HPV16 AND HPV52 E6-SPECIFIC IMMUNITY IN HIV-INFECTED ADULTS ON COMBINATION ANTIRETROVIRAL THERAPY

LENG CHAN YOON

FACULTY OF MEDICINE
UNIVERSITY OF MALAYA
KUALA LUMPUR

2017

UNIVERSITY OF MALAYA

ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: Leng Chan Yoon [redacted]

Registration/Matric No: MGN130020

Name of Degree: Master of Medical Science

Title of Project Dissertation:

HPV16 AND HPV52 E6-SPECIFIC IMMUNITY IN HIV-INFECTED ADULTS ON COMBINATION ANTIRETROVIRAL THERAPY

Field of Study:

I do solemnly and sincerely declare that:

(1) I am the sole author/writer of this Work;
(2) This Work is original;
(3) Any use of any work in which copyright exists was done by way of fair dealing and for permitted purposes and any excerpt or extract from, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the title of the Work and its authorship have been acknowledged in this Work;
(4) I do not have any actual knowledge nor do I ought reasonably to know that the making of this work constitutes an infringement of any copyright work;
(5) I hereby assign all and every rights in the copyright to this Work to the University of Malaya ("UM"), who henceforth shall be owner of the copyright in this Work and that any reproduction or use in any form or by any means whatsoever is prohibited without the written consent of UM having been first had and obtained;
(6) I am fully aware that if in the course of making this Work I have infringed any copyright whether intentionally or otherwise, I may be subject to legal action or any other action as [redacted]ermined by UM.

Candidate's Signature [redacted] Date: 3/4/2017

Subscribed and solemnly declared before,

Witness's Signature [redacted] Date: 5.4.2017.

Name: WOO YIN LING

Designation: PROFESSOR OF O+G

ABSTRACT

Human papillomavirus (HPV) infection and its associated cancers disproportionately affect those infected with human immunodeficiency virus (HIV) despite effective combination antiretroviral therapy (cART). Cell-mediated immune (CMI) responses against HPV E6 antigen have been associated with clearance of HPV infection or its associated diseases. This study aims to investigate the presence of HPV16 and 52 E6-specific T cell responses in HIV+ patients on cART with suboptimal (sIR) and optimal (oIR) immune reconstitution. A cross-sectional cohort (n= 67) consisting of patients with sIR (CD4+T cell <350cells/mm^3) and oIR (CD4+T cell >500cells/mm^3) after a minimum of 2-years on cART with sustained suppressive viral load RNA <50copies/ml over a year following cART were recruited from Infectious Diseases clinic in University of Malaya Medical Centre (UMMC). A detailed questionnaire on socio-demographic information was administered. Specimens obtained from each participant included whole blood, an anal swab and oral rinse. HPV DNA genotyping test was performed on anal and oral specimens. Anal cytology was also undertaken. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood for interferon (IFN)-γ ELISPOT and flow cytometry. IFNγ ELISPOT was used to detected HPV16 and HPV52 E6-specific T cell immune responses whereas flow cytometry was to identify the immunophenotypes of CD4+ and CD8+T cells at systemic level. All HIV+ individuals received non-nucleoside reverse transcriptase inhibitors (NNRTI)-based cART. As compared to oIR group, sIR group exhibited lower baseline of CD4+ T cell count (28 vs 190cells/mm^3, p<0.001), CD8+ T cell count (473 vs 711cells/mm^3, p=0.011) and CD4/CD8 ratio (0.07 vs 0.22, p<0.001). Those in the sIR group had more AIDS-defining illnesses compared to oIR group (84.4% vs 54.3%, p=0.008). Despite suppressive viral load, sIR showed higher percentage of CD4+ central memory T cell (T_{CM}) (46.2% vs 38.1%, p<0.05) and effector memory T cell (T_{EM}) in both CD4+ (26.8% vs 19.0%, p=0.001) and CD8+ (56.1% vs

