summary of the visit schedule and of the samples collected at each visit is shown in Table 1.

### Inclusion visit (V1)

This visit takes place at the CeGIDD and is scheduled by the Clinical Research Technician



(CRT) via phone or email.

Women meet a study investigator, who explains the goals and requirements of the study and checks that the inclusion criteria are met. If so, after a general discussion, the informed consent forms are signed.

A female physician/midwife performs a general exam and then a gynaecological exam during which the following samples are taken:

- vaginal pH cotton swab (EcoCare™),
- vaginal swab (Copan ESwab™) in 1mL Amies liquid for DNA extraction and microbiota analysis,
- vaginal swab (Copan ESwab™) in 1mL of RNA preservation medium,
- ophthalmic sponge (Weck-cel®) to collect cervical secretions for cytokines analysis,
- cervical smear in 20mL of Thinprep® (Preservcyt® liquid) for HPV and HSV assays, and cytology evaluation.

Following the gynaecological consultation, the participant meets with a nurse to measure body temperature, blood pressure and draw 20mL of blood (a 5mL tube for SNPs sequencing, a 10mL tube for immunophenotyping and a 5mL tube for HPV antibody titration). For the longitudinal study, the nurse provides the participant with 3 self-sampling kits, 3 pH strips, a freezer box to bring back to the next visit, as well as instructions on how to perform the home sampling and store the samples in her personal freezer until the next visit.

If the participant has not been tested for a STI in the last 3 months, the nurse draws an additional blood tube of 5mL to test for STIs (HIV, HCV, HBV) and collects vaginal self-samples for chlamydiae and gonorrhoea detection. Syphilis testing is prescribed to participants who meet the STI clinic's guidelines.

Finally, the participant meets with the CRT to fill in questionnaires #1 (inclusion visit) and #3 (home). The CRT answers any remaining questions, explains how to fill the home

questionnaires (#3) and sets an appointment for the Results visit.

### Results visit (V2)

During this visit, the participants are given the result of cervical lesion screening using the liquid cytology (normal, ASCUS, LSIL or HSIL). Participants with a high-grade lesion (HSIL) exit the study and are referred to the gynaecology service of the CHU of Montpellier.

During this visit, the physician/midwife collects additional samples: 2 vaginal swabs for DNA and RNA analysis, and a cervical smear in 10mL of PBS (to confirm HPV status and perform flow cytometry analyses).

The participant fills in questionnaires #2 (for return visits) and #3 (home). An appointment for the next visit is set and swabs for home self-sampling are given.

### Return visits (Vi)

These visits only occur in the longitudinal study.

**HPV- arm.** Participants uninfected by HPV visit the clinic every 4 months until month 26. During these visits, the same samples as in the inclusion visit (V1) are collected by the physician/midwife except for the cervical smear, which is put in PBS instead of Thinprep.

The nurse only draws blood if a screening test for STIs other than HPV is required. The participant then fills in questionnaires #2 and #3 and an appointment is set for the next visit in 16 weeks.

If an HPV infection is detected in the cervical smear collected during this visit, the participant moves to the HPV+ arm and the CRT contacts the participant to move her appointment forward.

**HPV+ arm.** Participants infected by HPV visit the clinic every 2 months. They cannot switch arm and will remain in the HPV+ arm until clearance or the end of the study. During the visits, the same samples as in the inclusion visit (V0) are collected by the physician/midwife except for the cervical smear, which is put in PBS instead of Thinprep.

1 The nurse then draws 5mL of blood for HPV antibody titration. If this is the first HPV+ visit  
2 following an HPV- visit, the nurse also draws 10mL of blood for immunophenotyping. Finally, if a  
3 test for additional STIs is needed, the nurse draws 5mL of blood and collects vaginal self-  
4 samples for STI detection.  
5  
6  
7

8  
9  
10 Importantly, if the participant has been infected by a HR-HPV for more than 12 months and  
11 cytology has not been performed within the last 12 months, the cervical smear is put in  
12 Thinprep<sup>®</sup> fixation medium, instead of PBS, for cytological analysis (cervical lesion screening).  
13  
14  
15

16  
17  
18 Finally, the participant fills in questionnaires #2 and #3, receives self-samples for home  
19 collection and an appointment is set for the next visit in 8 weeks.  
20  
21  
22  
23  
24  
25  
26

## 27 **Endpoints**

28  
29  
30 The primary endpoint for the study is the inclusion and follow-up of HPV-infected women in  
31 order to describe the kinetics of HPV virus load, and the associated immune response.  
32  
33  
34

35  
36 Secondary endpoints are the characterisation of the interactions between the course of the  
37 infection (e.g. duration), the HPV type(s), the abundance and taxonomic diversity of bacteria,  
38 fungi and viruses in the vaginal microbiota, human genetics (SNPs) and basal immunological  
39 status.  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49

## 50 **Technical procedures**

### 51 **DNA extraction**

52  
53  
54 DNA extraction from cervical smears will be performed using Nuclisens EasyMAG from  
55 Biomerieux or an equivalent protocol. For the microbiota analyses, special kits involving  
56 physical (via beads) and/or enzymatic breaking of the cellular barrier will be favoured following  
57 standard protocols to study the vaginal microbiome [36], e.g. the MagAttract<sup>®</sup>  
58  
59  
60

PowerMicrobiome<sup>®</sup> DNA/RNA kit from Qiagen.

### HPV detection, typing and quantification

The participants' infection status (HPV+ or HPV-) will be assessed using the DEIA test, which is based on a PCR of the short SPF10 amplicon [37] and detects all *Alphapapillomaviruses* with great sensitivity.

If the DEIA test is positive, HPVs will be typed using the LiPA25 kit, which is based on the same SPF10-PCR, and has a lower detection threshold compared to other hybridisation-based typing methods [38].

The reason for basing the detection on the DEIA rather than the LiPA25 is that some *Alphapapillomavirus* may be detected by DEIA but not genotyped by LiPA and also that the DEIA is more sensitive than the LiPA. If the DEIA is positive and the LiPA25 is negative, typing will be performed by sequencing the product of a PGMY09/11 PCR [39], which targets another region of the HPV genome than the SPF10 PCR.

The quantification of HPV DNA genome copy number in the samples will be performed using the protocol set up by Micalessi et al. [40].

### Cytokine titration

Cytokines can be used as markers of immune activation or immunosuppression and can also inform us on which components of the immune system are involved. Cervical sponges are centrifuged after the addition of 175 $\mu$ L of PBS. Cervical secretions are analysed for a set of 5 to 6 cytokines levels using the Meso Scale Discovery (MSD) Multiplex ELISA platform, which has a low detection threshold and a slowly saturating dose-response curve. Based on earlier results [41, 42], we will first investigate a large panel of 20 cytokines (IFN- $\alpha$ 2a, IFN- $\gamma$ , IL-1 $\alpha$ , IL-5, IL-6, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-23, IL-25, IP-10, MCP-1, MIP-1 $\alpha$ , MIP-3 $\alpha$ , MIP-3 $\beta$ , TNF- $\alpha$ , TNF- $\beta$ ) to choose the most relevant ones for a longitudinal follow-up.

### Flow cytometry

Analysing immune cells via flow cytometry is extremely challenging on cells as fragile as the

ones from cervical smears. However, several studies suggest that this is feasible [41–43]. Here, we follow the protocol described in [44].

Staining is performed using a Duraclone custom mix targeting CD45, CD3, CD4, CD8, CD16, CD56, CD69, CD161 and TCR $\gamma\delta$ . The last marker, Live&Dead tests for cellular viability.

Samples are acquired using a Navios flow cytometer (Beckman Coulter, three-laser configuration).

### Sequencing

Sequencing will be performed for microbiota profiling. It involves PCR amplification of the V3-V4 region of 16S RNA for bacteria [45] and ITS1 for fungi [46]. We anticipate that the bacteria should belong to the operational taxonomic units (OTU) described in the five community state types found in vaginal communities [47, 48]. The virome will also be explored using shotgun sequencing and rolling circle PCR amplification [49]. Human genetics are explored using chip sequencing for SNPs.

