Prospective longitudinal study on immunogenicity, induction of cellular immune responses and safety of vaccination against HPV with the 9valent vaccine in HIV-positive women

The Papillon study

French title:

Etude longitudinale et prospective sur l'immunogénicité, l'induction d'immunité cellulaire et la sécurité de la vaccination contre le HPV par le vaccin 9valent chez les femmes VIH-positives

Etude Papillon

Phase IV prospective study evaluating the reactogenicity and immunogenicity (both humoral and cellular responses) and the safety of the 9valent vaccine against HPV (Gardasil9®Merck) in HIV-positive women aged 15-40 years with fully suppressed HIV viremia on combined antiretroviral therapy. After a first open phase evaluating tolerability of Gardasil9, women will be randomized between two different doses schedules: in the first schedule, women will receive 2 doses at time 0 and 6 months and a third dose between 18-48 months if their antibody levels are insufficient; the second schedule will be 3 doses at 0, 2 and 6 months. Primary outcome is the non-inferiority of the rate of seroconversion against each HPV vaccine genotypes in women seronegative at baseline after either 2 or 3 doses of vaccination (month 7). Secondary outcomes are rate of seroconversion after 3 doses if they have received a third dose, completion of vaccine schedule, vaccine safety, antibody titles, and induction of cellular immunity against HPV contained in the vaccine, incidence of cervical HPV infection and incidence of abnormal cytology after vaccination.

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The Papillon study

Executive summary

Design

Phase IV prospective randomized study comparing the reactogenicity and the immunogenicity (neutralizing antibody titles against each HPV vaccine genotype) of the 9valent vaccine against HPV (Gardasil9®Merck) in HIV-positive women aged 15-40 years with fully suppressed HIV viremia on combined antiretroviral therapy receiving two different vaccination schedules: 0, 2 and 6 months or 0, 6 months and a third later booster if needed. The safety of the vaccination (local or systemic reaction and impact on HIV viral control and immunodeficiency level) will be assessed.
The cellular immune response will be assessed in a subgroup of patients.

Inclusion criteria

- HIV-positive woman
- Age 15-40 years
- Undetectable HIV viral load (VL) <400 cp/ml) for at least 6 months (i,e: having at least two separate VL<400 cp/ml at 6 months intervals; the most recent VL <400 cp/ml may be the screening CV for the study).
- No planned pregnancy foreseen for the next 7 first months and use of contraception such as condom, hormonal contraception or intrauterine device
- IC signed

Exclusion criteria

- Previous hysterectomy or conisation
- Previous or current biopsy-proven cervical, vulvar or vaginal HPV-associated lesion defined as ≥CIN2, VIN2, VaIN2 or invasive carcinoma
- Previous vaccination against HPV (at least one dose)
- Ongoing or planned pregnancy foreseen in the next 7 months
- Other immunodeficiency conditions such as ongoing or previous (within 6 months) chemotherapy against cancer or chronic systemic corticosteroids treatment or immunosuppressive therapy after transplantation
- Any condition contraindicating intramuscular injection such as warfarin therapy.

Number of patients

Study: 200 patients

First phase: open label: 3 doses of vaccine at 0, 2 and 6 months

Second phase (150 patients): randomized study comparing two arms:

- ARM A schedule of vaccination (75 patients): 0 and 6 months and a facultative later booster dose if antibodies level insufficient at month 7

- ARM B schedule of vaccination(75 patients): 0,2 and 6 months

Substudy on cellular immune responses in a subset of patients: 40 patients and separated informed consent

Amendment (February 2021) : Introduction of a third phase of the study

At the time of the start of the Papillon study (June 2018), there was no experience nor publication of vaccination with Gardasil9 in women living with HIV (WLHIV). We have then started our study by a first open label part including 45 patients to observe if tolerance and safety was equal to what was described in HIV-negative women. At the end of this open label part (from June 2018 to January 2019), our data on safety were reassuring and we proceeded to the randomized phase as described upper (from January 2019 to February 2021).

Because of the SARS-CoV2 pandemic, the enrollment had been slowed down and by February 2021, we had randomized 102 WLHIV instead of 150.

As we needed to end the study enrolment by September 2019 because of budget constraints, we have introduced an amendment to give two doses (as in ARM A) to all the patients enrolled from February 25th 2021.

We plan to analyze the results in intention-to-treat on the randomized cohort (n=102), and in On-treatment on the whole cohort (n=169).

Amendment (December 2023) :

In order to investigate the role of basal immune activation on the seroconversion and long-term persistence of HPV-specific antibody responses, we wish to extend the follow up to the following time points :

- **24 months**
- **36 months**
- **48 months**
- **60 months**

After completion of the vaccine administration (2 or 3 doses).

Women included will be contacted in order to collect a serum sample and will sign a new informed consent. If the time point is exceeded, we will retrieved plasma samples collected during routine care for HIV viral load determination (stored at the AIDS Reference Laboratory-LHUB ULB).

Primary outcome

Rate of seroconversion of neutralizing antibodies against each HPV vaccine genotypes namely 6/11/16/18/31/33/45/52/58 among women seronegative at baseline for HPV vaccine genotypes, by measuring neutralizing antibody against the 9 vaccine genotypes of HPV at baseline, month 7. Comparison of that rate at month 7 (non inferiority defined as at least 80% of seroconversion in ARM A).

Secondary outcomes

- Completion of vaccine schedule.
- Safety and clinical tolerance of the vaccine administration.
- Impact on T-lymphocytes CD4+ counts and HIV viremia at month 7.
- Rate of seroconversion of neutralizing antibodies against each HPV vaccine genotypes namely 6/11/16/18/31/33/45/52/58 among women seronegative at baseline for HPV vaccine genotypes, by measuring neutralizing antibody against the 9 vaccine genotypes of HPV at Month 18**, 24, 36, 48 and 60** compared to baseline.
- Measure of neutralizing antibodies title against each HPV vaccine types at baseline, month 7 and 18
- Assessment of long term persistence of HPV-specific antibody responses at 24,36,48 and 60 months after completion of the last vaccine administration dose.
- The cellular immune response against HPV 16/18/31/52/58 will be assessed in a subgroup of patients (n=40, 20 in each arm) on PBMC samples tested. For this analysis, a separated IC should be first signed by the patient.
- Incidence and prevalence of HPV infections and of abnormal cervical cytology performed by cytology and PCR on cervical swab at baseline and 12 months after the end of vaccination.