43.6%, p<0.05) subsets, with lower naïve CD4+ (24.9% vs 36.4%, p<0.05) and CD8+ (8.1% vs 15.7%, p<0.05) T cells compared to those with oIR. In addition, higher percentage of regulatory T cells (Treg), activated and senescent CD4+ and CD8+ T cells were observed in sIR compared to oIR groups. The prevalence of oral (16.7 vs 11.1%, p=0.57) and anal (57.1% vs 56.5%, p=0.97) HPV DNA between sIR and oIR groups were similar. In multivariate analysis, age of cART initiation (OR=1.21 [95% CI 1.04-1.40], p=0.01), and serum HsCRP level (OR=908 [95% CI 4.3-191720], p=0.01) were risk factors correlating to anal HPV carriage. Furthermore, sIR (2/32, 6.3%) had significant lower HPV16 and 52 E6-specific CMI responses compared to oIR (9/34, 26.5%) (p=0.028) irrespective of anal and oral HPV carriage. Apart from CD4+T cell counts, no other immunological correlates to HPV16 and 52 E6-specific T cell responses. As a conclusion, a longitudinal study to evaluate the effect of HPV16 and 52-specific CMI responses among HIV+ adult with optimal immune reconstitution is recommended. Clinically, anal screening is recommended for HIV+ population, particularly those with poor immune reconstitution.

ABSTRAK

Penyakit berkaitan human papillomavirus (HPV) memberi kesan kepada pesakit human immunodeficiency virus (HIV) meskipun telah menerima gabungan terapi antiretroviral berkesan (cART). Tindak balas sel-pengantara imun terhadap antigen HPV E6 telah dikaitkan dengan pembersihan jangkitan HPV atau penyakit yang berkaitan dengan HPV. Kajian ini bertujuan untuk mengkaji kehadiran tindak balas spesifik E6 imun sel (CMI) terhadap HPV16 dan 52 atas kumpulan pesakit yang mencapai penyusunan semula imun yang suboptimal (sIR) dan optimum (oIR). Kohort (n = 67) yang terdiri daripada pesakit sIR (CD4 + T sel <350cells / mm^3) dan oIR (CD4 +T sel> 500cells /mm^3) yang menerima rawatan cART sekurang-kurangnya 2 tahun dengan berterusan beban virus RNA tertindas yang berterusan <50copies/ml selama lebih setahun telah direkrut daripada klinik penyakit berjangkit klinik di Pusat Perubatan Universiti Malaya, PPUM. Soal selidik yang terperinci mengenai maklumat sosio-demografik pesakit telah dijalankan. Spesimen yang diperolehi daripada setiap peserta termasuk darah, swab dubur dan bilas mulut. Ujian HPV DNA genotip telah dilakukan ke atas spesimen swab dubur dan bilas mulut. Spesimen swab dubur juga diproses untuk penilaian sitologi. Sel-sel mononuklear darah periferi (PBMCs) telah diasingkan daripada darah untuk ujian Inteferon (IFN)-γ ELISPOT dan "flow cytometry". IFNγ ELISPOT telah digunakan untuk mengesan tindak balas spesifik-E6 HPV16 dan 52 CMI manakala "flow cytometry" adalah untuk mengenal pasti immunofenotip bagi CD4+ dan CD8+T sel pada tahap sistemik. Semua HIV + individu telah menerima non-nucleoside reverse transcriptase inhibitor (NNRTI) berasaskan cART. Berbanding dengan kumpulan oIR, sIR menunjukkan garis dasar bilangan CD4+ T sel (28 vs 190cells/mm^3, p <0.001), CD8+ T sel (473 vs 711cells/mm^3, p = 0.011) dan nisbah CD4/CD8 (0.07 vs 0.22, p <0.001) yang lebih rendah. Individu daripada kumpulan sIR dijangkiti penyakit khusus AIDS berbanding dengan oIR (84.4% vs 54.3%, p = 0.008). Walaupun beban virus ditindas, sIR menunjukkan peratusan CD4+

