Received Date : 06-Dec-2015

Revised Date : 16-May-2016

Accepted Date : 08-Jun-2016

Article type : Original research

Title

HPV16 and 52 E6-Specific Immunity in HIV-Infected Adults on Combination Antiretroviral Therapy (cART)

Running title

HPV16 and 52 E6 immunity in HIV-infected patients

Authors

Chan Yoon Leng,¹ Huey Chi Low,¹ Ling Ling Chua,² Meng Li Chong,¹ Helmi Sulaiman,^{1,3} Iskandar Azwa,^{1,3} Jennifer M. Roberts,⁴ Adeeba Kamarulzaman,^{1,3} Reena Rajasuriar,^{1,5,6} Yin Ling Woo^{1, 2, 7}

Affiliations of authors

¹Centre of Excellence for Research in AIDS (CERiA), Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.

²University of Malaya Cancer Research Institute (UMCRI), Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.

³Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.

⁴Douglass Hanly Moir Pathology, New South Wales, Australia.

⁵Department of Pharmacy, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/hiv.12432](https://doi.org/10.1111/hiv.12432)

This article is protected by copyright. All rights reserved

⁶Peter Doherty Institute for Infection and Immunity, University of Melbourne, Victoria, Australia.

⁷Department of Obstetrics and Gynaecology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.

Corresponding author's contact information:

Name: Yin Ling Woo

Address: Department of Obstetrics and Gynaecology
Faculty of Medicine
University of Malaya
50603 Kuala Lumpur
Malaysia

Office phone number: +60 3 7949 2462

Fax number: +60 3 2246 3385

Email address: ylwoo@um.edu.my

Abstract

Objectives

Human papillomavirus (HPV)-associated cancers disproportionately affect those infected with human immunodeficiency virus (HIV) despite effective combination antiretroviral therapy (cART). The primary aim of this study was to quantify HPV16 and 52 E6-specific interferon (IFN)- γ ELISPOT T-cell responses, a correlate of protective immunity in the first year following cART and subsequently in those patients with suboptimal (sIR) and optimal immune reconstitution (oIR).

Methods

94 HIV-infected patients were recruited; a longitudinal cohort just prior to commencing cART and followed-up for 48 weeks (n=27), and a cross-sectional cohort (n= 67) consisting of patients with sIR (CD4+ T-cell <350cells/ μ l) and oIR (CD4+ T-cell >500cells/ μ l) after a minimum of two years on cART. Controls (n=29) consisted of HIV-negative individuals. Interferon- γ ELISPOT responses against HPV16 and 52 E6 were correlated to clinical

characteristics, anal and oral HPV carriage, T-cell maturational subsets, markers of activation, senescence and T-regulatory cells.

Results

HPV16 and 52 E6-specific T-cell responses were detected in only 1/27 (3.7%) patients during the initial phase of immune recovery. After at least 2 years of cART, those who achieved oIR had significantly higher responses (9/34, 26.5%) compared to sIR (2/32, 6.3%) ($p=0.029$). Apart from higher CD4⁺ T-cell counts and lower CD4⁺ T-cell activation, no other immunological correlates were associated with the detection of HPV16 and 52 E6-specific responses.

Conclusions

HPV16 and 52 E6-specific IFN- γ T-cell responses, a correlate of protective immunity, was detected more frequently among HIV patients who achieved optimal immune recovery on cART (26.5%) compared to those with suboptimal recovery (6.3%).

Word count: 248 words

Key words

Human papillomavirus (HPV), HPV-specific immune responses, human immunodeficiency virus (HIV), immune recovery, cancer, combination antiretroviral therapy (cART)

BACKGROUND

Effective combination antiretroviral therapy (cART) has led to a dramatic increase in the life expectancy among individuals living with human immunodeficiency virus (HIV) (1, 2). While opportunistic infections have reduced significantly among those living with HIV, the incidence of human papillomavirus (HPV)-associated diseases including cancers are increasing (3, 4). This is consistent with the rising burden of HPV-associated cancers even among those without HIV (5, 6). When compared to the general population in the USA, those living with HIV had a 38-fold higher risk of developing anal cancer and 2.7-fold increase risk of invasive cervical cancer (7). In addition, HPV-associated oral cancer is also 3-fold more common in HIV-positive men than in the general population (8). It is estimated that 15% of

all new cancer diagnosis among those living with HIV can be attributed to HPV infection (7) compared to 4.8% among the general population (9).

Cellular immune responses are central in balancing health and disease states associated with HPV. The role of cellular immune responses to HPV have been demonstrated in immune-histological studies where the regression of cervical intraepithelial neoplasia and genital warts have been associated with an influx of CD4⁺ T-cells, CD8⁺ cytotoxic T-cells and macrophages (10, 11), while HPV-specific T-cell responses to HPV E6 antigens have been consistently shown to correlate with virological clearance or clinical regression of HPV-associated diseases (12-15). This in turn has been exploited in the development of therapeutic vaccines where immunological responses to E6 was found to correlate with clinical outcomes (16-18). Therefore, systemic HPV-specific E6 responses can be used to identify those who will likely mount a protective cellular immune response against HPV infection and its related diseases compared to those who may fail. The higher prevalence of HPV infection (19) and cancers among individuals living with HIV suggest that despite apparently optimal immune reconstitution as assessed by CD4⁺ T-cell count and viral suppression, a protective immunological response against oncogenic HPV may either never or inadequately be mounted. A better understanding of the natural history of HPV infection and functional reconstitution of HPV-specific immunity in those with living with HIV would inform prevention and treatment strategies against HPV-associated diseases.

We therefore explored the association of HPV immunity in relation to CD4⁺ T-cell reconstitution among HIV-infected individuals receiving suppressive cART. Our primary aim was to define immunological correlates of HPV16 and 52 E6-specific immune responses following cART. We also explored the prevalence and risk factors associated with HPV DNA detection in the anogenital tract and oral cavity among this multi-ethnic Asian HIV-infected cohort receiving suppressive cART.

METHODS

Study population

Two cohorts of HIV-infected patients (longitudinal and cross-sectional) were recruited from the Infectious Diseases Unit (IDU), University Malaya Medical Centre (UMMC). The longitudinal cohort (n=27) comprised of patients commencing cART (t=0 weeks) and were

followed-up for 48 weeks (t=12, 24 and 48 weeks). The inclusion criteria were: men or women >18 years old, naïve to cART, no acute illness and not pregnant at recruitment. The cross-sectional cohort consisted of patients with suboptimal (sIR) and optimal (oIR) immune recovery. The inclusion criteria were: men >18 years old, receiving cART for a minimum of two years, CD4+ T-cell count persistently <350cells/μl (sIR) or >500cells/μl (oIR) on suppressive (HIV RNA <50copies/ml) cART. HIV-negative controls were recruited from a local community-based clinic. Samples collected were 40mls of blood, anal swab and oral rinse. Peripheral blood mononuclear cells (PBMCs) were isolated for interferon-γ (IFN-γ) ELISPOT assay and immunophenotyping. Clinical and detailed socio-demographic parameters were extracted from medical records and a self-administered questionnaire. All participants provided written informed consent and this study was approved by UMMC ethics review board (Medical Ethics Committee Reference: 865.18).