### Statistical analyses

#### Times series analyses

The core results of the study will come from the longitudinal follow-up of infected women, which will generate time series, i.e. a set of values collected from the same individual over time (Figure 2). There will be several time series per individual (virus load, number of immune cells, cytokine and antibody levels). These time series will be used to fit mathematical viral kinetics models that describe the interaction between viruses, host target cells (here, in the case of HPV, keratinocytes) and the immune response. These models are commonly developed for viral infections [13, 50–52], including those caused by HPVs [53]. We anticipate our follow-up to yield adequate data for such a fit based on the estimated duration of HPV infections (9 to 18 months [5, 7–9]). Furthermore, the weekly self-samples allow us to increase the resolution if necessary.

We will use non-linear mixed effect models [54] to jointly analyse time series from all

1 participants. More precisely, we will rely on *R* packages such as nlme [55] or lme4 [55]. Note  
2 that, in addition to estimating model parameters (e.g. life-expectancy of infected cells or virion  
3 production rate of infected cells), this approach also allows us to compare biological models  
4 using statistical tools based on model likelihood such as Akaike Information Criterion. For an  
5 example of such analysis in the case of HIV, see [51].  
6  
7  
8  
9

### 10 **Microbiota dynamics**

11 The composition of the vaginal microbiota has already been described and shown to exhibit  
12 considerably less diversity than the gut microbiota [47]. The dynamics of this microbiota has  
13 also been studied and shown to closely follow menstrual cycles [48].  
14  
15  
16  
17  
18  
19  
20  
21

22 Using the time series of OTU abundances (measured via 16S RNA sequencing and qPCR) we  
23 will infer interaction parameters by assuming an underlying Lotka-Volterra competition model  
24 [56]. This work will include time series analysis techniques (e.g. auto-correlation or local  
25 similarity analysis) and statistical inference methods in order to infer community structure and  
26 interactions from the next-generation sequencing (NGS) datasets [57]. Finally, statistical  
27 methods from ecology will also be used to study community diversity (e.g diversity indices) and  
28 community assembly, such as cluster and ordination analyses [58].  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38

### 39 **Genome Wide Association Studies**

40 We will use human single nucleotide polymorphisms (SNPs) inferred by chip sequencing to look  
41 for genetic determinants of key traits (e.g. microbiota composition or HPV infection duration).  
42 This is classically done by performing a Genome Wide Association Study (GWAS), which is a  
43 complex regression method designed for situations where there are many explanatory variables  
44 (here millions of SNPs) for a single trait of interest. GWAS will be performed using classical  
45 methods [59]. Earlier GWAS studies have been applied to HPV infections for instance to test for  
46 determinants to the ability to seroconvert following infection [60] and cervical cancer (see [61]  
47 for a review). Here, our expected sample ( $N = 300$  women) is limited but SNPs with large effects  
48 have been detected by studies with comparable sizes [62].  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

### Additional analyses

1 For all collected variables, descriptive statistics will be calculated according to the level of  
2  
3 measurement. For metric variables these statistics can be mean and standard deviation as well  
4  
5 as quantiles and more robust statistics [63]. In case of categorical variables group proportions  
6  
7 and contingency tables are prepared.  
8  
9

10  
11 Univariate inferential statistics follow a descriptive analysis. Generally, parametric testing  
12  
13 procedures are preferred to non-parametric tests, as the former have higher power. That is why,  
14  
15 for metric variables, we will first check whether the data can be assumed to be normally  
16  
17 distributed. For normally distributed variables, ANOVA statistics are done to detect differences  
18  
19 between groups. In case of significance, post-hoc analysis (Tukey test) are planned to reveal  
20  
21 pairwise differences. If the data are not normally distributed or ordinally scaled, non-parametric  
22  
23 analyses will be used. These contain the Kruskal-Wallis test and the Wilcoxon test as a post-  
24  
25 hoc test with an appropriate correction of the significance level. Since the cell counts are  
26  
27 expected to be small, Fisher's exact test will be performed for contingency tables instead of the  
28  
29 asymptotic  $\chi^2$  test for categorical variables.  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39

### Sample size calculation

40  
41 The study will enrol a total of  $N = 300$  women, with  $N = 150$  in a longitudinal study and  $N = 150$   
42  
43 in a cross-sectional study. The goal of the longitudinal study is to follow 75 women  
44  
45 longitudinally, preferentially before they are infected (see above). For the following calculations,  
46  
47 we assumed a high percentage of lost during follow-up (30%).  
48  
49  
50  
51  
52  
53  
54  
55

56 With 150 enrolments and considering that the prevalence of HPV infection in young women is  $\approx$   
57  
58 60% (based on our preliminary data) and 30% of lost to follow-up, we expect to detect (and  
59  
60 successfully follow) 63 infections at inclusion [CI95: 51–75, assuming a binomial distribution to  
calculate the 95% confidence interval].  
For peer review only - <http://bmjopen.bmj.com/site/about/guidelines.xhtml>

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Among women who are uninfected at the first visit and considering the yearly incidence being close to 30% [64], we expect 12 [CI95: 6–20] to be infected during the first year of follow-up (still assuming 30% of lost to follow-up).

In the end, with 150 enrolments and assuming a high percentage of lost to follow-up (30%), we expect to successfully follow 75 [CI95: 56–95] women infected at different stages of HPV infection: beginning, during and end.

This will be made possible by the probability of transmission of HPV, which is estimated to be  $\approx$  90% without condom use and still high with condom use ( $\approx$  40%) [34].

Finally, regarding potential interference with the HPV vaccines, we do not anticipate any significant problem for two reasons. First, as mentioned above, the vaccine coverage is low in France [32]. Second, and more importantly, the vaccines only target few HPV types, thus leaving open the possibility of infection by dozens of types. Furthermore, studying the kinetics of a non-vaccine HPV type in a vaccinated woman will be extremely informative, e.g. to detect any potential cross-reactivity [65].

To run cross-sectional analyses (especially on the microbiota and human genetics), we will enrol  $N = 150$  additional women who will only perform the inclusion and the results visits. This sample size was chosen to reach that of earlier GWAS studies [61, 62].

## **Trial governance**

### **Sponsor**

This study is sponsored by the Centre Hospitalier Universitaire (CHU) of Montpellier. The CHU is involved in the implementation of the trial, legal/ethical submissions (see below for details on Ethics approval) and implementing the clinical database (eCRF), which is hosted by Ennov-Clinical (ClinSight). The CHU is not involved in the analysis or interpretation of the data. The CHU of Montpellier performs regular quality control assessments. A clinical research assistant



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

will visit the CeGIDD every 4 months to ensure that implementation is in accordance with the protocol. The CHU has taken out insurance from the Société hospitalière d'assurances mutuelles, 18, rue Edouard Rochet-6 9372 Lyon cedex 08 (contract number 138983) through the full research period, covering its own civil liability and that of any agent (clinical or research staff), in accordance with article L.1121-10 of the French Public Health Code.

### **Scientific committee**

The scientific committee comprises the study investigators, clinicians, scientific experts and representatives of the sponsor. The committee meets yearly and is responsible for following research progress, monitoring compliance with good clinical practices and patient safety. It can also decide relevant modification of the protocol. Requests from third parties to access data collected during the study will be evaluated by the committee.

### **Monitoring**

Monitoring is performed during the whole study at CeGIDD according to the sponsor specific SOP. Routine monitoring visits are made by the monitors designated by the sponsor to check compliance with the protocol, the completeness, accuracy and consistency of the data, and adherence to GCP. The principal investigator ensures that eCRFs are completed in a timely manner and must allow periodical access to eCRFs, patient records, drug logs, and all other study-related documents and materials. The frequency of monitoring visits is determined by factors such as study design and the site enrolment requirements but visits will normally occur at least once every 4 months.

### **Trial registration**

The trial has been registered to ClinicalTrials.gov on 27 Oct 2016 with ID number NCT02946346.

## Ethics and Dissemination

1  
2  
3 The PAPCLEAR trial obtained favourable opinions from the Comité de Protection des  
4 Personnes (CPP) Sud Méditerranée I on May 11, 2016 (CPP number 16 42, reference number  
5 ID RCB 2016-A00712-49); from the Comité Consultatif sur le Traitement de l'Information en  
6 matière de Recherche dans le domaine de la Santé (CCTIRS) on July 12, 2016 (reference  
7 number 16.504); and from the Commission Nationale Informatique et Libertés (CNIL) on Dec  
8 16, 2016 (reference number MMS/ABD/AR1612278, decision number DR-2016-488). This trial  
9 was authorised by the Agence Nationale de Sécurité du Médicament et des Produits de Santé  
10 (ANSM) on July 20, 2016 (reference 20160072000007).