List of abbreviation

ACIP	Advisory Committee on immunization Practices cART
cART	combined antiretroviral therapy
CIN	Cervical Intraepithelial Neoplasia
HR	high risk
HRHPV	high risk HPV
HG	high grade
HGCIN	high grade cervical intraepithelial neoplasia
HGVAIN	high grade vaginal intraepithelial neoplasia
HGVIN	high grade vulvar intraepithelial neoplasia
HIV	human immunodeficiency virus
HPV	human papillomavirus
IC	informed consent
ICC	invasive cervical cancer
NCI	National Cancer Institute
PBMC	peripheral blood mononuclear cell
VaIN	Vaginal Intraepithelial neoplasia
VIN	Vulvar Intraepithelial neoplasia
VLP	Virus-like particle

Rationale

In women, persistent infection with high risk (HR) Human papillomavirus (HPV) is responsible for the development of high grade cervical, vulvar or vaginal intraepithelial neoplasia (HGCIN, HGVIN, HGVAIN) and invasive cervical cancer (ICC). Worldwide, HPV 16 and 18 represent respectively 50 and 20% of all HRHPV genotypes responsible for ICC [1]. Prophylactic vaccination against HPV 16 and 18 in adolescent and young women aged 16-26 years has been shown to be highly efficient in preventing both infection with these genotypes of HRHPV and HPV-associated lesions such as HGCIN, HGVIN, HGVAIN due to vaccine types (prevention of >90%) [2-4]. Vaccination of women aged 26-45 years is also efficient with around 80% of prevention for both infections and lesions related to vaccine HPV types [5-6].

HIV-positive women are more frequently infected with persistent HRHPV and suffer significantly more from HPV-associated diseases such as ICC (6 to 10 times more frequent than in the general population) and precancerous lesions. Their dysplastic lesions are more severe and they have a worst outcome after treatment [7-8].

We have conducted a prospective longitudinal study on HRHPV infection and HPV-associated diseases in a cohort of almost 900 HIV-positive women living in Belgium. We found that HRHPV infection was highly prevalent (43%) with a high incidence of new infections (13/100 women-year) [9-10]; 28% had cervical intraepithelial abnormal cytology and 3% had HGCIN compared to respectively 6% and 1.2% in the general Belgian population. We also showed that the most prevalent HPV genotypes were HPV 52 (20%), HPV 18 (15%), HPV 31/35/51/58 (12% each) and HPV 16% (9.5%)[11]. This genotypic distribution is different from the general population and is attributable first to HIV-infection which is a known risk factor for more frequent infection with HPV other than 16 or 18 and second with the African origin of most women followed for HIV in Belgium (similar genotype distribution is found in women living in sub-Saharan African countries).

Immunogenicity of the prophylactic vaccines against HPV 16 and 18 has been evaluated in several studies including almost 700 HIV-positive female between 8 and 45 years [12-17]. Most studies were done with the quadrivalent vaccine against HPV 6/11/16/18 (Gardasil®Merck). These studies showed a high seroconversion rate >90% for all HPV vaccine types in particular in patients with high CD4 cell count or under combined antiretroviral therapy (cART). These studies showed also that the presence of antibodies (seropositivity) against all vaccine-types HPV before vaccination was present in only 4-5% of women (median age 21 and 36 years) which suggest that in most HIV-positive adult women, there could be a benefit to vaccinate and protect against the HPV genotypes not yet encountered [14, 16]. Moreover, in patients seropositive for one or several HPV vaccine genotype(s), vaccination was able to induce a significant increase in these antibody titles (by 1.5 $\log_{10}$ mMU/ml) [17].

The ideal timing for vaccination against HPV is during adolescence however most adult women followed for HIV today had no opportunity to receive these vaccines during adolescence. The ACIP (Advisory Committee on immunization Practices), the British HIV Association and the Belgian Superior Health Council are recommending to vaccinate adult HIV-positive women against HPV [18, 19, 20]. This is partially based on the excellent results of the registration studies for the three different vaccines which demonstrated high efficacy in women up to 26 years. It is also based on later studies performed in HIV-negative women between 24 and 45 years old which demonstrate very good efficacy (between 88 and 91%) in the prevention of a combined endpoint (including persistent HRHPV infection, which is the preceding stage before precancerous lesions, external lesions such as genital warts and CIN1 due to HPV vaccinal types) [4-5].
In the USA, the ACIP recommends vaccinating HIV-positive patients against HPV as soon as possible starting from 11 and up to 26 and in the UK up to 40 years.

In Belgium, there are currently three available vaccines against HPV: the bivalent vaccine against HPV 16 and 18 (Cervarix®GSK), the quadrivalent vaccine against

HPV 6/11/16/18 (Gardasil®Merck) and the new ninevalent vaccine against HPV 6/11/16/18/31/33/45/52/58 (Gardasil9®Merck) available since June 2016. They are reimbursed in girls aged 12-18 years but are very expensive for women of 19 years or more: a course of 3 doses costs between 207 (Cervarix), 355 (Gardasil) and 404 euros (Gardasil9) (prices on October 19th 2017).

We have shown that the bi- or quadrivalent vaccines would cover at best 30% of our patients because they are infected with HPV genotypes other than 16 and 18; in comparison, vaccinating with the ninevalent vaccine would cover up to 82% of these women because it includes 5 additional HRHPV genotypes more frequently retrieved in our patients [11] . Other authors have shown similar results either in CIN3 from HIV-positive women or in AIN3 in HIV-positive man from the USA [21-22].

No data on the immunogenicity or safety of the ninevalent vaccine in HIV-positive patients are available so far. As the HRHPV genotypes included in this vaccine could better cover our patients than the bi- or quadrivalent vaccines, studying the immunogenicity and the safety of this vaccine in HIV-positive women is thus essential.