HPV DNA testing

Anal swabs were collected using a pre-moistened cervical brush (HybriBio Limited, China). Oral rinse was obtained by gargling 10ml of Listerine® mouthwash. DNA was extracted from both anal and oral samples, and HPV DNA typing was performed using the HybriBio Rapid GenoArray test kit (HybriBio Limited) (20). This kit detects 21 HPV genotypes, including 13 high-risk (HR) genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68), 6 low-risk genotypes (6, 11, 42, 43, 44, and CP8304 [81]), and 2 probable high-risk types (53 and 66). The tests were performed according to the manufacturer's protocol (20). Briefly, the extracted DNA was subjected to PCR amplification using HPV L1 consensus PCR primers. The PCR products were subsequently added to a probed membrane followed by flow-through hybridisation and colourimetric reaction for detection of amplified HPV DNA.

Anal cytology

The anal cytological samples were collected without anoscopic guidance using the pre-moistened brush into ThinPrep (Hologic, Inc., USA) liquid-based specimen and Papanicolaou stained. All slides were read by a single experienced pathologist, utilising the Bethesda System 2001 criteria and terminology (21).

IFN-γ ELISPOT

IFN-γ producing HPV-specific T-cells were quantified using ELISPOT as previously described (13, 22, 23). Briefly, PBMCs were suspended in Isocove's modified Dulbecco's

media enriched with 10% fetal bovine serum and seeded at 2×10^6 cells/ml/well in a 24-well plate. A set of HPV16 E6 peptides consisting of 37 15-mer peptides that overlap by 11 amino acids (Supplementary data. CTL, Germany) at 1 µg/ml/peptide (24) were pooled with a set of HPV52 E6 peptides consisting of 10 9-mer non-overlapping peptides (supplementary data) at 10 µg/ml/peptide (25). Memory recall mix containing 2 peptides from tetanus and 11 peptides from human Cytomegalovirus pp65 antigen (supplementary data)) at 10 µg/ml/peptide were used as positive controls. Following four days of incubation at 37°C, PBMCs were harvested, washed and seeded in triplicate at a density of 10^5 cells/well in a Multiscreen 96-well plate (Millipore, USA) coated with an IFN-γ capture antibody (Mabtech, Ireland). Further antibody incubations and development of the ELISPOT was performed as described. The measured HPV E6-specific IFN-γ responses were from a combination of CD4 and/ or CD8 T-cells against types HPV16 or 52. Spots were counted with a fully automated computer-assisted-video-imaging analysis system (CTL ImmunoSpot®, USA). Specific spots were calculated by subtracting the mean number of spots plus two times standard deviation of the medium control from the mean number of spots in experimental wells. Antigen-specific T-cell frequencies were considered to be positive when specific T-cell frequencies were $>1/10^4$ PBMCs.

T-cell immunophenotyping by flow cytometry

Briefly, 1×10^6 thawed PBMC were stained in two separate panels: 1) CD3 (PerCP-Cy5.5), CD4 (PE-Cy7), CD8 (APC-H7), CD28 (APC), CD57 (FITC), CD45RA (PE), CCR7 (BV421) and viability dye (FVS510) and 2) CD3, CD4, CD8, CD38, HLA-DR (BV421, clone G46-6), CD25 (BB515, clone 2A3), Foxp3 (Alexa Fluor® 647, clone 259D/C7) and viability stain. All antibodies were purchased from BD Pharmingen. PBMCs were stained for surface markers for 20 minutes at room temperature and washed twice before acquisition. PBMCs in the second panel were further fixed and permeabilized with Foxp3 staining buffer kit prior to intracellularly staining with anti-Foxp3 antibodies or isotype control IgG1 (Alexa Fluor® 647, clone MOP-21) for 30 mins. All samples were re-suspended in stabilizing fixative and acquired on BD FACS Canto II (BD Biosciences, USA) and analysed using FACS Diva v6. Following gating to exclude doublets and dead cells (FVS510+), CD3+ cells were selected and sequentially gated for CD4+ and CD8+ T-cells and subsequently for the maturational subsets of naïve (CD45RA+CCR7+), central memory, CM (CD45RA-CCR7+), effector memory, EM (CD45RA-CCR7-) and terminally-differentiated effector memory, TdEM (CD45RA+CCR7-) cells. The proportion of activation and senescence markers on

CD4⁺ and CD8⁺ T-cells were defined from the co-expression of CD38⁺HLA-DR⁺ and CD57⁺CD28⁻ cells, respectively. T-regulatory cells were reported as percentage of CD3⁺CD4⁺ T-cells expressing CD25⁺Foxp3⁺ (Figure 1).

Data analysis

Clinical and immunological characteristics were compared using Mann-Whitney U and Chi-square/Fisher's exact test. Logistic regression analysis was used to assess immunological factors associated with HPV carriage and HPV-specific responses. Co-variables with $p < 0.25$ and well established risk factors were included in the multivariate model where a p -value of < 0.05 was considered significant. Statistical analyses were performed using SPSS Statistics version 22 (IBM).

RESULTS

Patient characteristics

In total, 94 HIV-infected patients were recruited; 27 in the longitudinal arm and 67 in the cross-sectional arm, and 29 HIV-negative controls.

In the cross-sectional cohort, 32 patients displayed suboptimal immune recovery (sIR) while 35 had optimal immune recovery (oIR). The median age was 41 (interquartile range [IQR]: 39-48) years, with 40/63 (63.5%) smokers, 16/50 (32.0%) circumcised and 27/64 (42.2%) men who had sex with men (MSMs) (Table 1). The median duration on suppressive cART was 5 (IQR: 3-9) years (Table 2). The age, smoking history, circumcision rates, and sexual orientation, were not significantly different between sIR and oIR (Table 1) but baseline CD4⁺ T-cell counts was significantly higher in oIR compared to sIR (190 [IQR: 66-314] vs 28 [IQR: 10-59] cells/ μ l, $p < 0.001$) (Table 2).

In the longitudinal cohort, the median age was 35 (IQR: 30-41) years, with all other parameters including smoking history, circumcision rates and sexual orientation being comparable to the cross-sectional cohort (Table 1). The median baseline CD4⁺ T-cell count was 221 (IQR: 63-319) cells/ μ l (Table 2). Of interest, comparison of T-cell subsets between hetero- and homosexuals, demonstrated a significantly higher proportion of activated CD8⁺ T-cells (CD8⁺CD38⁺HLADR⁺) among the homosexuals. However, there were no

differences observed in proportions of T-regs, senescent CD4⁺ and CD8⁺ T-cells or activated CD4⁺ T-cells.

In the HIV-negative controls, the median age was 39 (IQR: 29-53) years, comparable to the HIV-positives ($p=0.314$). There were more individuals who were circumcised 17/28 (60.7%, $p=0.014$) and notably, 24/25 (96.0%) being heterosexuals compared to the HIV-infected individuals (Table 1).