sentral memori T sel (T_{CM}) (46.2% vs 38.1%, p <0.05) dan effector memori T sel (T_{EM}) dalam kedua-dua CD4+ (26.8% vs 19.0%, p = 0.001) dan CD8+ (56.1% vs 43.6%, p <0.05) subset yang lebih tinggi, dengan naif CD4+ (24.9% vs 36.4%, p <0.05) dan CD8+ T sel (8.1% vs 15.7%, p <0.05) yang lebih rendah berbanding oIR group. Di samping itu, kumpulan sIR juga menunjukkan peratusan sel-sel T regulatory (Treg), activated dan senescent CD4+ dan CD8+ yang lebih tinggi berbanding oIR. Prevalens oral (16.7 vs 11.1%, p = 0.57) dan dubur (57.1% vs 56.5%, p = 0.97) HPV DNA di antara kumpulan sIR dan oIR adalah lebih kurang sama. Berdasarkan analisis multivariat, umur bermula cART (OR=1.21 [95% CI 1.04-1.40], p=0.01), serum HsCRP level (OR=908 [95% CI 4.3-191720], p=0.01) adalah faktor risiko tak bersandar kepada dubur HPV. Tambahan pula, sIR (2/32, 6.3%) menunjukkan tindak balas spesifik-E6 terhadap HPV16 dan 52 CMI lebih rendah berbanding dengan oIR (9/34, 26.5%) (p = 0.028) tanpa mengira pembawaan HPV DNA dari dubur dan mulut. Selain daripada bilangan CD4+ T sel, tiada tindak balas imunologi yang lain dikaitkan dengan tindak balas T sel spesifik-E6 terhadap HPV16 dan 52. Kesimpulannya, jangka masa yang panjang diperlukan untuk pesakit HIV seperti yang perhatikan di kumpulan sIR adalah kumpulan yang berisiko tinggie dengan jangkitan HPV atau kanser berkaitannya. Oleh itu, pemeriksaan cytologi dubur adalah dicadangkan untuk mencegah penyakit ini di kalangan pesakit HPV. Kesimpulannya, kajian longitudinal untuk menilai kesan CMI khusus kepada HPV16 dan HPV52 di kalangan HIV + dewasa dengan optimum penyusunan semula imun adalah disyorkan. Secara klinikal, pemeriksaan dubur intensif dicadangkan untuk HIV + penduduk, terutamanya yang mempunyai penyusunan semula imun yang lemah.

ACKNOWLEDGEMENTS

Firstly, I would like to express my deepest gratitude to my supervisor, Prof. Dr. Woo Yin Ling from the Department of Obstetrics and Gynecology, Faculty of Medicine, University of Malaya (UM), who patiently provided me with guidance, assistance, and advice in completing this research project. I would like to acknowledge Dr. Reena Rajasuriar from the Department of Pharmacy, Faculty of Medicine, UM for her generous and helpful advice throughout my research project. Thank you to University of Malaya in providing financial support and the opportunity to conduct this project.

Special thanks to all my friends from Immunotherapeutic lab (ITL), Pediatric oncology lab (POL) and other seniors in Faculty of Medicine who have been so supportive, providing me with constructive suggestions throughout the research process. I am also very thankful to my ex-employers, Mr. Calvin Thien and Dr. Wong Yong Wee from DNA laboratories diagnostic lab who have shared their working experience and motivate me to pursue postgraduate studies. I would like to extend my gratitude to my beloved family for their support towards my postgraduate studies.

Last but not least, I deepest appreciate all the participants from University of Malaya Medical Center and Community Clinic for their willingness to participate. Hopefully, this research project would in turn be beneficial to the community.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

α	:	Alpha
aa	:	Amino acid
AIDS	:	Acquired immunodeficiency syndrome
Anal SILs	:	Anal squamous intraepithelial lesions
APC	:	Antigen-presenting cell
ASC-H	:	High-grade dysplasia cannot be excluded
ASCUS	:	Atypical squamous epithelial cell of undetermined significance
β	:	Beta
bp	:	Base pair
CART	:	Combination antiretroviral therapy
CCL	:	Chemokine (C-C motif) ligand
cDNA	:	Complementary DNA
cells/mm^3	:	Cells per milimeter
CIN	:	Cervical intraepithelial neoplasm
CMI	:	Cell-mediated immunity
CMV	:	Cytomegalovirus
Copies/ml	:	Copies per mililiter
CTL	:	Cytotoxic T lymphocyte
CXCL	:	Chemokine (C-X-C Motif) Ligand
DISC	:	Death-inducing signaling complex
DMSO	:	Dimethyl sulfoxide
DNA	:	Deoxyribonucleic acid
EBV	:	Epstein–Barr virus
EDTA	:	Ethylenediaminetetraacetic acid