Regarding safety, an analysis of seven trials over 15,000 individuals who received at least one dose of the ninevalent vaccine, showed that the most common adverse effects were mild or moderate injection site reactions (pain, erythema, and swelling), occurring slightly more frequently than with the quadrivalent vaccine. The frequency of systemic adverse effects (eg, headache, fever, nausea, dizziness) was similar to the quadrivalent vaccine. Serious adverse effects occurred in <0.1 percent [23,24].

Our objective is to provide data on the immunogenicity and safety of the ninevalent vaccine which has not yet been reported for HIV-positive women although they are at greater risk of HRHPV infection and associated cancerous diseases. Our results could impact not only on the management of the 5700 women living with HIV in Belgium [25] but also on the healthcare of millions across the world who still suffer

from an unacceptable rate of HPV-related cancer and precancerous lesions. In sub-Saharan Africa, ICC is the first most frequent cancers in HIV-positive young women (30-40 years) with an incidence of up to 450/100.000 women-year compared to 30-50 in Africa and 9 /100.000 women-year in Belgium, both in the general population [26].

We also aim to provide data on cellular immune response against HRHPV in HIV-positive women. Several studies in HIV positive patients have shown that HPV vaccination was able to increase the specific cellular immune responses against HPV such as a raise in CD4+ and CD8+ lymphocytes against HPV 16 and 18 [13, 15]. This analysis comparing the evolution between cellular immune response targeted to specific HRHPV genotypes before and after vaccination will be performed in a subset of 40 patients and will focus on the vaccine genotypes most frequently encountered in our cohort such as 16/18/31/52 and 58.

By December 2018, many questions have aroused about how many doses of vaccine against HPV should be given to be efficient. The registration studies for Cervarix and Gardasil were performed in the early 2000' with a three doses schedule (0, 1 or 2 and 6 months) based on previous studies with similar protein-based vaccine such as hepatitis B vaccine. However, all the vaccines against HPV are designed as virus-like particle (VLP) with unique immunogenic properties. Immunobridging studies have confirmed that a twodoses regimen (0 and 6 months) was as reactogenic (giving an antibody response in >95% of the vaccinated subjects that were seronegative at baseline) and as immunogenic (inducing an antibody response of the same magnitude) as a three-dose schedule in teenagers less than 15 years –old [27]. More recently several randomized-controlled studies have shown that one- versus two- versus three-doses schedule were equivalent in terms of clinical endpoint. The Costa Rica Vaccine Trial (CVT) and the Papilloma TRIal against Cancer In young Adults (PATRICIA Trial), both studies using bivalent vaccine, showed similar vaccine efficacy over four years among women who received one, two and three doses of the HPV16/18 vaccine, and stable antibody

responses have been observed throughout the seven years of follow-up accrued to date in CVT, suggesting durability of responses [28]. Additionally, 36-month preliminary analysis of a large, post-licensure trial of the quadrivalent vaccine in India showed similar protection against HPV16/18 cervical infection whether the women received one dose, two doses, or three doses [29]. However, vaccine recipients in these trials were not randomized to receive these fewer doses, and immunogenicity among one-dose recipients was lower than that observed following two- or three-doses, leading regulatory bodies to conclude that the level of evidence in support of single-dose HPV vaccination is insufficient to warrant changes in current recommendations for two- or three- dose schedules. Consequently, the American National Cancer Institute (NCI) has recently launched a large randomized study comparing the clinical efficacy of Cervarix in one or two doses to Gardasil9 in one or two doses.

Indeed, the possibility of giving less than three doses is important in terms of compliance to the full vaccination scheme, tolerability of the vaccine and last but not least in terms of costs which are important with these vaccines. In Belgium, these vaccines are not reimbursed after 18 years-old even in more fragile population such as HIV-positive persons and total cost for a three-dose vaccine is more than 400 Euros.

Up to now, there is no data for less than three doses in HIV-positive women or HIV-positive adolescents and all the recommendations from the European AIDS clinical society, or British HIV Association or American guidelines on HIV and on vaccination recommend a three doses schedules for HIV adolescent or adults.

In order to answer the question of whether a two-doses schedule is not inferior to a three-doses schedule in HIV-positive women, we propose to randomly evaluate the reactogenicity and the immunogenicity of 2 different vaccination schedules given according to randomization in HIV virally-suppressed women under cART: Arm A would receive vaccination with 9valent vaccine at time 0 and at month 6 and a later facultative booster if antibodies measurement at month 7 are insufficient. Arm B

would receive 9valent vaccine at 0, 2 and 6 months. We will measure the antibody responses in both arms at baseline and month 7 (one month after the second or the third dose) to determine if a good antibody response can be reached already after 2 doses or needs a third dose in ARM A.

Our study has started as a phase IV open study enrolling patients in March 2018 and vaccinating the first patient in June 2018: up to now, 42 women have received the first dose of the three-doses schedule (0,2 and 6) and 25 have also received the second dose at month 2. The intermediate evaluation shows that acceptability and tolerability are excellent. We did not encounter adverse event of moderate or severe grade.
We propose to continue the classical 3 doses schedule (0, 2 and 6 months) in the patients that have been already enrolled and to randomized all the new patients between ARM A and ARM B in order to continue our study as a randomized non inferiority trial including 150 patients (75 per arm).

Design of the study

The study started as an open study to evaluate the reactogenicity, the immunogenicity and the cellular immune response against each HPV vaccine genotypes (6/11/16/18/31/33/45/52/58) at month 7 which will be one month after completion of a three doses schedule (0, 2 and 6 months) in women seronegative at baseline for these antibodies.

After the December 2018 amendment, the study will enter a second phase as a non-inferiority study with randomization between two arms:

- **ARM A: vaccination at time 0 and 6 month and a later facultative booster if needed according to month 7 antibodies measurement**

- **ARM B: vaccination at time 0, 2 and 6 months**
- **Amendment: Introduction of a third phase of the study**
- **At the time of the start of the Papillon study (June 2018), there was no experience nor publication of vaccination with Gardasil9 in women living**

with HIV (WLHIV). We have then started our study by a first open label part including 45 patients to observe if tolerance and safety was equal to what was described in HIV-negative women. At the end of this open label part (from June 2018 to January 2019), our data on safety were reassuring and we proceeded to the randomized phase as described upper (from January 2019 to February 2021).