Prevalence and risk factors associated with HPV DNA detection

Overall, 25/44 (56.8%) and 7/51 (13.7%) of the cross-sectional cohort had HPV DNA detected in the anal and oral cavity respectively, with no significant differences between sIR and oIR ($p=0.604$ and $p=0.432$, respectively) (Table 3). In anal samples with detectable HPV DNA, 17/25 (68.0%) had two or more HPV types, with 21/25 (84.0%) having a HR-HPV. HPV16 and HPV52 were detected in 6/21 (28%) and 5/21 (23%) of the anal samples with multiple infections detected in all the samples carrying HPV16. The other commonly detected HPV types were 6,11,16,31 and 58. In oral samples with detectable HPV DNA, 6/7 (85.7%) had single HPV types, with 4/7 (57.1%) being HR-HPV.

In the longitudinal cohort, anal HPV DNA was detected in 19/26 (73.1%) at recruitment and went up to 16/20 (80.0%) at 48 weeks with 4/27 (14.8%) and 6/21 (28.6%) detected in the oral cavity during the same time-points (Table 3). 21/24 (87.5%) of the anal samples had two or more HPV types, with 23/24 (95.8%) having a HR-HPV type at any single time-point. The most common HR-HPV types present in this cohort at any single time-point were HPV16 (45.8%) and HPV52 (37.7%). For oral samples with detectable HPV DNA, 4/10 (40.0%) had two or more HPV types detected at any single time-point, all of whom had a HR-HPV type. The most common HR-HPV types present at any single time-point were HPV18 (50.0%) and HPV16 (40.0%). No decrease in anal and oral HPV carriage was observed over the 48-week period despite cART-induced CD4⁺ T-cell count increase.

There was a correlation between alcohol consumption with oral HPV carriage in the HIV-infected individuals ($p=0.042$) and a tendency of greater HPV anal carriage with engagement of anal sex ($p=0.055$) (Table 4). Both circumcision ($p=0.255$) and smoking history ($p=0.358$) did not influence anal and oral HPV carriage respectively. However, no

significant risk factors were found to be independently associated with either anal or oral HPV carriage in the multivariate analysis.

In the HIV-negative controls, detection of HPV DNA in the anal canal was 1/16 (6.3%) which was significantly lower than the HIV-infected cohorts ($p < 0.001$), while the detection in the oral cavity was comparable at 3/29 (10.3%), $p = 0.337$.

Correlation between anal cytology and HPV DNA detection

In the cross-sectional cohort, only 44/67 samples had sufficient cells for cytological assessment. When comparing the sIR and oIR groups, there was no difference in those with cytology $\geq$ atypical squamous cells of undetermined significance (ASC-US) (38.9% vs 25.0%, $p = 0.358$). While presence of anal HPV DNA carriage was positively associated with cytology $\geq$ ASC-US ($p = 0.001$), no correlation between multiple HPV types and the presence of HR-HPV was associated with cytology $\geq$ ASC-US ($p = 0.13$ and $p = 0.23$ respectively). No cytological trends were observed in the longitudinal cohort.

Immunological and clinical correlates of HPV16 and 52 E6-specific responses

Overall, 12/91 (13.2%) from the HIV-infected cohort exhibited HPV16 and 52 E6-specific responses (Table 3) compared to 2/28 (7.1%) in the HIV-negative cohort ($p = 0.385$).

In the cross-sectional cohort, regardless of HPV carriage and anal cytology, significantly lower HPV16 and 52 E6-specific responses were observed among the sIR (2/32, 6.3%) compared to oIR (9/34, 26.5%) ($p = 0.029$) (Table 3). The magnitude of the E6-specific responses between oIR (17.4, IQR: 13.2-18.9) were comparable to the sIR (19.3, IQR: 15.2-23.8) group ($p = 0.637$). The presence of HPV16 and/or 52 at anal and/or oral sites at the time of recruitment did not correspond to the HPV16 and 52 E6-specific immunity detected ($p = 0.339$). Baseline CD4+ T-cell counts and duration on cART were not different in individuals with and without HPV16 and 52 E6-specific responses ($p = 0.282$ and 0.965 , respectively). However, individuals with higher CD4+ T-cell counts at recruitment had significantly greater magnitude of HPV16 and 52 E6-specific responses ($p = 0.028$). While there were significant differences in the E6-specific responses between oIR and sIR, responses to MRM only trended towards significance ($p = 0.569$) (Table 3).

In the longitudinal cohort (n=25), only one patient displayed HPV16 and 52 E6-specific responses at weeks 12 and 24 after cART. This individual had HPV52 detected in the anal canal at weeks 12 and 24. While the immune responses to E6-peptides were barely detectable, approximately 50% of the patients mounted a response to MRM despite the low CD4+ T-cell counts (Table 3).

In the analysis of immune correlates of HPV16 and 52 E6-specific responses in cART-treated HIV-infected individuals, both the data for the cross-sectional and longitudinal (week 48) were combined (Table 5). In the univariate analysis, differences in T-cell maturational subsets (naïve, CM, EM and TdEM) were not associated with HPV16 and 52 E6-specific responses. However, higher CD4+ T-cell activation ($p=0.030$) was significantly associated with the lack of HPV16 and 52 E6-specific responses with only a tendency for higher CD8+ T-cell activation exhibiting a similar trend ($p=0.067$). Neither the proportion of CD4+ T-regulatory cells ($p=0.959$) nor the proportion of senescent CD4+ T-cells ($p=0.718$) and CD8+ T-cells ($p=0.433$) were associated with HPV16 and 52 E6-specific responses. Besides CD4+ T-cell counts at recruitment ($p=0.008$), there was a tendency for patients with a lower CD4:CD8 T-cell ratio ($p=0.072$) to fail in mounting a HPV16 and 52 E6-specific responses (Table 5).

In the multivariate analysis, none of the immunological parameters were independently associated with HPV16 and 52 E6-specific immune responses.

DISCUSSION

To date this is the largest study to have assessed the correlates of systemic HPV-specific immune responses to immune recovery following cART among those with HIV infection. We quantified HPV-specific immunity during the initial phase of immune recovery following cART and subsequently in patients with sub-optimal and optimal immune recovery (sIR and oIR). We showed that HPV-specific immune responses were rarely detected during the first year following cART but in individuals on cART for a longer duration, an optimal immune recovery $>500\text{cells}/\mu\text{l}$ was associated with significantly higher frequencies of HPV-specific responses compared to individuals with suboptimal recovery ($<350\text{cells}/\mu\text{l}$). When assessing the immunological correlates of HPV-specific responses, only higher CD4+ T-cell counts and lower CD4+ T-cell activation were found to be associated with HPV-specific responses.

The anal and oral HPV carriage rates in our HIV-infected cohort of 64.1% and 18.1%, respectively are consistent with the high prevalence of HPV infection reported among HIV-infected individuals (42-96% anal and 9.6-38.5% oral HPV). These studies also demonstrate HPV16 and 52 being the most common HPV types (26-29). The consistently higher prevalence of HR-HPV observed globally, despite effective cART and CD4+ T-cell recovery point towards an inadequate immune-mediated resolution of and protection against HPV infection.