EGFR	:	Epidermal growth factor receptor
ELISPOT	:	Enzyme-linked immunospot
≥	:	Equal or larger than
FBS	:	Fetal bovine serum
FLU	:	Influenza viruses
Interferon	:	Gamma
GI	:	Gastrointestinal tract
G2/M	:	Between Checkpoint 2 and mitosis phase in cell cycle
HBV	:	Hepatitis B virus
HGH	:	Human growth hormone
HIV	:	Human immunodeficiency virus
HLA		Human leukocyte antigen
HPV	:	Human papillomavirus
HR	:	High-risk
HsCRP	:	High sensitivity c-reactive protein
HSIL	:	High-grade cervical epithelial lesion
IFN	:	Interferon
IL	:	Interleukin
IMDM	:	Iscove's Modified Dulbecco's medium
IQR	:	Interquartile range
>	:	Larger than
LC	:	Langerhans cell
LCR	:	Long control region
LN_2	:	Liquid nitrogen
LPS	:	Lipopolysaccharides
LR	:	Low-risk

LSIL	:	Low-grade squamous intraepithelial lesions
MBT	:	Mycobacterium tuberculosis
MCP	:	Monocyte chemotactic protein
mg/dL	:	Milligram per deciliter
MIP	:	Macrophage inflammatory protein
MRM	:	Memory recall mix
mRNA	:	Messenger ribonucleic acid
MSM	:	Men who have sex with men
NC	:	Nitrocellulose
NK	:	Natural killer
NNRTI	:	Non-nucleoside reverse transcriptase inhibitors
NRTI	:	Nucleoside reverse transcriptase inhibitors
OR	:	Odd ratio
PBMC	:	Peripheral blood mononuclear cells
PCR	:	Polymerase chain reaction
PHA	:	Phytohaemagglutinin
oIR	:	Optimal immune reconstitution
ORF	:	Open reading frame
RLU	:	Relative light unit
RNA	:	Ribonucleic acid
RT-PCR	:	Reverse-transcription polymerase chain reaction
<	:	Smaller than
sIR	:	Suboptimal immune reconstitution
T_{CM}	:	Central memory T cell
TCR	:	T cell receptor
T_{dEM}	:	Differentiated effector T cell

T_{EM}	:	Effector memory T cell
Th	:	T-helper
TLR	:	Toll-like receptor
TNF	:	Tumour necrosis factor
Treg	:	Regulatory T cell
UMMC	:	University of Malaya Medical Centre
URR	:	Upstream regulatory region
UV	:	Ultraviolet
VL	:	Viral load

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Background of study

In the combination antiretroviral therapy (cART) era, the incidence of most acquired immunodeficiency syndrome (AIDS)-defining illnesses have decreased (Schwarcz, Chen, Vittinghoff, Hsu, & Schwarcz, 2013). In contrast, the impact of cART on human papillomavirus (HPV) infection or HPV-related diseases remains disproportionately high and appears to be increasing. Those living with human immunodeficiency virus (HIV) have 38-fold higher risk of developing anal cancer (Frisch, Biggar, & Goedert, 2000) and nearly 3-fold higher risk for oral cancer (Mellin, Friesland, Lewensohn, Dalianis, & Munck-Wikland, 2000) when compared to the general population. In particular, those with CD4+ T cell counts of less than 200cells/mm^3 (Ahdieh et al., 2000; Delmas et al., 2000; Rositch et al., 2013) were found to be at highest risk of HPV carriage.

In non HIV cohorts, studies have shown that poor functionality of poor functionality of HPV specific T-helper cells (de Jong et al., 2004; Luxton, Nath, Derias, Herbert, & Shepherd, 2003; Steele et al., 2005) and/ or increased regulatory T cells (Treg) (McCredie et al., 2008; Molling et al., 2007; Visser et al., 2007) were associated with the development of invasive cervical cancer. In addition, HPV-specific immune responses against E6 (one of the oncogenic genes in HPV genome) peptides are more frequently detected in the earlier stages of HPV associated lesion but not in most patients with advanced stages of HPV associated lesion including high-grade cervical epithelial lesion (HSIL) & invasive cancer (Woo et al., 2010). Together, the data suggest that the cellular immune responses play a crucial role to control the development of HPV associated diseases. However, whether the dynamics of HPV antigen-specific immunity in HIV-infected cohort is similar to that observed in non HIV cohort remains to be defined.