- **Because of the SARS-CoV2 pandemic, the enrollment had been slowed down and by February 2021, we had randomized 102 WLHIV instead of 150.**
- **As we needed to end the study enrolment by September 2019 because of budget constraints, we have introduced an amendment to give two doses (as in ARM A) to all the patients enrolled from February 25th 2021.**
- **We plan to analyze the results in intention-to-treat on the randomized cohort (n=102), and in On-treatment on the whole cohort (n=169).**

Antibodies title peaks one month after last vaccine dose than decrease to a plateau and stabilize around month 12 after receiving the last dose. In this study, antibodies against HPV will be measured at baseline, month 7 (that is to say 1 month after receiving 2 or 3 doses depending in which arm the patient has been randomized to) and month 18 (or 12 months after receiving the second or third dose, reflecting the stabilized antibodies level. We expect that two doses of vaccine in HIV-positive patients with well controlled HIV viremia under cART for at least 6 months could induce seroconversion in at least 80% of the patients seronegative at baseline whereas the seroconversion rate is expected to be 95% after 3 doses according to previous studies using the quadrivalent vaccine in HIV-positive patients. We find a minimum of 80% efficacy in the two doses arm acceptable as in this arm the vaccine costs would decrease by 33%, administration will be easier, the risk of local reaction after injection could also be decreased and compliance with the whole vaccine administration could be improved.

Regarding the number of patients that should be enrolled in the study, we considered a sample size calculation based on the SAS 9.4 program for comparing two independent proportions (1:1 allocation in a non-inferiority trial) by a Pearson Chi-square test. If we consider 95% rate of seroconversion for ARM B (3 doses vaccines) and 80% for ARM A (2 doses vaccines) with a significance level test of 0.05 (alpha) and an 80% statistical power (Beta), we found that 60 patients per

arm would be needed (120 patients in total). We would then need in total 150 women to be randomized taking into account the possibility of follow-up lost.

The purpose of vaccinating women is to induce antibody response against each HPV genotype contained in the vaccine and in particular against the 7 high-risk genotypes that induces cancer. If, after 2 doses of vaccine (ARM A), the month 7 antibodies measure shows absence of seroconversion against one of the HPV genotypes contained in the vaccine, the participant will receive a third booster dose of Gardasil9.

In order to assess long term persistence of HPV-specific antibody responses, the follow up will be prolonged to the following time points after completion of the vaccine administration : 24, 36, 48 and 60 months.
The role of basal immune activation on seroconversion rate and long terme persistence of antibody responses, will be assessed.

Primary outcome

The rate of seroconversion of specific neutralizing antibodies against each HPV vaccine genotypes (6/11/16/18/31/33/45/52/58) at month 7 (which will be one month after either two doses of vaccine in ARM A or completion of a three doses schedule (0, 2 and 6 months) in ARM B) in women seronegative at baseline for these antibodies. The measure will be performed on a 10 ml tube by cLIA technique in Merck laboratory (see material and methods) [30,31]. Non inferiority of ARM A compared to ARM B will be reached if for each HPV genotype contained in the vaccine, at least 80% of the patients have a seroconversion rate.**We will analyze the results in intention-to-treat on the randomized cohort (n=102), and in On-treatment on the whole cohort (n=169).**

Secondary outcomes

1/Safety and tolerability of the vaccines will be evaluated by a specific questionnaire on a phone call made by the research team to the participant and scheduled at least

48 hours and maximum 7 days after each vaccine dose; the questionnaire will evaluate whether there is any complain regarding local reaction (pain, redness, swelling, pruritus), systemic reaction (fever, malaise and fatigue) or other side effect. In case of any usual complain > mild stage, or the presence of an unsual complain, the patient will be assessed by a visit and physical examination performed by the research team. The questionnaire has been elaborated according to the published data on safety evaluation of the ninevalent vaccine [24].

2/The potential impact of vaccine administration on T-lymphocyte CD4+ cell count and HIV viremia will be assessed by measuring CD4 cell count and HIV viremia at baseline (any measure within 6 months before screening can be taken into account) and month 7. Any detectable HIVRNA >1000 cp/ml will be reassessed on a second samples taken 2-4 weeks later. Any significant decrease in T-lymphocyte CD4+ cell count (defined as a decrease by more of 5% in the percentage or >100 cells/µl) will be reassessed on a second sample taken 2-4 weeks later.

3/ measure of the title of specific neutralizing antibodies against each HPV vaccine genotypes (6/11/16/18/31/33/45/52/58), at baseline, month 7 (i.e., 1 month after 2 or 3 doses) by the cLIA technique and at month 18 (12 months after vaccination completion in 200 HIV-positive women by the IgG LIA technique [30].

4/The cellular immune response will be evaluated in a subset of 40 patients, 20 per arm, (aged 18-40 years old) by measuring specific CD4+T cells expressing CD40, IL2, IFN-g or TNF-alpha against HPV 16/18/31/52 and 58. The analysis will be performed on a PMBC sample of 30 to 50 ml taken at baseline and at month 7 (see Procedures for Collection and Handling of Study Specimens section); a separate IC has to be signed for this sub-analysis.

4/ The incidence and prevalence rates of HPV infections and of abnormal cervical cytology performed by PCR and cytology will be done on cervical swab taken by the gynecologist at baseline and month 18. Cervical cytology and HPV infection will be assessed by the national reference centre for HPV (AML, Antwerpen) [32]. The

baseline gynecological sample might have been taken up to 6 months before the vaccination. These swabs will be sampled in all participants with previous vaginal sexually activity. In case of no previous vaginal sexual intercourse, the samples will not be taken.

5/Completion of the vaccine schedule
Comparison of the proportion of women achieving full course of vaccine administration in each arm, namely for ARM A receiving 2 doses and for ARM B receiving 3 doses.

6/ Proportion of patients needing a booster dose (i.e..a third dose) in ARM A
If after 2 doses of vaccine (ARM A), the month 7 antibodies analysis shows absence of seroconversion against one of the HPV genotypes contained in the vaccine, the participant will receive a third booster dose.
7/ Impact of basal immune activation (levels of CD25, sCD14, IP-10, IL-6, TNF-α, d-Dimers, CRP) on the seroconversion rate and long term antibody persistance

Randomisation

We will generated a randomization schedule with the classical method of block randomization. In practice, fort this study with two arms, 75 patients each, we will decide a number of blocks of 25, with block size of 6. We will use SAS programming and SAS PROC PLAN for the generating randomization schedules (version 9.4 SAS institute, Cary North Carolina, USA).