11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23 The protocol has been modified since its initial version and the latest modification was accepted  
24 by the CPP on Dec 12, 2018.

25  
26  
27  
28 All participants in the study will sign an informed consent form prior to participation.

29  
30  
31  
32 The results will be published on preprint servers (e.g. BioRxiv), peer-reviewed journals, post-  
33 print servers (e.g. HAL) and disseminated through conferences.

## Discussion

### Expected results

34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47 Acute infections by HPVs are important to study because vaccination is most effective when  
48 performed before the first infection. However, we currently know very little about the early  
49 stages of HPV infections. This clinical study will give us an unprecedented level of detail into the  
50 natural history of HPV infections in young women. Variations in virus load over time have been  
51 studied but in the context of cervical cancer in older women [66]. In addition, we will also  
52 describe the nature and the dynamics of the immune response (local immune cells and  
53 cytokines, circulating anti-HPV antibodies) and of the vaginal microbiota. Beyond these kinetics,  
54 we will also have access to data such as infection clearance or not in 24 months, presence of  
55  
56  
57  
58  
59  
60

more than one HPV type or coinfection by other STIs.

1  
2  
3 These data will be analysed in the light of numerous cofactors. One of the most important will  
4  
5 be human genetics, with the sequencing of millions of SNPs. Others will be related to the sexual  
6  
7 behaviour (number of partners, contraception methods, sexual practices) and general lifestyle.  
8  
9 We, therefore, expect broader insights regarding sexual health in young women.  
10  
11  
12

### 13 **Practical and operational issues**

14  
15  
16 One of the main practical challenges resides in the analysis of cervical smears by flow  
17  
18 cytometry. Indeed, the tissues are known to be fragile, adhesive and auto-fluorescent. Even  
19  
20 though standard protocols now exist [44], they require processing fresh samples in less than 2  
21  
22 hours.  
23  
24

25  
26 Another potential issue has to do with contamination by HPV DNA between samples, which are  
27  
28 frequent in the HPV field due to the robustness of the virions and the sensitivity of the tests. To  
29  
30 certify our ability to control for these, we have entered the 2017 GLOBAL HPV DNA Proficiency  
31  
32 Panel from the WHO HPV LabNet [67].  
33  
34

35  
36 Regarding the enrolment of the participants, we do not expect issues with enrolling 150 women  
37  
38 in 28 months for the longitudinal study and 150 for the cross-sectional study. This is due to the  
39  
40 number of visitors of the centre who fit the inclusion criteria (more than 3,000 per year) and  
41  
42 because of earlier high participation rates in the same population ([33] enrolled 1381  
43  
44 participants in 5 months for their study).  
45  
46  
47

48  
49 Concerning the follow-up, the high incidence rate of HPV can also lead to transient carriage,  
50  
51 i.e. women who are positive for a type only at a single visit. This has been observed for instance  
52  
53 in longitudinal studies with a tight follow-up interval [21]. To control for this, we will run the HPV  
54  
55 detection test on the cells from the cervical smear after washing with RPMI.  
56  
57  
58  
59  
60

1  
2  
3  
4 **Table 1: Summary of the visit schedules and samples take.** The cross-sectional study only  
5 includes the first two columns (V1 and V2). The ∞ indicate samples taken at visits. + participants  
6 infected by a HR-HPV for 12 month will have one PBS smear replaced by a Thinprep® smear  
7 to perform a cytology and check for lesions. ◁ this sample is only taken at the first HPV+ visit of  
8 a formerly HPV- participant. \* STI detection will be performed at inclusion unless the participant  
9 has been tested within the last 3 months and during the study every 6 months if a new partner  
10 has been reported or upon request.  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

|                                          | Inclusion<br>(V1) | Results<br>(V2) | Return<br>(Vi, with $i > 2$ ) |            |
|------------------------------------------|-------------------|-----------------|-------------------------------|------------|
| Participants                             | all               | all             | HPV+                          | HPV-       |
| Time                                     | day 0             | + 4 weeks       | + 8 weeks                     | + 16 weeks |
| Eligibility                              | ☐                 |                 |                               |            |
| Consent                                  | ☐                 |                 |                               |            |
| Gynecological consult                    | ☐                 | ☐               | ☐                             | ☐          |
| Vaginal pH cotton swab                   | ☐                 | ☐               | ☐                             | ☐          |
| 2 vaginal swab samples (Copan<br>ESwab™) | ☐                 | ☐               | ☐                             | ☐          |
| 1 ophtalmological sponge sample          | ☐                 |                 | ☐                             | ☐          |
| 1 cervical smear in Thinprep® (cytology) | ☐                 |                 | +                             |            |
| 1 cervical smear in PBS                  |                   | ☐               | +                             | ☐          |
| Blood sampling (HPV antibodies)          | ☐                 |                 | ☐                             |            |
| Blood sampling (sequencing)              | ☐                 |                 |                               |            |
| Blood sampling (immunophenotyping)       | ☐                 |                 | △                             |            |
| Other STI detection                      | *                 | *               | *                             | *          |
| Questionnaire #1 (inclusion)             | ☐                 |                 |                               |            |
| Questionnaire #2 (visit)                 |                   | ☐               | ☐                             | ☐          |
| Questionnaire #3 (home)                  | ☐                 | ☐               | ☐                             | ☐          |
| Returning self-sampling samples          |                   | ☐               | ☐                             | ☐          |
| Serious Adverse Event collection         |                   | ☐               | ☐                             | ☐          |

## Abbreviations

1  
2  
3  
4  
5 STI: sexually transmitted infection  
6

7 TCR: T-cell receptor,  
8

9 WHO: World Health Organisation. CNRS: Centre National de la Recherche Scientifique,  
10

11 CRT: Clinical Research Technician,  
12

13 ELISA: enzyme-linked immunosorbent assay,  
14

15 GWAS: Genome Wide Association Study,  
16

17 HIV: Human Immunodeficiency Virus,  
18

19 HPV: Human Papillomavirus,  
20

21 HR: high-risk,  
22

23 ITS: Internal Transcribed Spacer,  
24

25 HSIL: High grade Squamous Intraepithelial Lesion,  
26

27 LR: low-risk,  
28

29 LSIL: Low grade Squamous Intraepithelial Lesion,  
30

31 NGS: Next Generation Sequencing,  
32

33 OTU: Operational Taxonomic Unit,  
34

35 PBMC: Peripheral Blood Mononuclear Cell,  
36

37 PBS: Phosphate Buffered Saline,  
38

39 RPMI: Roswell Park Memorial Institute medium,  
40

41 SNP: Single Nucleotide Polymorphism, ANOVA: Analysis of variance,  
42

43 ASC-US: Atypical squamous cells of undetermined significance,  
44

45 CD: Cluster of differentiation,  
46

47 CI95: 95% Confidence interval,  
48

49 CeGIDD: Centre Gratuit d'Information de Dépistage et de Diagnostic,  
50

51 CHU: Centre Hospitalier Universitaire,  
52

53 CIN: Cervical intraepithelial Neoplasia,  
54  
55  
56  
57  
58  
59  
60

## Trial status

1  
2  
3 The study began on Oct 1, 2016 and the first inclusion was on Nov 3, 2016. On Jun 23, 2018,  
4  
5 89 participants have been included in the longitudinal study. Inclusions in the longitudinal study  
6  
7 will continue until March 2019 and the study is expected to last until Aug 2021.  
8  
9

## Conflicts of interests

10  
11  
12  
13  
14 The authors have read and understood BMJ policy on declaration of interests and declare that  
15  
16 they have no competing interests.  
17  
18

## Funding

19  
20  
21  
22  
23 This project has received funding from the European Research Council (ERC) under the  
24  
25 European Union's Horizon 2020 research and innovation program (grant agreement No  
26  
27 648963). The authors acknowledge further support from the Centre National de la Recherche  
28  
29 Scientifique (CNRS), the Institute de Recherche pour le Développement (IRD) and the Centre  
30  
31 Hospitalier Universitaire (CHU) of Montpellier.  
32  
33  
34