Therefore, the aim of this study is to explore the associations of cellular immunity against HPV among HIV-infected individuals receiving suppressive cART.

1.2 Objectives

The objectives of the present study are

a) To compare the HPV carriage in both oral and anal compartment of cART-treated HIV males with suboptimal immune reconstitution, sIR (CD4+T cell count at study entry <350cells/mm^2) and optimal immune reconstitution, oIR (CD4+T cell count at study entry >500cells/mm^2).

b) To identify clinical correlates to HPV carriage.

c) To measure HPV16 and HPV52 E6-specific immune responses among those with sIR and oIR.

d) To define the correlations between systemic immunophenotypes to HPV16 and HPV52 E6- specific immune responses.

CHAPTER 2: LITERATURE REVIEW

2.1 Human papillomavirus (HPV)

2.1.1 Biology of HPV

HPV is a non-enveloped small virus with an intact protein coat consisting of 72 capsomeres (Stern & Kitchener, 2008) with the outermost layer shaped as a spherical particle with a diameter of 55nm. HPV genome is a double-stranded circular DNA of approximately 8,000bp in size. The genome of HPV is divided into three regions- Early gene (E1, E2, E4 and E5), Late genes (L1 and L2) and Long Control Region (LCR) or Upstream Regulatory Region (URR). LCR region is a non-protein coding nucleotide segment and contains most of the regulatory elements for viral DNA replication and transcription. Early genes encode proteins mainly involved in cell proliferation, transcriptional regulation and can lead to instability of host cell genetic leading to formation of malignancy whereas late genes is responsible for formation of viral capsid proteins (Table 2.1).

As L1 gene is highly conserved compared to other HPV genes, International Committee on the Taxonomy of Viruses (ICTV) uses L1 gene to group Human papillomaviruses (HPV) into five genera which are Alpha-, Beta-, Gamma-, Mu- and Nu-PVs (de Villiers, Fauquet, Broker, Bernard, & zur Hausen, 2004). Besides, HPVs are also classified based on the clinical outcomes caused by the particular HPV type. HPV types found mainly in skin or anogenital warts are classified as low-risk (LR) HPV (HPV type 6, 11, 40, 42, 43, 44, 54, 61, 72, 81) whereas the high-risk (HR) HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59) types were associated with the development of invasive cancer and several types of HPV are grouped as probable HR types (HPV type 26, 53, and 66) due to insufficient cases for the study (Munoz et al., 2003).

Basically, HPV establishes infection in its hosts by initially penetrating the epithelium through microabrasions and infect mitotically active epithelial stem cells that are located in the basal epithelial cell layer (Figure 2.1). At first, the viral capsid interacts with the cytoplasmic membrane of host cells. After binding, their genome is internalized via clathrin-dependent endocytic pathway (P. M. Day, Lowy, & Schiller, 2003) and then transferred into nucleus of host cells (Doorbar, 2005; Kines, Thompson, Lowy, Schiller, & Day, 2009). The virions in the infected basal cells replicate their DNA episomally at relatively low copy number of about 50 to 200 or so copies per cell (K. Kim & Lambert, 2002) and are highly dependents on the host cellular DNA-replication machinery (Frazer, 2004; Tommasino, 2013). All the non structural proteins (E1, E2, E5, E6 and E7) are expressed at this stage whereas the E1 and E2 proteins initiates the replication of viral episomes during host cell division.

As cells divide, the viral episome is distributed to all daughter cells. As a result, some daughter cells remain at the basal membrane and become reservoir of viral genome for further cell division (Stubenrauch & Laimins, 1999) whereas others are pushed vertically from the basal membrane to the superbasal layer of epithelium and proceed into terminally differentiated keratinocyte which then exit the cell cycle. The viral proteins E6 and E7 in terminally differentiated keratinocyte disturbs the cell-cycle control checkpoints in order to maintain the viral genome replication in the cells (Funk et al., 1997; Jones, Alani, & Münger, 1997). In this amplification stage, the number of HPV genome is amplified to more than 1000 copies per cell (Tommasino, 2013). In HR HPV infection, the E6 and E7 genes are commonly found to be tightly incorporated to host cell DNA with a portion of viral genome deleted (Schwarz et al., 1985) as HPV related disease progress to malignancy. In comparison, the function of E6 and E7 genes of LR HPV is less effective compared to the former (Klingelhutz & Roman, 2012).