Amendment after Coronavirus crisis

1.Due to the COVID-19 health crisis in Belgium including a lockdown between March 15th 2020 and June 2020, some patients could not attend their scheduled appointments for this study. Moreover, the routine gynecological consultation was closed during the two-months lockdown. Now that the routine gynecological has

reopened, it is busier than usual as patients that have missed their appointments need to be seen. The initial study protocol stated that the first vaccination dose should be performed within 6 months after the baseline cervical sample. In order not to repeat the baseline cervical sample in an already full consultation, we propose to extend this time lapse to 12 months.

In the same way, we propose to extend the last serum and cervical samples delay to 18 months after the last vaccine dose (initially to be performed 12 months after last vaccination dose) so the 18-months visit can be postponed up to 24 months after first vaccination dose.

2. Introduction of a third phase of the study

At the time of the start of the Papillon study (June 2018), there was no experience nor publication of vaccination with Gardasil9 in women living with HIV (WLHIV). We have then started our study by a first open label part including 45 patients to observe if tolerance and safety was equal to what was described in HIV-negative women. At the end of this open label part (from June 2018 to January 2019), our data on safety were reassuring and we proceeded to the randomized phase as described upper (from January 2019 to February 2021).

Because of the SARS-CoV2 pandemic, the enrollment had been slowed down and by February 2021, we had randomized 102 WLHIV instead of 150.

As we needed to end the study enrolment by September 2019 because of budget constraints, we have introduced an amendment to give two doses (as in ARM A) to all the patients enrolled from February 25th 2021.

We plan to analyze the results in intention-to-treat on the randomized cohort (n=102), and in On-treatment on the whole cohort (n=169).

3. Extension of the study follow-up

In order to assess long term persistence of antibody response, we will collect serum samples at different time points after completion of the vaccine schedule (24, 36, 48 and 60 months.). If the time point is exceeded, plasma samples collected during

routine care will be retrieved for the AIDS reference laboratory (VUB-LHUB ULB). Signature of a new informed consent will be required before collecting stored or new samples.

Inclusion criteria

- HIV-positive woman
- Age 15-40 years
- Undetectable HIV viral load (HIVRNA <400 cp/ml) for at least 6 months (i,e: having at least two separate VL<400 cp/ml at 6 months intervals; the most recent VL<400 cp/ml may be the screening CV for the study).
- No planned pregnancy foreseen for the next 7 months and use of contraception such as condom, hormonal contraception or intrauterine device
- IC signed

Exclusion criteria

- Previous hysterectomy or conisation
- Previous or current biopsy-proven cervical, vulvar or vaginal HPV-associated lesion defined as ≥CIN2, VIN2, VaIN2 or invasive carcinoma
- Previous vaccination against HPV (at least one dose)
- Ongoing or planned pregnancy foreseen in the next 7 months
- Other immunodeficiency conditions such as ongoing or previous (within 6 months) chemotherapy against cancer or chronic systemic corticosteroids treatment or immunosuppressive therapy after transplantation
- Any condition contraindicating intramuscular injection such as warfarin therapy.

Site of enrolment and study

AIDS reference center in Saint-Pierre University hospital

Ethical committee

Saint-Pierre University hospital

Informed consent

All patients have to read and sign the inform(s) consent(s) before entering the study.

For participants aged 18 years or more, there is one IC to be red and signed before entering the main study.
For participants aged less than 18 years old, there are two separated forms: one for the participant and one for her parents or legal representatives; these two forms have to be signed before entering the study.

For persons participating to the sub-study on cellular immune response to the vaccine (they will be aged 18-40), there will be another separated IC to be signed.

Flowchart

To be performed at baseline:

- Informed consent(s) signature
- Measure of serum antibodies against HPV 6/11/16/18/31/33/45/52/58 (1 10 ml tube) by 9cLIA by Merck USA.
- Measure of lymphocytes T CD4+ level and HIV viremia if there is no previous sample up to 6 months before baseline.
- Cervical smear on a liquid based samples (Cytoprep) taken by the gynecologist for cytology and HPV detection (to be performed by the national reference centre for HPV, AML, Antwerpen) if there is no previous sample up to 6-12 months before baseline. These samples will not be taken if the participant has never experienced vaginal sexual intercourse.
- ONLY FOR THE CELLULAR IMMUNE RESPONSE SUBSTUDY: an additional specific IC should be signed and a sample of 50-100 ml should be taken.
- Randomisation (after December 2018 amendment): patients will be allocated to one of the two arms by random randomization through a software program. The result of the allocation will be known to the patient, to his healthcare providers and to the study team.
    - ARM A: vaccination at time 0 and 6 and a later booster if needed
    - ARM B: vaccination at time 0, 2 and 6 months
    - **Vaccine administration after randomisation**

Gardasil9 (Merck®) first dose will be administrated intramuscularly in the deltoid while the patient is lying down. The patient will remain lying 15 minutes after the injection.

First safety assessment after vaccination
The patient will be contacted by phone by the research team scheduled at least 48 hours and maximum 7 days after vaccine dose to evaluate the safety and tolerance of the vaccine with a questionnaire on local reaction (size, redness, pain and swelling), systemic reaction (fever, malaise and fatigue) or any other side effect. In case of any usual complain > grade mild, or the presence of an unusual complain, the patient will be assessed by a visit and physical examination performed by the research team.

Month 2 (only for patients in ARM B)
Gardasil9 second dose will be administrated intramuscularly in the deltoid while the patient is lying down. The patient will remain lying 15 minutes after the injection.

Second safety assessment after vaccination
The patient will be contacted by phone by the research team scheduled at least 48 hours and maximum 7 days after vaccine dose to evaluate the safety and tolerance of the vaccine with a questionnaire on local reaction (size, redness, pain and swelling), systemic reaction (fever, malaise and fatigue) or any other side effect. In case of any usual complain > grade mild, or the presence of an unusual complain, the patient will be assessed by a visit and physical examination performed by the research team.