We investigated HPV-specific immune responses during the different phases of immune recovery following cART. In the longitudinal cohort, only 1/25 patient exhibited HPV-specific responses within the first 48 weeks of suppressive cART. In contrast, higher frequencies of HPV-specific responses were detected in the cross-sectional cohort with a median duration of 5 years on cART. More strikingly, individuals in the oIR group had significantly higher HPV-specific responses (26.5%) compared to the sIR group (6.3%). The lack of antigen-specific responses against pathogens during early recovery has previously been observed in other HIV co-infections (30, 31). It was therefore not unexpected for HPV-specific responses to be infrequently detected during the first year of treatment. Furthermore, HPV is exclusively epitheliotrophic where the majority of antigen-specific immune cells reside locally (reviewed in (32)) with low but detectable levels of circulating HPV-specific T-cells. The recovering immune system during cART initiation is also challenged by a larger load of other systemic antigens including human herpes viruses (33) as evidenced by a detectable MRM response in nearly half of the longitudinal cohort, 12 weeks after commencing cART. Taken together, the lack of HPV-specific responses in early phase of CD4+ T-cell recovery and subsequently, increased responses seen in oIR implies that a specific threshold of CD4+ T-cells is required before HPV-specific immunity can be detected. The concept of immune-competence as assessed by CD4+ T-cell counts and its association with virus-specific cellular immunity has also been observed in HIV/hepatitis B virus and CMV co-infected individuals (30, 34). Alternatively, the lack of HPV-specific responses in the early phase of immune recovery could be driven by the loss of memory-specific cells due to greater turnover as we observed a significant inverse correlation between CD4+ T-cell activation and HPV-specific responses in this cohort.

In our cohort, neither the extent of immune recovery nor the development of HPV16 and 52 E6-specific responses were associated with differences in HPV carriage or abnormal anal cytology. This is not surprising as histological outcomes (biopsies) have been shown to correlate better to the immunological markers compared to virological or cytological outcomes (13) particularly since this study was limited by its small numbers.

The largest study to date identifying HPV16 E6-specific responses was in a mixed HIV-infected/negative population of MSMs using a flow cytometry-based assay(24). In that study, 50% displayed HPV16 E6-specific responses, but this did not show any correlations with age, HIV status, or anal HPV16 status. However, a trend between recent anal high-grade squamous intraepithelial lesion (HSIL) regression and systemic E6-specific CD4+ T-cell responses was demonstrated, confirming the superiority of tissue biopsies over HPV DNA detection as a surrogate marker for clinical outcomes.

The observed HPV16 and 52 E6-specific responses among the HIV-negative controls (7.1%) was lower than that expected of around 30-50%. The discrepancy could be explained by differences in the study populations. Historically, studies demonstrating high proportions of protective immunity associated with E6-specific responses have been predominantly in women with cervical disease with regressing lesions or low-grade disease that did not progress to high-grade lesions (12-15). In contrast, our controls were healthy males with low anal HPV carriage rates.

The strength of the study is its well-characterized clinical population and correlating it to immunological outcomes in HIV-infected individuals. It also utilises a sensitive T-cell assay (IFN- γ ELISPOT) that has been validated and used in various populations (12, 13, 22, 23) to detect low levels of antigen-specific immunity. A major limitation of this assay is that IFN- γ was the only parameter measured, discounting the effects of other regulatory cytokines. Other limitations of the study include small sample numbers in the longitudinal cohort and the lack of immunological correlates to long-term clinical outcomes.

Despite effective viral suppression on cART, 1/3 of patients will not achieve optimal CD4+ T-cell recovery, especially in those starting treatment late (35, 36). They are also particularly susceptible to non-AIDS associated co-morbidities (37, 38). This study has demonstrated that those with suboptimal immune recovery also lacks HPV protective

immunity and are possibly at greater risk of developing serious HPV-associated diseases. We propose that this group should be considered for more intensive screening and should be encouraged to consider HPV vaccination. There is good evidence to suggest that HPV vaccination among those who have previously received treatment for pre-malignant disease of the cervix (cervical intra-epithelial neoplasia) significantly reduced the incidence of subsequent HPV-related disease (39). This suggests that those infected with HIV on cART exhibiting HPV-associated diseases may also potentially benefit. This is supported by immunogenicity studies among those who are HIV-infected (40) and studies that have shown that vaccinating men with a history of high-grade intraepithelial neoplasia reduces the recurrence rates by up to 50% (41), and that this strategy is cost effective even among the HIV-infected (42).

CONCLUSIONS

HPV-related pathology remains a significant cause of morbidity and mortality among those living with HIV despite effective viral suppressive therapy. The failure to mount an effective HPV-immune response among those with suboptimal CD4⁺ T-cell recovery may increase their susceptibility to HPV-associated cancers. Patients on cART with suboptimal CD4⁺ T-cell recovery should therefore be considered for increased surveillance and HPV vaccination in the prevention of HPV-associated cancers.

Word count: 3483 words

Acknowledgements

This study was funded by the Ministry of Higher Education, Malaysia, under the High Impact Research/Ministry of Higher Education Grant [UM.C/625/1/HIR account E-000001-20001], and by the University of Malaya's University of Malaya Research Grant [UMRG RP029A-14HTM].

Author contributions: RR, AK and YLW designed this study. MLC, HS and IA recruited patients, collected samples and data. CYL, HCL, LLC, RR, JMR and YLW analysed patient samples, generated, compiled and analysed the results. All authors have reviewed, commented, and approved the approved the final manuscript. All authors report no conflicts of interest.

References

1. Lohse N, Hansen AB, Pedersen G, Kronborg G, Gerstoft J, Sorensen HT, et al. Survival of persons with and without HIV infection in Denmark, 1995-2005. *Ann Intern Med.* 2007;146(2):87-95.
2. Life expectancy of individuals on combination antiretroviral therapy in high-income countries: a collaborative analysis of 14 cohort studies. *Lancet.* 2008;372(9635):293-9.
3. Hoots BE, Palefsky JM, Pimenta JM, Smith JS. Human papillomavirus type distribution in anal cancer and anal intraepithelial lesions. *International journal of cancer.* 2009;124(10):2375-83.
4. Chaturvedi AK, Madeleine MM, Biggar RJ, Engels EA. Risk of human papillomavirus-associated cancers among persons with AIDS. *Journal of the National Cancer Institute.* 2009;101(16):1120-30.
5. Forte T, Niu J, Lockwood GA, Bryant HE. Incidence trends in head and neck cancers and human papillomavirus (HPV)-associated oropharyngeal cancer in Canada, 1992-2009. *Cancer causes & control : CCC.* 2012;23(8):1343-8.
6. Hartwig S, Syrjänen S, Dominiak-Felden G, Brotons M, Castellsagué X. Estimation of the epidemiological burden of human papillomavirus-related cancers and non-malignant diseases in men in Europe: a review. *BMC Cancer.* 2012;12:30-.
7. Robbins HA, Pfeiffer RM, Shiels MS, Li J, Hall HI, Engels EA. Excess cancers among HIV-infected people in the United States. *Journal of the National Cancer Institute.* 2015;107(4).
8. Mellin H, Friesland S, Lewensohn R, Dalianis T, Munck-Wikland E. Human papillomavirus (HPV) DNA in tonsillar cancer: Clinical correlates, risk of relapse, and survival. *International journal of cancer.* 2000;89(3):300-4.
9. Forman D, de Martel C, Lacey CJ, Soerjomataram I, Lortet-Tieulent J, Bruni L, et al. Global burden of human papillomavirus and related diseases. *Vaccine.* 2012;30 Suppl 5:F12-23.
10. Coleman N, Birley HD, Renton AM, Hanna NF, Ryait BK, Byrne M, et al. Immunological events in regressing genital warts. *American journal of clinical pathology.* 1994;102(6):768-74.
11. Woo YL, Sterling J, Damay I, Coleman N, Crawford R, van der Burg SH, et al. Characterising the local immune responses in cervical intraepithelial neoplasia: a cross-sectional and longitudinal analysis. *BJOG.* 2008;115(13):1616-21; discussion 21-2.
12. de Jong A, van Poelgeest MI, van der Hulst JM, Drijfhout JW, Fleuren GJ, Melief CJ, et al. Human papillomavirus type 16-positive cervical cancer is associated with impaired CD4+ T-cell immunity against early antigens E2 and E6. *Cancer research.* 2004;64(15):5449-55.
13. Woo YL, van den Hende M, Sterling JC, Coleman N, Crawford RA, Kwappenberg KM, et al. A prospective study on the natural course of low-grade squamous intraepithelial lesions and the