Thereafter, virus L1 and L2 proteins are expressed, allowing assembly of viral capsid and genome. Finally, the viral particles are produced and released only when the infected cells reach the epithelial surface as HPVs are non-lytic (Kadaja, Silla, Ustav, & Ustav, 2009). This is known as the productive phase of the infective cycle.

Table 2.1: HPV functional genes

Gene	Main function	References
E1	1) A DNA helicase/ATPase 2) Interact with E2 gene to facilitate viral DNA replication.	(Chiang et al., 1992; Hughes & Romanos, 1993)
E2	1) Forms a complex with E1 for efficient origin recognition and activate viral genome replication. 2) Tethers viral genome to mitotic chromosomes segregation during host cell division. 3) As transcriptional repressor to regulate expression of HPV E6 and E7 proteins.	(Bernard et al., 1989; Chiang et al., 1992; McPhillips, Oliveira, Spindler, Mitra, & McBride, 2006)
E4	1) Interacts with cytokeratins and induces collapse of the intermediate filament network to release viral particles. 2) Suppresses cell growth by arresting the cell cycle at G_2/M boundary	(Doorbar, Ely, Sterling, Mclean, & Crawford, 1991)
E5	1) Delays endosomal acidification in keratinocytes resulted in enhancement of the activity of the epidermal growth factor receptor (EGFR) in the presence of ligand. 2) Prevents cell apoptosis during early stages of viral infection by downregulating the amount of tumor necrosis factor (Fas) receptor and altering the formation of Death-Inducing Signalling Complex (DISC). 3) Helps in immune evasion by down-regulating expression of HLA class. I	(Ashrafi, Haghshenas, Marchetti, O'Brien, & Campo, 2005; Kabsch & Alonso, 2002; Straight, Hinkle, Jewers, & Mccance, 1993)
E6	1) Degrades p53 and Bak to resist apoptosis and chromosomal instability. 2) Telomerase activation. 3) With expression of E7 to stimulate cell cycle progression.	(Doorbar et al., 2012; Havre, Yuan, Hedrick, Cho, & Glazer, 1995; Jackson, Harwood, Thomas, Banks, & Storey, 2000; Veldman, Horikawa, Barrett, & Schlegel, 2001)

Table 2.1: HPV functional genes (Continued)

Gene	Main function	References
E7	1) Degrade and associate with cellular tumour suppressor, pRb. 2) With expression of E6 to progress the neoplasia to invasive cancer stage.	(Doorbar et al., 2012; Dyson, Howley, Munger, & Harlow, 1989)
L1	1) Major capsid protein. 2) Facilitates the viral particle infect host cells.	(Buck, Day, & Trus, 2013; Giroglou, Florin, Schäfer, Streeck, & Sapp, 2001)
L2	1) Minor capsid protein. 2) Facilitates the capsid assembly. 3) Enhances the internalization of viral into host cells.	(Holmgren, Patterson, Ozbun, & Lambert, 2005; Ishii, Ozaki, Tanaka, & Kanda, 2005)