Month 6
Gardasil9 second (ARM A) or third (ARM B) dose will be administered intramuscularly in the deltoid while the patient is lying down. The patient will remain lying 15 minutes after the injection.

Second (ARM A) or third (ARM B) safety assessment after vaccination
The patient will be contacted by phone by the research team scheduled at least 48 hours and maximum 7 days after vaccine dose to evaluate the safety and tolerance of the vaccine with a questionnaire on local reaction (size, redness, pain and swelling), systemic reaction (fever, malaise and fatigue) or any other side effect. In case of any usual complain > mild, or the presence of an unusual complain, the patient will be assessed by a visit and physical examination performed by the research team.

Month 7
Measure of lymphocytes T CD4+ level and HIV viremia.
Measure of serum antibodies against HPV 6/11/16/18/31/33/45/52/58 (1 10 ml tube)

ONLY FOR THE CELLULAR IMMUNE RESPONSE SUBSTUDY: a sample of 50-100 ml will be taken.

Month 18-24

- Measure of serum antibodies against HPV 6/11/16/18/31/33/45/52/58 (1 10 ml tube) by IgG LIA by Merck USA.

- Cervical smear on a liquid based samples (Cytoprep) taken by the gynecologist for cytology and HPV detection. These samples will not be taken if the participant has never experienced vaginal sexual intercourse.

Procedures for Collection and Handling of Study Specimens:

1/Measure of serum antibodies against HPV 6/11/16/18/31/33/45/52/58 (one 10 ml tube)

For each visit that requires a serum specimen for anti-HPV measurements, a 10 mL (nonheparinized, non-serum separator, red-top tube) blood specimen will be collected and will be separated to avoid hemolysis. A minimum of 3 mL of serum will be aliquoted to a vial. An additional 1.5 mL of serum (Retention Serum) will be aliquoted to a vial.
After the serum has been processed, the vials will be transfered to the freezer (-20°C or lower) without delay. All serum samples will be kept in the freezer until they are shipped on dry ice; the transport tube will be a 2ml cryovials.

Centrifuging will be done per protocol with sera frozen.

"Serum" vials will be stored at the site at -20°C (or lower) until shipped on dry ice to the lab. The freezer used to store the vials must be a non-frost-free freezer. All available serum will be used for conducting assays specified in the clinical protocol. Serum may also be used during the clinical trial, for further HPV immunologic testing in addition to tests specified in the protocol.

Retention Serum vials will remain stored at the site at -20°C (or lower). The freezer used to store the vials must be a non-frost-free freezer. The site will ship "Retention Serum" separately from the "Serum" sample.

The rest of the sample will be kept on site frozen as a backup sample.

Booster dose in ARM A

A third booster dose of Gardasil9 will be given in women from ARM A if they fail to develop antibodies against any of the HPV genotypes contained in the vaccine. As the analysis of the antibodies will be performed in MERCK laboratory in the USA in batches, the booster dose will be given as soon as the results of the antibodies measurement will be known which is predicted to be as soon as possible just after Month 7 of the last enrolled patient.

2/ Immune cellular response: PBMC procedure

Abbreviations and codes

- LAF – Laminar air flow
- RT – Room temperature
- PBMC – Peripheral Blood Mononuclear Cell
- EDTA – Ethylenediaminetetraacetic acid
- PBS – Phosphate buffered saline
- RPMI – Roswell Park Memorial Institute
- FCS – Fetal calf serum
- DMSO – Dimethyl sulfoxide

1 Material

Equipment

- Laminar air flow (LAF)
- Centrifuge for large tubes and small eppendorf tubes
- Pipette boy
- Pipettes: P20, P200, P1000
- Light microscope
- Refrigerator

Material

- Gloves
- Laboratory wipes
- 70% cleaning ethanol
- Pipettes: 5mL, 10mL, 25mL
- 15mL Falcon tubes
- 50mL Falcon tubes
- Cryovials
- Appropriate label for cryovial *(see SOP01 labelling)*
- 1,5mL eppendorf tubes
- 2x 2ml EDTA tubes *(only for visit one of each patient for genetic testing)*
- Lymphoprep
- Leucosep tubes
- CryoStor®complete cryopreservation medium
- PBS or RPMI 1640 (without **EDTA**)
- Trypan blue
- Counting chamber
- Cryostor (Freezing medium)
- Freezing container (precooled)

2 Method

- Switch on LAF and clean surface using 70% ethanol.
- *The first visit:* of each patient after inclusion (M0) demands a whole blood sample for genetic testing. Therefore fill two EDTA tubes of 2ml with 1ml blood, label and store at -80°C prior to continue with PBMC isolation.
- Add 15mL Lymphoprep solution on the polyethylene membrane of a 50mL leucosep tube.
- Centrifuge 2 min at 200 x g at RT.
- Add EDTA blood on the membrane of a Leucosep tubes (normally 3x9ml EDTA blood in one tube).
- Centrifuge for 20 min at 880 x g at RT (switch off breaks in the centrifuge for proper separation!).
- Aspirate the plasma (top layer) and transfer to a 15mL tube. The plasma should be divided in 5 fractions of 2 ml and the rest in fractions of 14 ml.
    - Prepare 5 aliquots of 2 mL plasma in 2mL tubes and aliquots of 14 ml in 15 ml tubes and store at -80°C.
    - Label with patient study number, study visit code and date of sampling *(see SOP01 labelling)*.
- Aspirate the white ring of PBMCs and transfer to a 50mL tube.
- Top up with RPMI or PBS (*without EDTA!*) until the 50mL mark to wash the sample.
- Centrifuge for 10 min at 880 x g at RT.
- Discard the supernatant (SN) without disturbing the pellet.
- Resuspend the pellet in 10 ml PBS or RPMI (without **EDTA**).
- Count PBMCs with a counting chamber.
- Continue with section below (preparation of cells in CryoStor®)

Preparation of cells in CryoStor®

- Take cell material and centrifuge for 10 min at 880 x g.
- Resuspend the pellet at 20 x 10^6 cells per ml in *CryoStor®* complete freezing medium
- Aliquot 4 x 500ul (= 10 x 10^6 cells) into cryotubes and top with 500 ul of *CryoStor®* (equals 10x10^6 cell aliquots). Distribute the rest of cells by adding 1 ml (= 20 x 10^6 cells) of the cell suspension per cryovial.