- presence of HPV16 E2-, E6- and E7-specific T-cell responses. International journal of cancer. 2010;126(1):133-41.
14. Nakagawa M, Spencer HJ, Coleman HN, Greenfield WW. Distribution of human papillomavirus (HPV) types and anti-HPV T-cell immune responses among different racial/ethnic groups in Central Arkansas. The Journal of the Arkansas Medical Society. 2013;109(8):160-3.
15. Farhat S, Nakagawa M, Moscicki AB. Cell-mediated immune responses to human papillomavirus 16 E6 and E7 antigens as measured by interferon gamma enzyme-linked immunospot in women with cleared or persistent human papillomavirus infection. International journal of gynecological cancer : official journal of the International Gynecological Cancer Society. 2009;19(4):508-12.
16. de Vos van Steenwijk PJ, Ramwadhoebe TH, Lowik MJ, van der Minne CE, Berends-van der Meer DM, Fathors LM, et al. A placebo-controlled randomized HPV16 synthetic long-peptide vaccination study in women with high-grade cervical squamous intraepithelial lesions. Cancer Immunol Immunother. 2012;61(9):1485-92.
17. Bissa M, Illiano E, Pacchioni S, Paolini F, Zanotto C, De Giuli Morghen C, et al. A prime/boost strategy using DNA/fowlpox recombinants expressing the genetically attenuated E6 protein as a putative vaccine against HPV-16-associated cancers. J Transl Med. 2015;13:80.
18. Kenter GG, Welters MJ, Valentijn AR, Lowik MJ, Berends-van der Meer DM, Vloon AP, et al. Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. N Engl J Med. 2009;361(19):1838-47.
19. Mendez-Martinez R, Rivera-Martinez NE, Crabtree-Ramirez B, Sierra-Madero JG, Caro-Vega Y, Galvan SC, et al. Multiple human papillomavirus infections are highly prevalent in the anal canal of human immunodeficiency virus-positive men who have sex with men. BMC infectious diseases. 2014;14:671.
20. Liu SS, Leung RC, Chan KK, Cheung AN, Ngan HY. Evaluation of a newly developed GenoArray human papillomavirus (HPV) genotyping assay and comparison with the Roche Linear Array HPV genotyping assay. Journal of clinical microbiology. 2010;48(3):758-64.
21. Nayar R, Solomon D. Second edition of 'The Bethesda System for reporting cervical cytology' - atlas, website, and Bethesda interobserver reproducibility project. CytoJournal. 2004;1(1):4.
22. Welters MJ, de Jong A, van den Eeden SJ, van der Hulst JM, Kwappenberg KM, Hassane S, et al. Frequent display of human papillomavirus type 16 E6-specific memory t-Helper cells in the healthy population as witness of previous viral encounter. Cancer research. 2003;63(3):636-41.

23. Welters MJ, van der Logt P, van den Eeden SJ, Kwappenberg KM, Drijfhout JW, Fleuren GJ, et al. Detection of human papillomavirus type 18 E6 and E7-specific CD4+ T-helper 1 immunity in relation to health versus disease. *International journal of cancer*. 2006;118(4):950-6.
24. Tong WW, Shepherd K, Garland S, Meagher A, Templeton DJ, Fairley CK, et al. Human papillomavirus 16-specific T-cell responses and spontaneous regression of anal high-grade squamous intraepithelial lesions. *The Journal of infectious diseases*. 2015;211(3):405-15.
25. Chan PK, Liu SJ, Cheung JL, Cheung TH, Yeo W, Chong P, et al. T-cell response to human papillomavirus type 52 L1, E6, and E7 peptides in women with transient infection, cervical intraepithelial neoplasia, and invasive cancer. *Journal of medical virology*. 2011;83(6):1023-30.
26. Palefsky JM, Holly EA, Ralston ML, Jay N. Prevalence and risk factors for human papillomavirus infection of the anal canal in human immunodeficiency virus (HIV)-positive and HIV-negative homosexual men. *The Journal of infectious diseases*. 1998;177(2):361-7.
27. Parisi SG, Cruciani M, Scaggiante R, Boldrin C, Andreis S, Dal Bello F, et al. Anal and oral human papillomavirus (HPV) infection in HIV-infected subjects in northern Italy: a longitudinal cohort study among men who have sex with men. *BMC infectious diseases*. 2011;11:150.
28. Phanuphak N, Teeratakulpisarn N, Pankam T, Kerr SJ, Barisri J, Deesua A, et al. Anal human papillomavirus infection among Thai men who have sex with men with and without HIV infection: prevalence, incidence, and persistence. *Journal of acquired immune deficiency syndromes*. 2013;63(4):472-9.
29. Videla S, Darwich L, Canadas MP, Coll J, Pinol M, Garcia-Cuyas F, et al. Natural history of human papillomavirus infections involving anal, penile, and oral sites among HIV-positive men. *Sexually transmitted diseases*. 2013;40(1):3-10.
30. Keane NM, Price P, Lee S, Almeida CA, Stone SF, James I, et al. Restoration of CD4 T-cell responses to cytomegalovirus is short-lived in severely immunodeficient HIV-infected patients responding to highly active antiretroviral therapy. *HIV medicine*. 2004;5(6):407-14.
31. Burgess K, Price P, James IR, Stone SF, Keane NM, Lim AY, et al. Interferon-gamma responses to *Candida* recover slowly or remain low in immunodeficient HIV patients responding to ART. *Journal of clinical immunology*. 2006;26(2):160-7.
32. Stanley MA. Epithelial cell responses to infection with human papillomavirus. *Clinical microbiology reviews*. 2012;25(2):215-22.
33. Komanduri KV, Viswanathan MN, Wieder ED, Schmidt DK, Bredt BM, Jacobson MA, et al. Restoration of cytomegalovirus-specific CD4+ T-lymphocyte responses after ganciclovir and highly active antiretroviral therapy in individuals infected with HIV-1. *Nature medicine*. 1998;4(8):953-6.