## Data statement

35  
36  
37  
38  
39 All personal and identifying information collected from participants are kept in a secure place at  
40  
41 the CeGIDD during the duration of the trial and will be destroyed at the end of the study. The  
42  
43 final raw dataset will be accessible only to the sponsor (CHU) and the chief scientist's (SA)  
44  
45 team. Anonymous data will be available to external parties upon approval of both the sponsor  
46  
47 and the scientific committee. All publications will be made green or gold open access and the  
48  
49 corresponding data will be provided as supplementary material or via a public repository  
50  
51 (e.g. Zenodo), provided that there is no conflict with ethical guidelines.  
52  
53  
54  
55

## Author contributions

56  
57  
58  
59 Samuel Alizon, Carmen Lia Murall and Massical Rahmoun were the major contributors in the  
60  
conception of the protocol. Samuel Alizon wrote the initial version of the manuscript. Christian

1 Selinger, Monique Baldellou, Claire Bernat, Marine Bonneau, Vanina Boué, Mathilde Buisson,  
2 Guillaume Christophe, Giuseppe D'Auria, Florence De Taroni, Vincent Foulongne, Rémy  
3  
4 Froissart, Christelle Graf, Sophie Grasset, Soraya Groc, Christophe Hirtz, Audrey Jausset,  
5  
6 Julie Lajoie, Frédérique Lorcy, Eric Picot, Marie-Christine Picot, Jacques Ravel, Jacques  
7  
8 Reynes, Thérèse Rousset, Aziza Seddiki, Martine Teirlinck, Vincent Tribout, Édouard Tuillon,  
9  
10 Tim Waterboer, Nathalie Jacobs, Ignacio G Bravo, Michel Segondy and Natalie Boulle were  
11  
12 involved in the conception of the protocol, in the implementation of the study and read and  
13  
14 approved the final manuscript.  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32

## 33 **Acknowledgements**

34  
35 We thank all the study participants and the CeGIDD staff for their commitment to the study. We  
36  
37 also thank the reviewers and, in particular, Dr. Andrew Brouwer for his meticulous reading of the  
38  
39 manuscript.  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



## References

1. Tota, J.E., Chevarie-Davis, M., Richardson, L.A., Devries, M., Franco, E.L.: Epidemiology and burden of HPV infection and related diseases: implications for prevention strategies. *Prev Med* **53 Suppl 1**, 12–21 (2011). doi:[10.1016/j.ypmed.2011.08.017](https://doi.org/10.1016/j.ypmed.2011.08.017)
2. Monsonego, J., Zerat, L., Syrjänen, K., Zerat, J.C., Smith, J.S., Halfon, P.: Prevalence of genotype-specific HPV infection among women in France: implications for screening and vaccination. *Gynecol Obstet Fertil* **41**(5), 305–313 (2013). doi:[10.1016/j.gyobfe.2013.03.003](https://doi.org/10.1016/j.gyobfe.2013.03.003)
3. Brun-Micaleff, E., Coffy, A., Rey, V., Didelot, M.-N., Combecal, J., Doutre, S., Daurès, J.-P., Segondy, M., Boulle, N.: Cervical cancer screening by cytology and human papillomavirus testing during pregnancy in french women with poor adhesion to regular cervical screening. *J Med Virol* **86**(3), 536–45 (2014). doi:[10.1002/jmv.23764](https://doi.org/10.1002/jmv.23764)
4. Bruni, L., Diaz, M., Castellsagué, X., Ferrer, E., Bosch, F.X., de Sanjosé, S.: Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. *J Infect Dis* **202**(12), 1789–99 (2010). doi:[10.1086/657321](https://doi.org/10.1086/657321)
5. Insinga, R.P., Dasbach, E.J., Elbasha, E.H., Liaw, K.-L., Barr, E.: Incidence and duration of cervical human papillomavirus 6, 11, 16, and 18 infections in young women: an evaluation from multiple analytic perspectives. *Cancer Epidemiol Biomarkers Prev* **16**(4), 709–15 (2007). doi:[10.1158/1055-9965.EPI-06-0846](https://doi.org/10.1158/1055-9965.EPI-06-0846)
6. Woodman, C.B.J., Collins, S.I., Young, L.S.: The natural history of cervical HPV infection: unresolved issues. *Nat Rev Cancer* **7**(1), 11–22 (2007). doi:[10.1038/nrc2050](https://doi.org/10.1038/nrc2050)
7. Rodríguez, A.C., Schiffman, M., Herrero, R., Wacholder, S., Hildesheim, A., Castle, P.E., Solomon, D., Burk, R., Proyecto Epidemiológico Guanacaste Group: Rapid clearance of human papillomavirus and implications for clinical focus on persistent infections. *J Natl Cancer Inst* **100**(7), 513–7 (2008). doi:[10.1093/jnci/djn044](https://doi.org/10.1093/jnci/djn044)

- 1  
2  
3  
4  
5  
6  
7  
8  
9
8. Trottier, H., Mahmud, S., Prado, J.C.M., Sobrinho, J.S., Costa, M.C., Rohan, T.E., Villa, L.L., Franco, E.L.: Type-Specific Duration of Human Papillomavirus Infection: Implications for Human Papillomavirus Screening and Vaccination. *J Infect Dis* **197**(10), 1436–1447 (2008). doi:[10.1086/587698](https://doi.org/10.1086/587698)
- 10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24
9. Ramanakumar, A.V., Naud, P., Roteli-Martins, C.M., de Carvalho, N.S., de Borja, P.C., Teixeira, J.C., Blatter, M., Moscicki, A.-B., Harper, D.M., Romanowski, B., Tying, S.K., Ramjattan, B., Schuind, A., Dubin, G., Franco, E.L.: Incidence and duration of type-specific human papillomavirus infection in high-risk HPV-naïve women: results from the control arm of a phase II HPV-16/18 vaccine trial. *BMJ Open* **6**(8), 011371 (2016). doi:[10.1136/bmjopen-2016-011371](https://doi.org/10.1136/bmjopen-2016-011371)
- 25  
26  
27  
28  
29  
30  
31  
32  
33  
34
10. Houlihan, C.F., Baisley, K., Bravo, I.G., Kapiga, S., de Sanjosé, S., Changalucha, J., Ross, D.A., Hayes, R.J., Watson-Jones, D.: Rapid acquisition of HPV around the time of sexual debut in adolescent girls in Tanzania. *Int J Epidemiol* **45**(3), 762–773 (2016). doi:[10.1093/ije/dyv367](https://doi.org/10.1093/ije/dyv367)
- 35  
36  
37  
38  
39  
40
11. Alizon, S., Murall, C.L., Bravo, I.G.: Why Human Papillomavirus Acute Infections Matter. *Viruses* **9**(10), 293 (2017). doi:[10.3390/v9100293](https://doi.org/10.3390/v9100293)
- 41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54
12. Herrero, R., Wacholder, S., Rodríguez, A.C., Solomon, D., González, P., Kreimer, A.R., Porras, C., Schussler, J., Jiménez, S., Sherman, M.E., Quint, W., Schiller, J.T., Lowy, D.R., Schiffman, M., Hildesheim, A., Costa Rica Vaccine Trial Group: Prevention of persistent human papillomavirus infection by an HPV16/18 vaccine: a community-based randomized clinical trial in Guanacaste, Costa Rica. *Cancer Discov* **1**(5), 408–19 (2011). doi:[10.1158/2159-8290.CD-11-0131](https://doi.org/10.1158/2159-8290.CD-11-0131)
- 55  
56  
57  
58  
59  
60
13. Canini, L., Perelson, A.S.: Viral kinetic modeling: state of the art. *J Pharmacokinet Pharmacodyn* **41**(5), 431–443 (2014). doi:[10.1007/s10928-014-9363-3](https://doi.org/10.1007/s10928-014-9363-3)
14. Stanley, M.: Immune responses to human papillomavirus. *Vaccine* **24**(S1), 16–22 (2006).

doi:[10.1016/j.vaccine.2005.09.002](https://doi.org/10.1016/j.vaccine.2005.09.002)