- Label cryovials with from LIH provided labels (*for details see labelling SOP 01*)
    1. Patient study number
    2. PhenoCure study visit
    3. Amount of cells 10 x 10^6 PBMCs or 20 x 10^6 PBMCs
    4. Date of sampling (dd/mm/yyyy)
- Make sure that the patient specific study number on the label correlates with the eCRF by making a note to the clinician!
- Put the vials in a pre-cooled (4°C) Mr. Frosty and immediately put the Mr. Frosty at -80°C.
- Leave the samples at -80°C overnight and transfer them to liquid nitrogen the next morning.

Samples in Cryostor® should **not** be kept at -80°C for longer than 24h!

References

1. Arbyn M, Tommasino M, Depuydt C , Dillner J. Are twenty human papillomavirus types causing cervical cancer? J Pathol. 2014 Aug 15. Bruni L, Diaz M, Castellsagué X, Ferrer E, Bosch X, de Sanjosé S. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. J Infect Dis. 2010 Dec 15;202(12):1789-99.
2. FUTURE II Study Group. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. N Engl J Med. 2007 May 10;356(19):1915-27.
3. Paavonen J, Naud P, Salmerón J, Wheeler CM, Chow SN, Apter D, Kitchener H, Castellsague X, Teixeira JC, Skinner SR, Hedrick J, Jaisamrarn U, Limson G, Garland S, Szarewski A, Romanowski B, Aoki FY, Schwarz TF, Poppe WA, Bosch FX, Jenkins D, Hardt K, Zahaf T, Descamps D, Struyf F, Lehtinen M, Dubin G; HPV PATRICIA Study Group. Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. Lancet. 2009 Jul 25;374(9686):301-14.
4. Castellsagué X, Muñoz N, Pitisuttithum P, Ferris D, Monsonego J, Ault K, Luna J, Myers E, Mallary S, Bautista OM, Bryan J, Vuocolo S, Haupt RM, Saah A. End-of-study safety, immunogenicity, and efficacy of quadrivalent HPV (types 6, 11, 16, 18) recombinant vaccine in adult women 24-45 years of age. Br J Cancer. 2011 Jun 28;105(1):28-37.
5. Wheeler C , Skinner S, Del Rosario-Raymundo M, et al. Efficacy, safety, and immunogenicity of the human papillomavirus 16/18 AS04-adjuvanted vaccine in women older than 25 years: 7-year follow-up of the phase 3, double-blind, randomised controlled VIVIANE study. Lancet Infect Dis. 2016 Oct;16(10):1154-68.

6. Castellsagué X, Muñoz N, Pitisuttithum P, Ferris D, Monsonego J, Ault K, Luna J, Myers E, Mallary S, Bautista OM, Bryan J, Vuocolo S, Haupt RM, Saah A. End-of-study safety, immunogenicity, and efficacy of quadrivalent HPV (types 6, 11, 16, 18) recombinant vaccine in adult women 24-45 years of age.Br J Cancer. 2011 Jun 28;105(1):28-37.
7. Gilles C, Manigart Y, Konopnicki D, Barlow P, Rozenberg S. Management and outcome of cervical intraepithelial neoplasia lesions: a study of matched cases according to HIV status. Gynecol Oncol 2005;96(1):112-8.
8. Konopnicki D, Manigart Y, Gilles C, Barlow P, de Marchin J, Feoli F et al. High-risk human papillomavirus infection in HIV-positive African women living in Europe. J Int AIDS Soc. 2013 Feb 14;16:18023.
9. Konopnicki D, Manigart Y, Gilles C, Barlow P, de Marchin J, Feoli F et al. Sustained viral suppression and higher CD4+ T-cell count reduces the risk of persistent cervical high-risk human papillomavirus infection in HIV-positive women. J Infect Dis. 2013 Jun 1;207(11):1723-9.
10. Konopnicki D, Manigart Y, Gilles C, Barlow P, Feoli F, Delforge M, Clumeck N and De Wit S. Long lasting viral suppression and immune reconstitution of great magnitude reduce the risk of cervical dysplasia in HIV-positive women. 15th European AIDS Clinical Conference, October 21-24th October 2015, Barcelona. Oral presentation PS5/4.
11. Konopnicki D, Manigart Y, Gilles C, Barlow P, De Marchin J, Feoli F, Delforge M, Clumeck N, De Wit S. High-risk human papillomavirus genotypes distribution in a cohort of HIV-positive women living in Europe: epidemiological implication for vaccination against human papillomavirus. AIDS. 2016 Jan 28;30(3):425-33.
12. Levin MJ, Moscicki AB, Song LY, Fenton T, Meyer WA 3rd, Read JS, Handelsman EL, Nowak B, Sattler CA, Saah A, Radley DR, Esser MT, Weinberg A; IMPAACT P1047 Protocol Team. Safety and immunogenicity of a quadrivalent human papillomavirus (types 6, 11, 16, and 18) vaccine in HIV-infected children 7 to 12 years old. J Acquir Immune Defic Syndr. 2010 Oct;55(2):197-204.
13. Denny L, Hendricks B, Gordon C, Thomas F, Hezareh M, Dobbelaere K, Durand C, Hervé C, Descamps D. Safety and immunogenicity of the HPV-16/18 AS04-adjuvanted vaccine in HIV-positive women in South Africa: a partially-blind randomised placebo-controlled study.Vaccine. 2013 Nov 19;31(48):5745-53.
14. Kahn JA, Xu J, Kapogiannis BG, Rudy B, Gonin R, Liu N, Wilson CM, Worrell C, Squires KE. Immunogenicity and safety of the human papillomavirus 6, 11, 16, 18 vaccine in HIV-infected young women. Clin Infect Dis. 2013 Sep;57(5):735-44.
15. Giacomet V, Penagini F, Trabattoni D, Viganò A, Rainone V, Bernazzani G, Bonardi CM, Clerici M, Bedogni G, Zuccotti GV. Safety and immunogenicity of a quadrivalent human papillomavirus vaccine in HIV-infected and HIV-negative adolescents and young adults.Vaccine. 2014 Sep 29;32(43):5657-61. /S1470-2045(15)00414-3. Epub 2015 Dec 2.
16. Toft L, Storgaard M, Müller M, Sehr P, Bonde J, Tolstrup M, Østergaard L, Søgaard OS. Comparison of the immunogenicity and reactogenicity of Cervarix and Gardasil human papillomavirus vaccines in

HIV-infected adults: a randomized, double-blind clinical trial. J Infect Dis. 2014 Apr 15;209(8):1165-73.