34. Chang JJ, Sirivichayakul S, Avihingsanon A, Thompson AJ, Revill P, Iser D, et al. Impaired quality of the hepatitis B virus (HBV)-specific T-cell response in human immunodeficiency virus type 1-HBV coinfection. *Journal of virology*. 2009;83(15):7649-58.
35. Onen NF, Overton ET, Presti R, Blair C, Powderly WG, Mondy K. Sub-optimal CD4 recovery on long-term suppressive highly active antiretroviral therapy is associated with favourable outcome. *HIV medicine*. 2009;10(7):439-46.
36. Torti C, Prosperi M, Motta D, Digiambenedetto S, Maggiolo F, Parainfo G, et al. Factors influencing the normalization of CD4+ T-cell count, percentage and CD4+/CD8+ T-cell ratio in HIV-infected patients on long-term suppressive antiretroviral therapy. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*. 2012;18(5):449-58.
37. Lewden C, Chene G, Morlat P, Raffi F, Dupon M, Dellamonica P, et al. HIV-infected adults with a CD4 cell count greater than 500 cells/mm³ on long-term combination antiretroviral therapy reach same mortality rates as the general population. *Journal of acquired immune deficiency syndromes*. 2007;46(1):72-7.
38. Marin B, Thiebaut R, Bucher HC, Rondeau V, Costagliola D, Dorrucchi M, et al. Non-AIDS-defining deaths and immunodeficiency in the era of combination antiretroviral therapy. *AIDS (London, England)*. 2009;23(13):1743-53.
39. Joura EA, Garland SM, Paavonen J, Ferris DG, Perez G, Ault KA, et al. Effect of the human papillomavirus (HPV) quadrivalent vaccine in a subgroup of women with cervical and vulvar disease: retrospective pooled analysis of trial data 2012 2012-03-27 22:34:19.
40. Toft L, Storgaard M, Muller M, Sehr P, Bonde J, Tolstrup M, et al. Comparison of the immunogenicity and reactogenicity of Cervarix and Gardasil human papillomavirus vaccines in HIV-infected adults: a randomized, double-blind clinical trial. *The Journal of infectious diseases*. 2014;209(8):1165-73.
41. Swedish KA, Factor SH, Goldstone SE. Prevention of recurrent high-grade anal neoplasia with quadrivalent human papillomavirus vaccination of men who have sex with men: a nonconcurrent cohort study. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2012;54(7):891-8.
42. Deshmukh AA, Chhatwal J, Chiao EY, Nyitray AG, Das P, Cantor SB. Long-Term Outcomes of Adding HPV Vaccine to the Anal Intraepithelial Neoplasia Treatment Regimen in HIV-Positive Men Who Have Sex with Men. *Clinical Infectious Diseases*. 2015.

564 **Table 1** Demographic characteristics of HIV-infected patients and HIV-negative controls

565

	Cross-sectional		Longitudinal (n=27)	Control (n=29)
	sIR (n=32)	oIR (n=35)		
Age, median (IQR)	42 (39-50)	40 (38-46)	35 (30-41)	39 (29-53)
Ethnicity (%)				
Malay	2 (6.2)	7 (20.0)	6 (22.2)	12 (41.4)
Chinese	29 (90.6)	21 (60.0)	15 (55.6)	9 (31.0)
Indian	1 (3.2)	7 (20.0)	1 (3.7)	8 (27.6)
Others	0 (0)	0 (0)	2 (7.4)	0 (0)
Smoking history (%)				
Yes	20/30 (66.7)	20/33 (60.6)	12 (44.4)	18/28 (64.3)
Never	10/30 (33.3)	13/33 (39.4)	15 (55.6)	10/28 (35.7)
Circumcised (%)				
Yes	6/24 (25.0)	10/26 (38.5)	5/14 (35.7)	17/28 (60.7)
Sexual orientation (%)				
Heterosexual	18/30 (60.0)	16/34 (47.1)	6/26 (23.1)	24/25 (96.0)
Homosexual	11/30 (36.7)	16/34 (47.1)	19/26 (73.1)	0/25 (0)
Bisexual	1/30 (3.3)	2/34 (5.8)	1/26 (3.8)	1/25 (4.0)
Average number of lifetime sexual partners (%)				
1	2/24 (8.3)	4/26 (15.4)	1/15 (6.7)	6/25 (24.0)

≥2	15/24 (62.5)	17/26 (65.4)	10/15 (66.7)	17/25 (68.0)
Do not remember	7/24 (29.2)	5/26 (19.2)	4/15 (26.7)	2/25 (8.0)
Sexual practices (%)				
Oral sex	17/24 (70.8)	22/26 (84.6)	13/15 (86.7)	12/25 (48.0)
Anal sex	10/24 (41.7)	12/26 (46.2)	12/15 (80.0)	2/25 (8.0)
HSV-1 antibody positive (%)	12/19 (63.2)	13/22 (59.1)	12/20 (60.0)	--
HSV-2 antibody positive (%)	13/19 (68.4)	9/22 (40.9)	4/20 (20.0)	--

HSV, herpes simplex virus; IQR, interquartile range; oIR, optimal immunological responder; sIR, suboptimal immunological responder.

Table 2 Clinical and immunological parameters in the HIV-infected patients and HIV-negative controls

	Cross-sectional		Longitudinal (n=27)				Control
	sIR (n=32)	oIR (n=35)	Week 0	Week 12	Week 24	Week 48	(n=29)
Years on cART, median (IQR)	5 (3-9)	5 (3-9)	--	--	--	--	--
cART regimen							--
NNRTI-based	32 (100)	35 (100)	--	26 (96.3)	26 (96.3)	25 (92.6)	
PI-based	0 (0)	0 (0)	--	0 (0)	0 (0)	1 (3.7)	
Integrase inhibitor-based	0 (0)	0 (0)	--	1 (3.7)	1 (3.7)	1 (3.7)	
CD4+ T-cell count (cells/μl), median (IQR)							--
Baseline	28 (10-59)	190** (66-314)	221 (63- 319)	--	--	--	
At recruitment	285	733**	--	291	310	410	

	(224-332)	(671-945)		(186-406)	(207-462)	(268-481)	
CD8+ T-cell count (cells/μl) , median (IQR)							--
Baseline	473 (237-876)	710.5* (492.3-1391)	745 (414-1020)	--	--	--	
At recruitment	799 (560-982)	950* (723-1421)	--	883 (596-1271)	809 (539-1118)	952 (668-1297)	
CD4:CD8 ratio, median (IQR)							--
Baseline	0.07 (0.03-0.12)	0.22** (0.09-0.31)	0.29 (0.16-0.38)	--	--	--	
At recruitment	0.37 (0.31-0.45)	0.78** (0.57-1.09)	--	0.38 (0.25-0.54)	0.44 (0.29-0.61)	0.39 (0.29-0.55)	
HIV viral load (copies/ml), median (IQR)							
Baseline	194245.5 (97525-405805)	92461* (37599-233000)	54401 (27649-155050)	--	--	--	--
At recruitment	Below limit of detection	Below limit of detection	--	Below limit of detection	Below limit of detection	Below limit of detection	
Anal cytology (%)							
Technically unsatisfactory	3/21 (14.3)	3/23 (13.0)	11/24	4/16 (25.9)	5/20 (25.0)	3/14 (21.4)	26/29