- 1  
2  
3 15. Ferenczy, A., Franco, E.: Persistent human papillomavirus infection and cervical  
4  
5 neoplasia. *Lancet Oncol* **3**(1), 11–6 (2002)  
6  
7
- 8  
9 16. zur Hausen, H.: Review: Papillomaviruses — to Vaccination and Beyond. *Biochemistry*  
10  
11 **73**(5), 498–503 (2008). doi:[10.1134/S0006297908050027](https://doi.org/10.1134/S0006297908050027)  
12  
13
- 14  
15 17. Einstein, M.H., Schiller, J.T., Viscidi, R.P., Strickler, H.D., Coursaget, P., Tan, T., Halsey,  
16  
17 N., Jenkins, D.: Clinician's guide to human papillomavirus immunology: knowns and unknowns.  
18  
19 *Lancet Infect Dis* **9**(6), 347–56 (2009). doi:[10.1016/S1473-3099\(09\)70108-2](https://doi.org/10.1016/S1473-3099(09)70108-2)  
20  
21
- 22  
23 18. Van Hede, D., Langers, I., Delvenne, P., Jacobs, N.: Origin and immunoescape of uterine  
24  
25 cervical cancer. *Presse Med* **43**(12P2), 413–421 (2014). doi:[10.1016/j.lpm.2014.09.005](https://doi.org/10.1016/j.lpm.2014.09.005)  
26  
27
- 28  
29 19. Stanley, M.: Immunology of HPV infection. *Curr Obstet Gynecol Rep* **4**(4), 195–200  
30  
31 (2015). doi:[10.1007/s13669-015-0134-y](https://doi.org/10.1007/s13669-015-0134-y). Accessed 2017-03-20  
32  
33
- 34  
35 20. Gao, W., Weng, J., Gao, Y., Chen, X.: Comparison of the vaginal microbiota diversity of  
36  
37 women with and without human papillomavirus infection: a cross-sectional study. *BMC Infect*  
38  
39 *Dis* **13**, 271 (2013). doi:[10.1186/1471-2334-13-271](https://doi.org/10.1186/1471-2334-13-271)  
40  
41
- 42  
43 21. Brotman, R.M., Shardell, M.D., Gajer, P., Tracy, J.K., Zenilman, J.M., Ravel, J., Gravitt,  
44  
45 P.E.: Interplay between the temporal dynamics of the vaginal microbiota and human  
46  
47 papillomavirus detection. *J Infect Dis* **210**(11), 1723–33 (2014). doi:[10.1093/infdis/jiu330](https://doi.org/10.1093/infdis/jiu330)  
48  
49
- 50  
51 22. Koutsky, L.A., Ault, K.A., Wheeler, C.M., Brown, D.R., Barr, E., Alvarez, F.B.,  
52  
53 Chiacchierini, L.M., Jansen, K.U., Proof of Principle Study Investigators: A controlled trial of a  
54  
55 human papillomavirus type 16 vaccine. *N Engl J Med* **347**(21), 1645–51 (2002).  
56  
57 doi:[10.1056/NEJMoa020586](https://doi.org/10.1056/NEJMoa020586)  
58  
59
- 60  
23. Riethmuller, D., Jacquard, A.-C., Lacau St Guily, J., Aubin, F., Carcopino, X., Pradat, P.,  
Dahlab, A., Pr  tet, J.-L.: Potential impact of a nonavalent hpv vaccine on the occurrence of hpv-

related diseases in france. BMC Public Health **15**, 453 (2015). doi:[10.1186/s12889-015-1779-1](https://doi.org/10.1186/s12889-015-1779-1)

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
24. Joura, E.A., Giuliano, A.R., Iversen, O.-E., Bouchard, C., Mao, C., Mehlsen, J., Moreira, E.D. Jr, Ngan, Y., Petersen, L.K., Lazcano-Ponce, E., Pitisuttithum, P., Restrepo, J.A., Stuart, G., Woelber, L., Yang, Y.C., Cuzick, J., Garland, S.M., Huh, W., Kjaer, S.K., Bautista, O.M., Chan, I.S.F., Chen, J., Gesser, R., Moeller, E., Ritter, M., Vuocolo, S., Luxembourg, A., Broad Spectrum HPV Vaccine Study: A 9-valent HPV vaccine against infection and intraepithelial neoplasia in women. N Engl J Med **372**(8), 711–23 (2015). doi:[10.1056/NEJMoa1405044](https://doi.org/10.1056/NEJMoa1405044)
25. Murall, C.L., Bauch, C.T., Day, T.: Could the human papillomavirus vaccines drive virulence evolution? Proc Biol Sci **282**, 20141069 (2015). doi:[10.1098/rspb.2014.1069](https://doi.org/10.1098/rspb.2014.1069)
26. Alizon, S., Méthot, P.-O.: Reconciling Pasteur and Darwin to control infectious diseases. PLoS Biol **16**(1), 2003815 (2018). doi:[10.1371/journal.pbio.2003815](https://doi.org/10.1371/journal.pbio.2003815)
27. Moscicki, A.-B., Ma, Y., Wibbelsman, C., Darragh, T.M., Powers, A., Farhat, S., Shiboski, S.: Rate of and Risks for Regression of CIN-2 in adolescents and young women. Obstet Gynecol **116**(6), 1373–1380 (2010). doi:[10.1097/AOG.0b013e3181fe777f](https://doi.org/10.1097/AOG.0b013e3181fe777f)
28. Buck Jr., H.W.: Warts (genital). BMJ Clin Evid **2015**, 1602 (2015)
29. Herrero, R., González, P., Markowitz, L.E.: Present status of human papillomavirus vaccine development and implementation. Lancet Oncol **16**(5), 206–16 (2015). doi:[10.1016/S1470-2045\(14\)70481-4](https://doi.org/10.1016/S1470-2045(14)70481-4)
30. Maver, P.J., Poljak, M.: Progress in prophylactic human papillomavirus (HPV) vaccination in 2016: A literature review. Vaccine (2018). doi:[10.1016/j.vaccine.2017.07.113](https://doi.org/10.1016/j.vaccine.2017.07.113)
31. Fagot, J.-P., Boutrelle, A., Ricordeau, P., Weill, A., Allemand, H.: HPV vaccination in France: uptake, costs and issues for the National Health Insurance. Vaccine **29**(19), 3610–6 (2011). doi:[10.1016/j.vaccine.2011.02.064](https://doi.org/10.1016/j.vaccine.2011.02.064)

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

32. Ben Hadj Yahia, M.-B., Dervaux, B., Duport, N., Floret, D., Gaillot, J., Heard, I., Jacquet, A., Le Goaster, C., Lévy-Bruhl, D., Morer, I., Parent du Chatelet, I., Peigue-Lafeuille, H., Rumeau-Pichon, C.: Vaccination contre les infections à papilloamvirus. Technical report, Haut Conseil de la Santé Publique, Paris, France (2014).

<https://www.hcsp.fr/Explore.cgi/avisrapportsdomaine?clefr=454>

33. Clarivet, B., Picot, E., Marchandin, H., Tribout, V., Rachedi, N., Schwartzenruber, E., Ledésert, B., Dereure, O., Guillot, B., Picot, M.-C.: Prevalence of Chlamydia trachomatis, Neisseria gonorrhoeae and Mycoplasma genitalium in asymptomatic patients under 30 years of age screened in a French sexually transmitted infections clinic. *Eur J Dermatol* **24**(5), 611–6 (2014). doi:[10.1684/ejd.2014.2413](https://doi.org/10.1684/ejd.2014.2413)

34. Winer, R.L., Hughes, J.P., Feng, Q., O'Reilly, S., Kiviat, N.B., Holmes, K.K., Koutsky, L.A.: Condom use and the risk of genital human papillomavirus infection in young women. *N Engl J Med* **354**(25), 2645–54 (2006). doi:[10.1056/NEJMoa053284](https://doi.org/10.1056/NEJMoa053284)

35. Winer, R.L., Hughes, J.P., Feng, Q., Stern, J.E., Xi, L.F., Koutsky, L.A.: Incident Detection of High-Risk Human Papillomavirus Infections in a Cohort of High-Risk Women Aged 25-65 Years. *J Infect Dis* **214**(5), 665–75 (2016). doi:[10.1093/infdis/jiw074](https://doi.org/10.1093/infdis/jiw074)

36. Ravel, J., Brotman, R.M., Gajer, P., Ma, B., Nandy, M., Fadrosh, D.W., Sakamoto, J., Koenig, S.S., Fu, L., Zhou, X., Hickey, R.J., Schwebke, J.R., Forney, L.J.: Daily temporal dynamics of vaginal microbiota before, during and after episodes of bacterial vaginosis. *Microbiome* **1**(1), 29 (2013). doi:[10.1186/2049-2618-1-29](https://doi.org/10.1186/2049-2618-1-29)