17. Kojic EM, Kang M, Cespedes MS, Umbleja T, Godfrey C, Allen RT, Firnhaber C, Grinsztejn B, Palefsky JM, Webster-Cyriaque JY, Saah A, Aberg JA, Cu-Uvin S. Immunogenicity and safety of the quadrivalent human papillomavirus vaccine in HIV-1-infected women. Clin Infect Dis. 2014 Jul 1;59(1):127-35.
18. Human Papillomavirus Vaccination: Recommendations of the Advisory Committee on Immunization Practices (ACIP) *MMWR*, August 29, 2014, Vo1 63, #RR05.
19. The British HIV Association guidelines on the use of vaccines in HIV-positive adults 2015. https://www.google.be/search?q=BIVHA+vaccination+guidelines&ie=utf-8&oe=utf-8&client=firefoxb&gfe_rd=cr&ei=mO1KV_S5Jo3FaJzQtYAJ#q=BHIVA+AND+vaccination+guidelines accessed on May 29th, 2016.
20. Vaccination contre le papillomavirus humain (HPV). Recommandations du Conseil Supérieur de la Santé. Fiche 9181. https://www.health.belgium.be/sites/default/files/uploads/fields/fpshealth_theme_file/9181_hpv_fr_27092017_0.pdf. accessed on October 18th 2017.
21. Massad L , Xianhong X, Burk R and al. Association of cervical precancer with human papillomavirus types other than 16 among HIV co-infecetd women. Am J Obstet gynecol. March 2016;214(3):354.
22. Sahasrabuddhe VV, Castle PE, Follansbee S, Borgonovo S, Tokugawa D, Schwartz LM et al. Human papillomavirus genotype attribution and estimation of preventable fraction of anal intraepithelial neoplasia cases among HIV-infected men who have sex with men. J Infect Dis. 2013 Feb 1;207(3):392-401.
23. Joura EA, Giuliano AR, Iversen OE, Bouchard C, Mao C, Mehlsen J et al.; Broad Spectrum HPV Vaccine Study. A 9-valent HPV vaccine against infection and intraepithelial neoplasia in women. N Engl J Med. 2015 Feb 19;372(8):711-23.
24. Moreira ED Jr, Block SL, Ferris D, Giuliano AR, Iversen OE, Joura EA, Kosalaraksa P, Schilling A, Van Damme P, Bornstein J, Bosch FX, Pils S, Cuzick J, Garland SM, Huh W, Kjaer SK, Qi H, Hyatt D, Martin J, Moeller E, Ritter M, Baudin M, Luxembourg A. Safety Profile of the 9-Valent HPV Vaccine: A Combined Analysis of 7 Phase III Clinical Trials. Pediatrics. 2016 Aug;138(2).
25. Epidémiologie du SIDA et de l'infection à VIH en Belgique. Situation au 31/12/2012. Rapport ISP. www.wiv-isp.be/Documents/WIV-ISP_Rapport_VIH-SIDA_2012.pdf.
26. Eliane Ronher and al. Global burden in cervical cancer in HIV-positive women on antiretroviral therapy. 23rd Conference on Retrovirus and Opportunistic Infection. February 22-25th 2016, Boston. Abstract 617.
27. Ogilvie G[1], Sauvageau C[2], Dionne M[2], McNeil S[3], Krajden M[1], Money D[1], Dobson S[1]. Immunogenicity of 2 vs 3 Doses of the Quadrivalent Human Papillomavirus Vaccine in Girls Aged 9 to 13 Years After 60 Months. JAMA. 2017 Apr 25;317(16):1687-1688.
28. Kreimer AR1, Struyf F2, Del Rosario-Raymundo MR3,and al. The Costa Rica Vaccine Trial Study Group Authors, González P, Jiménez S, Lowy DR, and al; HPV PATRICIA Principal Investigators/Co-

Principal Investigator Collaborators; GSK Vaccines Clinical Study Support Group.
Efficacy of fewer than three doses of an HPV-16/18 AS04-adjuvanted vaccine: combined analysis of data from the Costa Rica Vaccine and PATRICIA Trials. Lancet Oncol. 2015 Jul;16(7):775-86.

29. Sankaranarayanan R1, Prabhu PR2, Pawlita M3 and al. Indian HPV Vaccine Study GroupImmunogenicity and HPV infection after one, two, and three doses of quadrivalent HPV vaccine in girls in India: a multicentre prospective cohort study. Lancet Oncol. 2016 Jan;17(1):67-77. doi: 10.1016Roberts C, Green T, Hess E, Matys K, Brown MJ, Haupt RM, Luxembourg A, Vuocolo S, Saah A, Antonello J. Development of a human papillomavirus competitive luminex immunoassay for 9 HPV types. Hum Vaccin Immunother. 2014;10(8):2168-74.
30. Dias D, Van Doren J, Schlottmann S, Kelly S, Puchalski D, Ruiz W, Boerckel P, Kessler J, Antonello JM, Green T, Brown M, Smith J, Chirmule N, Barr E, Jansen KU, Esser MT.Optimization and validation of a multiplexed luminex assay to quantify antibodies to neutralizing epitopes on human papillomaviruses 6, 11, 16, and 18.
31. Depuydt CE, Benoy IH, Beert JF, Criel AM, Bogers JJ, Arbyn M.Clinical validation of a type-specific real-time quantitative human papillomavirus PCR against the performance of hybrid capture 2 for the purpose of cervical cancer screening. Clin Diagn Lab Immunol. 2005 Aug;12(8):959-69. J Clin Microbiol. 2012 Dec;50(12):4073-7.