			(45.8)			(89.7)	
Normal	11/21 (52.4)	15/23 (65.2)	4/24 (16.7)	4/16 (25.0)	9/20 (45.0)	6/14 (42.9)	3/29 (10.3)
ASC-US	2/21 (9.5)	1/23 (4.4)	3/24 (12.5)	0/16 (0)	0/20 (0)	2/14 (14.3)	0/29 (0)
LSIL	2/21 (9.5)	3/23 (13.0)	4/24 (16.7)	5/16 (31.3)	2/20 (10.0)	1/14 (7.1)	0/29 (0)
ASC-H	3/21 (14.3)	0/23 (0)	2/24 (8.3)	1/16 (6.3)	3/20 (15.0)	0/14 (0)	0/29 (0)
HSIL	0/21 (0)	1/23 (4.0)	0/24 (0)	2/16 (12.5)	1/20 (5.0)	1/14 (7.1)	0/29 (0)
T-cell immunophenotyping							
CD4+ T-cells, median % (IQR)							
Naïve	25 (15-31)	36 (24-50)	28 (14-39)	23 (6-34)	28 (17-41)	35 (22-46)	43 (35-47)**
CM	46 (39-51)	38 (34-48)	31 (23-41)	35 (28-45)	40 (33-44)	35 (31-38)	37 (31-43)
EM	27 (21-36)	19 (11-26)	28 (20-49)	27 (19-54)	22 (17-36)	23 (17-35)	15 (13-20)**
TdEM	1 (1-3)	2 (1-5)	4 (1-11)	2 (1-8)	2 (1-6)	1 (1-5)	1 (1-4)
Activation	8 (6-11)	5 (4-7)	29 (15-50)	25 (13-40)	16 (13-29)	13 (10-21)	3 (3-5)**
Senescence	3 (1-7)	4 (1-10)	11 (2-28)	8 (2-16)	6 (1-13)	5 (1-14)	1 (0-3)*
T-regulatory cells	7 (6-9)	6 (5-7)	7 (5-10)	6 (5-12)	7 (5-11)	7 (5-10)	6 (5-6)
CD8+ T-cells, median % (IQR)							
Naïve	8 (5-15)	16 (10-25)	8 (4-11)	9 (4-15)	12 (8-22)	14 (7-17)	21 (9-28)*
CM	5 (3-8)	7 (5-9)	2 (1-3)	3 (1-4)	4 (3-5)	3 (2-5)	6 (4-9)

EM	56 (44-62)	44 (37-53)	57 (45-67)	49 (43-55)	43 (34-53)	47 (36-52)	46 (34-51)
TdEM	28 (20-35)	30 (20-38)	29 (23-41)	38 (29-44)	37 (29-45)	35 (27-44)	31 (23-40)
Activation	22 (13-30)	14 (11-19)	73 (58-77)	62 (50-71)	48 (45-53)	42 (29-51)	14 (8-21)*
Senescence	44 (32-55)	30 (22-43)	43 (37-51)	50 (43-58)	49 (37-57)	48 (43-58)	33 (26-43)*

569 ASC-H, atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion; ASC-US, atypical squamous cells of undetermined
570 significance; cART, combined antiretroviral therapy; CM, central memory; EM, effector memory; HIV, human immunodeficiency virus; HSIL,
571 high-grade squamous intraepithelial lesion; IQR, interquartile range; LSIL, low-grade squamous intraepithelial lesion; NNRTI, non-nucleoside
572 reverse transcriptase inhibitor; oIR, optimal immunological responder; PI, protease inhibitor; sIR, suboptimal immunological responder; TdEM,
573 terminally-differentiated effector memory.

574 * p -value <0.05 and ** p -value <0.001.

575 **Table 3** Detection of HPV DNA and HPV16 and 52 E6-specific immune responses in the HIV-infected cohort

576

	Cross-sectional		Longitudinal (n=27)			
	sIR (n=32)	oIR (n=35)	Week 0	Week 12	Week 24	Week 48
Anal HPV carriage						
Any HPV positive (%)	12/21 (57.1)	13/23 (56.5)	19/26 (73.1)	14/19 (73.7)	16/21 (76.2)	16/20 (80.0)
Any HR-HPV positive (%)	10/12 (83.3)	11/13 (84.6)	19/19 (100)	14/14 (100)	15/16 (93.8)	14/16 (80.0)
HPV16 positive (%)	3/12 (75.0)	4/13 (30.8)	8/19 (42.1)	5/14 (35.7)	6/16 (37.5)	6/16 (37.5)
HPV52 positive (%)	4/12 (33.3)	1/13 (7.7)	4/19 (21.1)	6/14 (42.9)	5/16 (31.3)	5/16 (31.3)
Number of types detected per sample						
1	3/12 (25.0)	5/13 (38.5)	5/19 (26.3)	3/14 (21.4)	4/16 (25.0)	6/16 (37.5)

≥2	9/12 (75.0)	8/13 (61.5)	14/19 (73.7)	11/14 (78.6)	12/16 (75.0)	10/16 (62.5)
Oral HPV carriage						
Any HPV positive (%)	4/24 (16.7)	3/27 (11.1)	4/27 (14.8)	4/20 (20.0)	5/22 (22.7)	6/21 (28.6)
Any HR-HPV positive (%)	2/4 (50.0)	2/3 (66.7)	4/4 (100)	4/4 (100)	5/5 (100)	6/6 (100)
HPV16 positive (%)	1/4 (25.0)	0/3 (0)	3/4 (75.0)	2/4 (50.0)	2/5 (40.0)	1/6 (16.7)
HPV52 positive (%)	0/4 (0)	0/3 (0)	0/4 (0)	0/4 (0)	0/5 (0)	0/6 (0)
Number of types detected per sample						
1	4/4 (100)	2/3 (66.7%)	2/4 (50.0)	3/4 (75.0)	3/5 (60.0)	6/6 (100)
≥2	0/4 (0)	1/3 (33.3%)	2/4 (50.0)	1/4 (25.0)	2/5 (40.0)	0/6 (0)
Cervical HPV carriage						
Any HPV positive (%)	--	--	2/2 (100)	2/2 (100)	2/2 (100)	1/2 (50.0)
Any HR-HPV positive (%)	--	--	2/2 (100)	2/2 (100)	2/2 (100)	1/1 (100)
HPV16 positive (%)	--	--	1/2 (50.0)	1/2 (50.0)	1/2 (50.0)	1/1 (100)
HPV52 positive (%)	--	--	1/2 (50.0)	1/2 (50.0)	1/2 (50.0)	0/1 (0)
Number of types detected per sample						
1	--	--	1/2 (50.0)	1/2 (50.0)	1/2 (50.0)	0/1 (0)
≥2	--	--	1/2 (50.0)	1/2 (50.0)	1/2 (50.0)	1/1 (100)
HPV16 and 52 E6-specific immune responses ¹ (%)	2/32 (6.3)	9/34 (26.5)	0/25 (0)	1/19 (5.3)	1/20 (5.0)	0/20 (0)
Responses to memory recall mix (MRM) containing tetanus and CMV peptides (%)	21/32 (65.6)	20/34 (58.8)	10/25 (40.0)	8/19 (42.1)	10/20 (50.0)	9/20 (45)

577 HIV, human immunodeficiency virus; HPV, human papillomavirus; HR-HPV, high-risk HPV genotypes; oIR, optimal immunological
578 responder; sIR, suboptimal immunological responder.¹HPV16 and HPV52 E6 antigen-specific immune responses as determined by IFN- γ
579 ELISPOT.