37. Kleter, B., van Doorn, L.-J., ter Schegget, J., Schrauwen, L., van Krimpen, K., Burger, M.,

- ter Harmsel, B., Quint, W.: Novel Short-Fragment PCR Assay for Highly Sensitive Broad-Spectrum Detection of Anogenital Human Papillomaviruses. *Am J Pathol* **153**(6), 1731–1739 (1998). doi:[10.1016/S0002-9440\(10\)65688-X](https://doi.org/10.1016/S0002-9440(10)65688-X)
38. Geraets, D.T., Struijk, L., Kleter, B., Molijn, A., van Doorn, L.-J., Quint, W.G.V., Colau, B.: The original SPF10 LiPA25 algorithm is more sensitive and suitable for epidemiologic HPV research than the SPF10 INNO-LiPA Extra. *J Virol Meth* **215-216**, 22–29 (2015). doi:[10.1016/j.jviromet.2015.01.001](https://doi.org/10.1016/j.jviromet.2015.01.001)
39. Gravitt, P.E., Peyton, C.L., Alessi, T.Q., Wheeler, C.M., Coutlée, F., Hildesheim, A., Schiffman, M.H., Scott, D.R., Apple, R.J.: Improved amplification of genital Human Papillomaviruses. *J Clin Microbiol* **38**(1), 357–361 (2000)
40. Micalessi, I.M., Boulet, G.A.V., Bogers, J.J., Benoy, I.H., Depuydt, C.E.: High-throughput detection, genotyping and quantification of the human papillomavirus using real-time PCR. *Clin Chem Lab Med* **50**(4), 655–661 (2012). doi:[10.1515/cclm.2011.835](https://doi.org/10.1515/cclm.2011.835)
41. Hunter, P.J., Sheikh, S., David, A.L., Peebles, D.M., Klein, N.: Cervical leukocytes and spontaneous preterm birth. *Journal of Reproductive Immunology* **113**, 42–49 (2016). doi:[10.1016/j.jri.2015.11.002](https://doi.org/10.1016/j.jri.2015.11.002)
42. Shannon, B., Yi, T.J., Perusini, S., Gajer, P., Ma, B., Humphrys, M.S., Thomas-Pavanel, J., Chieza, L., Janakiram, P., Saunders, M., Tharao, W., Huibner, S., Shahabi, K., Ravel, J., Rebbapragada, A., Kaul, R.: Association of HPV infection and clearance with cervicovaginal immunology and the vaginal microbiota. *Mucosal Immunology* **10**(5), 1310–1319 (2017). doi:[10.1038/mi.2016.129](https://doi.org/10.1038/mi.2016.129)
43. Lajoie, J., Juno, J., Burgener, A., Rahman, S., Mogk, K., Wachihi, C., Mwanjewe, J., Plummer, F.A., Kimani, J., Ball, T.B., Fowke, K.R.: A distinct cytokine and chemokine profile at the genital mucosa is associated with HIV-1 protection among HIV-exposed seronegative commercial sex workers. *Mucosal Immunol* **5**(3), 277–287 (2012). doi:[10.1038/mi.2012.7](https://doi.org/10.1038/mi.2012.7)

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
44. Juno, J.A., Boily-Larouche, G., Lajoie, J., Fowke, K.R.: Collection, isolation, and flow cytometric analysis of human endocervical samples. *J Vis Exp* **89**, 51906 (2014).  
doi:[10.3791/51906](https://doi.org/10.3791/51906)
45. Frank, J.A., Reich, C.I., Sharma, S., Weisbaum, J.S., Wilson, B.A., Olsen, G.J.: Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Appl Environ Microbiol* **74**(8), 2461 (2008). doi:[10.1128/AEM.02272-07](https://doi.org/10.1128/AEM.02272-07)
46. Findley, K., Oh, J., Yang, J., Conlan, S., Deming, C., Meyer, J.A., Schoenfeld, D., Nomicos, E., Park, M., NIH Intramural Sequencing Center Comparative Sequencing Program, Kong, H.H., Segre, J.A.: Topographic diversity of fungal and bacterial communities in human skin. *Nature* **498**(7454), 367–370 (2013). doi:[10.1038/nature12171](https://doi.org/10.1038/nature12171). Accessed 2017-09-13
47. Ravel, J., Gajer, P., Abdo, Z., Schneider, G.M., Koenig, S.S.K., McCulle, S.L., Karlebach, S., Gorle, R., Russell, J., Tacket, C.O., Brotman, R.M., Davis, C.C., Ault, K., Peralta, L., Forney, L.J.: Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci USA* **108**, 4680–7 (2011). doi:[10.1073/pnas.1002611107](https://doi.org/10.1073/pnas.1002611107)
48. Gajer, P., Brotman, R.M., Bai, G., Sakamoto, J., Schütte, U.M.E., Zhong, X., Koenig, S.S.K., Fu, L., Ma, Z.S., Zhou, X., Abdo, Z., Forney, L.J., Ravel, J.: Temporal dynamics of the human vaginal microbiota. *Sci Transl Med* **4**(132), 132–52 (2012).  
doi:[10.1126/scitranslmed.3003605](https://doi.org/10.1126/scitranslmed.3003605)
49. Johne, R., Müller, H., Rector, A., van Ranst, M., Stevens, H.: Rolling-circle amplification of viral DNA genomes using phi29 polymerase. *Trends Microbiol* **17**(5), 205–211 (2009).  
doi:[10.1016/j.tim.2009.02.004](https://doi.org/10.1016/j.tim.2009.02.004)
50. Nowak, M.A., May, R.M.: *Virus Dynamics: Mathematical Principles of Immunology and Virology*. Oxford University Press, Oxford, USA (2000)
51. Stafford, M.A., Corey, L., Cao, Y., Daar, E.S., Ho, D.D., Perelson, A.S.: Modeling plasma virus concentration during primary HIV infection. *J. theor. Biol.* **203**(3), 285–301 (2000).

doi:[10.1006/jtbi.2000.1076](https://doi.org/10.1006/jtbi.2000.1076)

1  
2  
3 52. Perelson, A.S.: Modelling viral and immune system dynamics. *Nat. Rev. Immunol.* **2**(1),  
4 28–36 (2002). doi:[10.1038/nri700](https://doi.org/10.1038/nri700)

5  
6  
7  
8  
9 53. Murall, C.L., Jackson, R., Zehbe, I., Boulle, N., Segondy, M., Alizon, S.: Epithelial  
10 stratification shapes infection dynamics. *PLoS Comput Biol* **15**(1), 1006646 (2019).  
11  
12  
13 doi:[10.1371/journal.pcbi.1006646](https://doi.org/10.1371/journal.pcbi.1006646)

14  
15  
16  
17 54. Steimer, J.L., Vozech, S., Racine Poon, A., Holford, N., O'Neil, R.: The population  
18 approach: rationale, methods and applications in clinical pharmacology and drug development.  
19 In: Balant, P.G.W..L. (ed.) *Handbook of Experimental Pharmacology*, vol. 110, pp. 405–451.  
20  
21  
22  
23 Springer, Berlin (1994)

24  
25  
26  
27 55. Bates, D., Mächler, M., Bolker, B., Walker, S.: Fitting linear mixed-effects models using  
28 lme4. *Journal of Statistical Software* **67**(1) (2015). doi:[10.18637/jss.v067.i01](https://doi.org/10.18637/jss.v067.i01)

29  
30  
31  
32  
33 56. Bucci, V., Tzen, B., Li, N., Simmons, M., Tanoue, T., Bogart, E., Deng, L., Yeliseyev, V.,  
34 Delaney, M.L., Liu, Q., Olle, B., Stein, R.R., Honda, K., Bry, L., Gerber, G.K.: MDSINE:  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65  
66  
67  
68  
69  
70  
71  
72  
73  
74  
75  
76  
77  
78  
79  
80  
81  
82  
83  
84  
85  
86  
87  
88  
89  
90  
91  
92  
93  
94  
95  
96  
97  
98  
99  
100  
101  
102  
103  
104  
105  
106  
107  
108  
109  
110  
111  
112  
113  
114  
115  
116  
117  
118  
119  
120  
121  
122  
123  
124  
125  
126  
127  
128  
129  
130  
131  
132  
133  
134  
135  
136  
137  
138  
139  
140  
141  
142  
143  
144  
145  
146  
147  
148  
149  
150  
151  
152  
153  
154  
155  
156  
157  
158  
159  
160  
161  
162  
163  
164  
165  
166  
167  
168  
169  
170  
171  
172  
173  
174  
175  
176  
177  
178  
179  
180  
181  
182  
183  
184  
185  
186  
187  
188  
189  
190  
191  
192  
193  
194  
195  
196  
197  
198  
199  
200  
201  
202  
203  
204  
205  
206  
207  
208  
209  
210  
211  
212  
213  
214  
215  
216  
217  
218  
219  
220  
221  
222  
223  
224  
225  
226  
227  
228  
229  
230  
231  
232  
233  
234  
235  
236  
237  
238  
239  
240  
241  
242  
243  
244  
245  
246  
247  
248  
249  
250  
251  
252  
253  
254  
255  
256  
257  
258  
259  
260  
261  
262  
263  
264  
265  
266  
267  
268  
269  
270  
271  
272  
273  
274  
275  
276  
277  
278  
279  
280  
281  
282  
283  
284  
285  
286  
287  
288  
289  
290  
291  
292  
293  
294  
295  
296  
297  
298  
299  
300  
301  
302  
303  
304  
305  
306  
307  
308  
309  
310  
311  
312  
313  
314  
315  
316  
317  
318  
319  
320  
321  
322  
323  
324  
325  
326  
327  
328  
329  
330  
331  
332  
333  
334  
335  
336  
337  
338  
339  
340  
341  
342  
343  
344  
345  
346  
347  
348  
349  
350  
351  
352  
353  
354  
355  
356  
357  
358  
359  
360  
361  
362  
363  
364  
365  
366  
367  
368  
369  
370  
371  
372  
373  
374  
375  
376  
377  
378  
379  
380  
381  
382  
383  
384  
385  
386  
387  
388  
389  
390  
391  
392  
393  
394  
395  
396  
397  
398  
399  
400  
401  
402  
403  
404  
405  
406  
407  
408  
409  
410  
411  
412  
413  
414  
415  
416  
417  
418  
419  
420  
421  
422  
423  
424  
425  
426  
427  
428  
429  
430  
431  
432  
433  
434  
435  
436  
437  
438  
439  
440  
441  
442  
443  
444  
445  
446  
447  
448  
449  
450  
451  
452  
453  
454  
455  
456  
457  
458  
459  
460  
461  
462  
463  
464  
465  
466  
467  
468  
469  
470  
471  
472  
473  
474  
475  
476  
477  
478  
479  
480  
481  
482  
483  
484  
485  
486  
487  
488  
489  
490  
491  
492  
493  
494  
495  
496  
497  
498  
499  
500  
501  
502  
503  
504  
505  
506  
507  
508  
509  
510  
511  
512  
513  
514  
515  
516  
517  
518  
519  
520  
521  
522  
523  
524  
525  
526  
527  
528  
529  
530  
531  
532  
533  
534  
535  
536  
537  
538  
539  
540  
541  
542  
543  
544  
545  
546  
547  
548  
549  
550  
551  
552  
553  
554  
555  
556  
557  
558  
559  
560  
561  
562  
563  
564  
565  
566  
567  
568  
569  
570  
571  
572  
573  
574  
575  
576  
577  
578  
579  
580  
581  
582  
583  
584  
585  
586  
587  
588  
589  
590  
591  
592  
593  
594  
595  
596  
597  
598  
599  
600  
601  
602  
603  
604  
605  
606  
607  
608  
609  
610  
611  
612  
613  
614  
615  
616  
617  
618  
619  
620  
621  
622  
623  
624  
625  
626  
627  
628  
629  
630  
631  
632  
633  
634  
635  
636  
637  
638  
639  
640  
641  
642  
643  
644  
645  
646  
647  
648  
649  
650  
651  
652  
653  
654  
655  
656  
657  
658  
659  
660  
661  
662  
663  
664  
665  
666  
667  
668  
669  
670  
671  
672  
673  
674  
675  
676  
677  
678  
679  
680  
681  
682  
683  
684  
685  
686  
687  
688  
689  
690  
691  
692  
693  
694  
695  
696  
697  
698  
699  
700  
701  
702  
703  
704  
705  
706  
707  
708  
709  
710  
711  
712  
713  
714  
715  
716  
717  
718  
719  
720  
721  
722  
723  
724  
725  
726  
727  
728  
729  
730  
731  
732  
733  
734  
735  
736  
737  
738  
739  
740  
741  
742  
743  
744  
745  
746  
747  
748  
749  
750  
751  
752  
753  
754  
755  
756  
757  
758  
759  
760  
761  
762  
763  
764  
765  
766  
767  
768  
769  
770  
771  
772  
773  
774  
775  
776  
777  
778  
779  
780  
781  
782  
783  
784  
785  
786  
787  
788  
789  
790  
791  
792  
793  
794  
795  
796  
797  
798  
799  
800  
801  
802  
803  
804  
805  
806  
807  
808  
809  
810  
811  
812  
813  
814  
815  
816  
817  
818  
819  
820  
821  
822  
823  
824  
825  
826  
827  
828  
829  
830  
831  
832  
833  
834  
835  
836  
837  
838  
839  
840  
841  
842  
843  
844  
845  
846  
847  
848  
849  
850  
851  
852  
853  
854  
855  
856  
857  
858  
859  
860  
861  
862  
863  
864  
865  
866  
867  
868  
869  
870  
871  
872  
873  
874  
875  
876  
877  
878  
879  
880  
881  
882  
883  
884  
885  
886  
887  
888  
889  
890  
891  
892  
893  
894  
895  
896  
897  
898  
899  
900  
901  
902  
903  
904  
905  
906  
907  
908  
909  
910  
911  
912  
913  
914  
915  
916  
917  
918  
919  
920  
921  
922  
923  
924  
925  
926  
927  
928  
929  
930  
931  
932  
933  
934  
935  
936  
937  
938  
939  
940  
941  
942  
943  
944  
945  
946  
947  
948  
949  
950  
951  
952  
953  
954  
955  
956  
957  
958  
959  
960  
961  
962  
963  
964  
965  
966  
967  
968  
969  
970  
971  
972  
973  
974  
975  
976  
977  
978  
979  
980  
981  
982  
983  
984  
985  
986  
987  
988  
989  
990  
991  
992  
993  
994  
995  
996  
997  
998  
999  
1000

58. Fox, G.A., Negrete-Yankelevich, S., Sosa, V.J.: *Ecological Statistics: Contemporary Theory and Application*. Oxford University Press, Oxford, USA (2015)

59. Shi, Y., Li, L., Hu, Z., Li, S., Wang, S., Liu, J., Wu, C., He, L., Zhou, J., Li, Z., Hu, T., Chen, Y., Jia, Y., Wang, S., Wu, L., Cheng, X., Yang, Z., Yang, R., Li, X., Huang, K., Zhang, Q., Zhou, H., Tang, F., Chen, Z., Shen, J., Jiang, J., Ding, H., Xing, H., Zhang, S., Qu, P., Song, X., Lin,



1 Z., Deng, D., Xi, L., Lv, W., Han, X., Tao, G., Yan, L., Han, Z., Li, Z., Miao, X., Pan, S., Shen, Y.,  
2 Wang, H., Liu, D., Gong, E., Li, Z., Zhou, L., Luan, X., Wang, C., Song, Q., Wu, S., Xu, H.,  
3 Shen, J., Qiang, F., Ma, G., Liu, L., Chen, X., Liu, J., Wu, J., Shen, Y., Wen, Y., Chu, M., Yu, J.,  
4 Hu, X., Fan, Y., He, H., Jiang, Y., Lei, Z., Liu, C., Chen, J., Zhang, Y., Yi, C., Chen, S., Li, W.,  
5 Wang, D., Wang, Z., Di, W., Shen, K., Lin, D., Shen, H., Feng, Y., Xie, X., Ma, D.: A genome-  
6 wide association study identifies two new cervical cancer susceptibility loci at 4q12 and 17q12.  
7 Nat Genet **45**(8), 918–22 (2013). doi:[10.1038/ng.2687](https://doi.org/10.1038/ng.2687)  
8  
9