580 **Table 4** Univariate analysis correlating HPV carriage to risk factors and clinical parameters

581

Parameter	Coefficient	OR (95% CI)	<i>p</i> -value
Anal HPV carriage			
Age	-0.011	0.99 (0.93-1.06)	0.757
Smoking	-0.241	0.79 (0.28-2.23)	0.651
Engagement in anal sex	1.088	2.97 (0.98-9.02)	0.055
Number of lifetime sexual partners	0.272	1.31 (0.23-7.38)	0.758
1	0	1	
≥2	0.272	1.31 (0.23-7.38)	0.758
Sexual orientation			
Heterosexual	0	1	
Homosexual	0.981	2.7 (0.90-7.89)	0.077
Bisexual	0.693	2 (0.16-24.87)	0.590
Circumcised	0.713	2.04 (0.59-6.96)	0.255
HSV-1 antibody			
Not detected	0	1	
Detected	-0.229	0.80 (0.20-3.13)	0.743
HSV-2 antibody			
Not detected	0	1	
Detected	0.229	1.26 (0.32-4.94)	0.743
Oral HPV carriage			
Age	-0.057	0.94 (0.87-1.03)	0.173
Smoking history	0.603	1.83 (0.51-6.62)	0.358
Alcohol consumption	2.228	9.28 (1.09-79.40)	0.042
Engagement in oral sex	0.264	1.30 (0.32-5.26)	0.711
Number of lifetime sexual partners			
1	0	1	
≥2	19.439	276938527.31 (0)	0.999
Sexual orientation			
Heterosexual	0	1	
Homosexual	-2.708	5.19 (1.03-26.23)	0.046

Bisexual	2.015	7.5 (0.46-122.70)	0.158
HSV-1 antibody			
Not detected	0	1	
Detected	-1.609	0.2 (0.034-1.19)	0.076
HSV-2 antibody			
Not detected	0	1	
Detected	-0.580	0.56 (0.096-3.28)	0.521

CI, confidence interval; HPV, human papillomavirus; HSV, herpes simplex virus; OR, odds ratio.

**p*-value <0.05 is considered statistically significant different. *p*-value of >0.25 were included into a multivariate analysis where no statistically significant relationship was demonstrated.

Table 5 Univariate analysis correlating the presence of HPV16 and 52 E6-specific immune responses to T-cell subsets

T-cell subsets	HPV16 and 52 E6-specific immune responses ¹		<i>p</i> -value
	Coefficient	OR (95% CI)	
CD4+ at recruitment (cells/μl)	0.002	1.00 (1.001-1.004)	0.01*
CD4:CD8 ratio	0.901	2.46 (0.65-9.34)	0.07
% CD4+CD45RA+CCR7+	0.005	1.01 (0.96-1.05)	0.83
% CD4+CD45RA-CCR7+	0.009	1.01 (0.95-1.08)	0.79
% CD4+CD45RA-CCR7-	-0.008	0.99 (0.94-1.05)	0.99
% CD4+CD45RA+CCR7-	-0.053	0.95 (0.80-1.13)	0.55
% CD8+CD45RA+CCR7+	0.002	1.00 (0.95-1.06)	0.94
% CD8+CD45RA-CCR7+	0.080	1.04 (0.89-1.22)	0.59
% CD8+CD45RA-CCR7-	-0.022	0.99 (0.93-1.03)	0.43
% CD8+CD45RA+CCR7-	0.017	1.02 (0.96-1.08)	0.54
% CD4+CD38+HLA-DR+	-0.248	0.78 (0.61-1.00)	0.03*
% CD8+CD38+HLA-DR+	-0.065	0.94 (0.88-1.00)	0.07
% CD4+CD28-CD57+	-0.032	0.97 (0.89-1.06)	0.72
% CD8+CD28-CD57+	-0.014	0.99 (0.95-1.03)	0.43
% CD4+CD25+Foxp3+	-0.059	0.94 (0.72-1.23)	0.96

CI, confidence interval; HPV, human papillomavirus; OR, odds ratio.

**p*-value <0.05 is considered statistically significant different. Univariate analysis showed a positive correlation between HPV16 and 52-E6 responses and CD4+ T-cells at recruitment, but inverse correlation with CD4+CD38+HLA-DR+ T-cells. *p*-value of >0.25 were included into a multivariate analysis where no statistically significant relationship was demonstrated. ¹HPV16 and HPV52 E6 antigen-specific immune responses as determined by IFN- γ ELISPOT.

Figure legends

Fig 1 Flow cytometry gating strategy for T-cell subsets. Panel A: Following gating to exclude doublets, CD3+ cells were selected and sequentially gated for CD4+ and CD8+ T- cells and subsequently for the maturational subsets of naïve (CD45RA+CCR7+), central memory, CM (CD45RA-CCR7+), effector memory, EM (CD45RA-CCR7-) and terminally-differentiated effector memory, TdEM (CD45RA+CCR7-). Panel B: Following gating to exclude doublets, CD3+ cells were selected and sequentially gated for CD4+ and CD8+ T-cells and subsequently for activation (CD38+HLA-DR+) and senescence markers (CD57+CD28-) cells, respectively. T-regulatory cells were reported as percentage of CD3+CD4+ T-cells expressing CD25+Foxp3+.

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Leng, CY;Low, HC;Chua, LL;Chong, ML;Sulaiman, H;Azwa, I;Roberts, JM;Kamarulzaman, A;Rajasuriar, R;Woo, YL

Title:

Human papillomavirus 16 (HPV16) and HPV52 E6-specific immunity in HIV-infected adults on combination antiretroviral therapy

Date:

2017-05-01

Citation:

Leng, C. Y., Low, H. C., Chua, L. L., Chong, M. L., Sulaiman, H., Azwa, I., Roberts, J. M., Kamarulzaman, A., Rajasuriar, R. & Woo, Y. L. (2017). Human papillomavirus 16 (HPV16) and HPV52 E6-specific immunity in HIV-infected adults on combination antiretroviral therapy. HIV MEDICINE, 18 (5), pp.321-331. <https://doi.org/10.1111/hiv.12432>.

Persistent Link:

<http://hdl.handle.net/11343/291750>