10  
11  
12  
13  
14  
15  
16  
17 60. Chen, D., Gaborieau, V., Zhao, Y., Chabrier, A., Wang, H., Waterboer, T., Zaridze, D.,  
18 Lissowska, J., Rudnai, P., Fabianova, E., Bencko, V., Janout, V., Foretova, L., Mates, I.N.,  
19 Szeszenia-Dabrowska, N., Boffetta, P., Pawlita, M., Lathrop, M., Gyllensten, U., Brennan, P.,  
20 McKay, J.D.: A systematic investigation of the contribution of genetic variation within the MHC  
21 region to HPV seropositivity. Hum Mol Genet **24**(9), 2681–2688 (2015).  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

60. Chen, D., Gyllensten, U.: Lessons and implications from association studies and post-  
GWAS analyses of cervical cancer. Trends Genet **31**(1), 41–54 (2015).  
doi:[10.1016/j.tig.2014.10.005](https://doi.org/10.1016/j.tig.2014.10.005)

62. Fellay, J., Shianna, K.V., Ge, D., Colombo, S., Ledergerber, B., Weale, M., Zhang, K.,  
Gumbs, C., Castagna, A., Cossarizza, A., Cozzi-Lepri, A., De Luca, A., Easterbrook, P.,  
Francioli, P., Mallal, S., Martinez-Picado, J., Miro, J.M., Obel, N., Smith, J.P., Wyniger, J.,  
Descombes, P., Antonarakis, S.E., Letvin, N.L., McMichael, A.J., Haynes, B.F., Telenti, A.,  
Goldstein, D.B.: A whole-genome association study of major determinants for host control of  
HIV-1. Science **317**(5840), 944–947 (2007). doi:[10.1126/science.1143767](https://doi.org/10.1126/science.1143767)

63. Huber, P.J.: The 1972 Wald Lecture Robust Statistics: A Review. Ann Math Stat **43**(4),  
1041–1067 (1972). doi:[10.1214/aoms/1177692459](https://doi.org/10.1214/aoms/1177692459)

64. Winer, R.L., Feng, Q., Hughes, J.P., O'Reilly, S., Kiviat, N.B., Koutsky, L.A.: Risk of  
female human papillomavirus acquisition associated with first male sex partner. J Infect Dis

1  
2  
3 **197**(2), 279–82 (2008). doi:[10.1086/524875](https://doi.org/10.1086/524875)  
4

5 65. Herrero, R.: Human Papillomavirus (HPV) Vaccines: Limited Cross-Protection against  
6 Additional HPV Types. *J Infect Dis* **199**(7), 919–922 (2009). doi:[10.1086/597308](https://doi.org/10.1086/597308)  
7

8 66. Depuydt, C.E., Verstraete, L., Berth, M., Beert, J., Bogers, J.-P., Salembier, G.,  
9 Vereecken, A.J., Bosmans, E.: Human papillomavirus positivity in women undergoing  
10 intrauterine insemination has a negative effect on pregnancy rates. *Gynecol Obstet Invest*  
11 **81**(1), 41–6 (2016). doi:[10.1159/000434749](https://doi.org/10.1159/000434749)  
12  
13  
14  
15  
16  
17

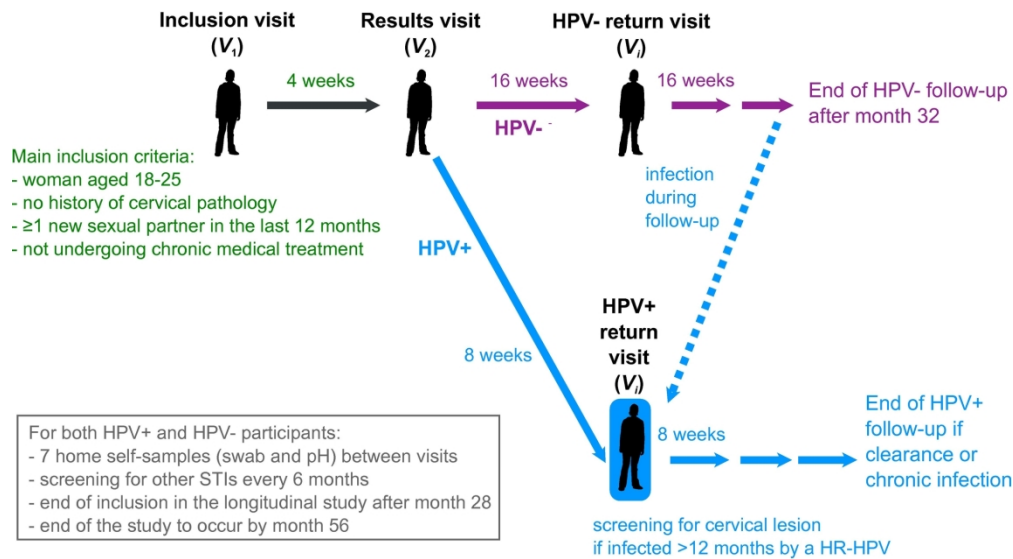
18 67. WHO HPV LabNet. World Health Organization.  
19

20 [http://www.who.int/biologicals/areas/human\\_papillomavirus/WHO\\_HP\\_V\\_LabNet/en/](http://www.who.int/biologicals/areas/human_papillomavirus/WHO_HP_V_LabNet/en/)  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## Figure captions

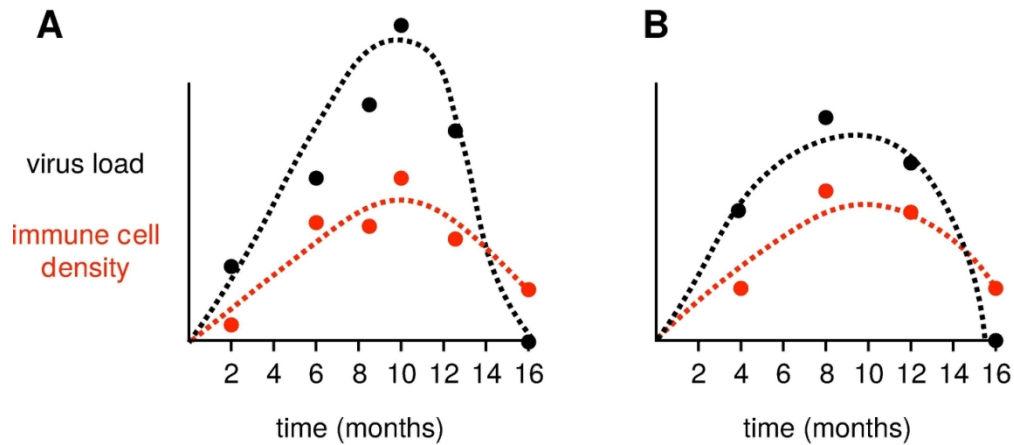
1  
2  
3 **Figure 1: General structure of the PAPCLEAR study.** For the longitudinal study, participants  
4 have an inclusion visit ( $V_1$ ), a results visit ( $V_2$ ) and then return visits ( $V_i$  with  $i > 2$ ). For the cross-  
5  
6 sectional study, participants only have  $V_1$  and  $V_2$ .  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21

22 **Figure 2: Fitting viral kinetics models to within-host times series.** Dashed lines indicate a  
23 model fitted using virus load (in black) or immune cells (in red) time series. In panel A, the  
24 follow-up is bi-monthly with 2 missing visits and several delayed visits, whereas in panel B the  
25 follow-up is every 4 months without any missing or delayed visits. In spite of missing data this,  
26 the situation shown in panel A is clearly the best for inferring parameter values and for fitting the  
27 underlying dynamics.  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



General structure of the PAPCLEAR study. For the longitudinal study, participants have an inclusion visit (V<sub>1</sub>), a results visit (V<sub>2</sub>) and then return visits (V<sub>i</sub> with  $i > 2$ ). For the cross-sectional study, participants only have V<sub>1</sub> and V<sub>2</sub>.

190x104mm (300 x 300 DPI)



Fitting kinetics dynamical models to within-host times series. Dashed lines indicate a model fitted using virus load (in black) or immune cells (in red) time series. In panel A, the follow-up is bi-monthly with 2 missing visits and several delayed visits, whereas in panel B the follow-up is every 4 months without any missing or delayed visits. In spite of missing data this, the situation shown in panel A is clearly the best for inferring parameter values and for fitting the underlying dynamics.

120x52mm (300 x 300 DPI)