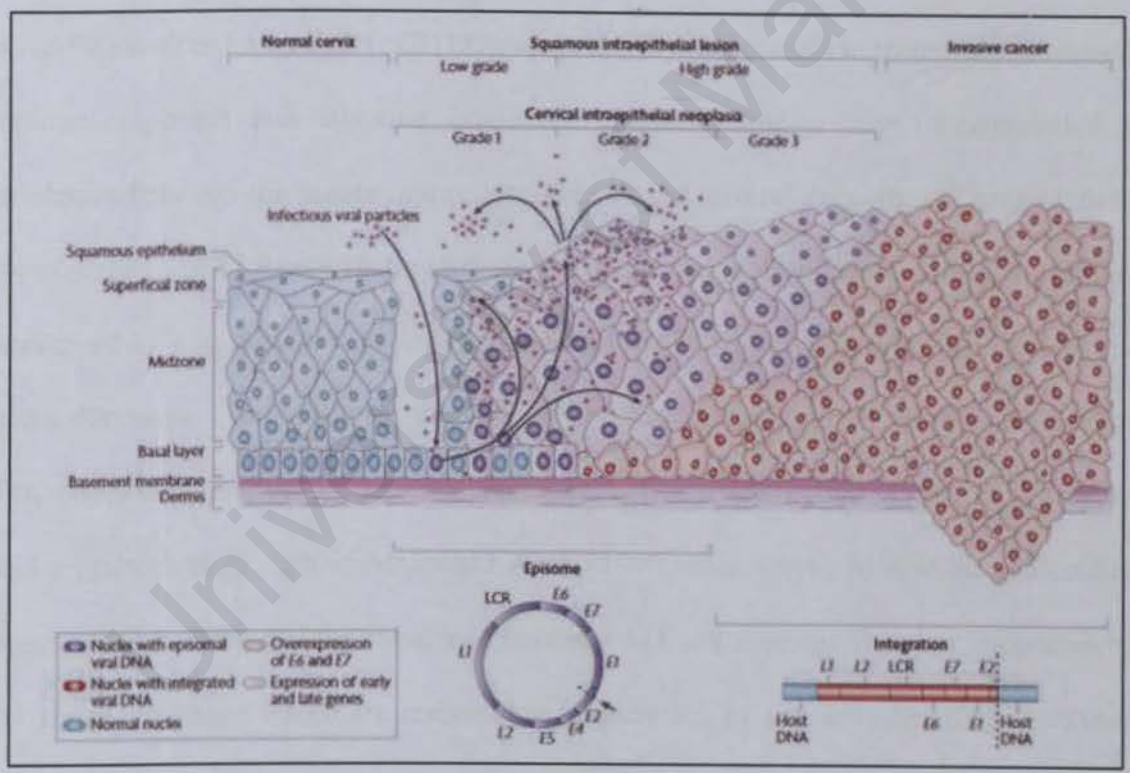

Figure 2.1: Life cycle of HPV
(Woodman, Collins, & Young, 2007)

2.1.2 The innate and adaptive immunity against HPV

In the general population, about 90% of HPV infections spontaneously clear while 10% persists with less than 1% progressing to cancer (Gadducci, Barsotti, Cosio, Domenici, & Riccardo Genazzani, 2011). Patients with spontaneous clearance of HPV infection or regression of HPV-associated diseases (warts and premalignant lesions) are usually characterized by high level of cellular immune responses compared to patients with persistence of HPV infection and progressive lesions (de Jong et al., 2002; van Esch et al., 2015; Woo et al., 2010). These observations point toward the important roles of cellular immunity against HPV infection.

As the HPV life cycle is confined to the epithelium, professional antigen-presenting cell (APC) such as Langerhans cell (LC), are primarily responsible in triggering the innate immune responses and adaptive immune responses. Studies have demonstrated a correlation between the innate immune responses and clinical outcomes. These include low expression of TLRs correlating to infection persistence (Daud et al., 2011), decreased number of LCs in cervical intraepithelial neoplasia (CIN) compared to normal cervix (Mota, Rayment, Chong, Singer, & Chain, 1999), downregulation of receptor on natural killer (NK) cells leading to decrease cytotoxic activity, (Arreygue-Garcia et al., 2008; Garcia-Iglesias et al., 2009; Majewski et al., 1990; Malejczyk, Majewski, Jablonska, Rogozinski, & Orth, 1989), imbalance between M1 macrophage (tumour suppressive) and M2 macrophage which are responsible in secretion of anti-inflammatory cytokines observed in HPV-induced vulvar or cervical carcinoma (Ambarus et al., 2012; van Esch et al., 2015). However, many details underlying the mechanisms on how innate immunity cells in respond to HPV infection remains unclear.

The adaptive immunity on the other hand, comprises of pathogen-specific T lymphocytes, HPV antigen-specific memory CD4+ T helper (Th) cells and CD8+ cytotoxic T lymphocyte (CTL) are generated in response to HPV infection and detected at systemic level as well as the local infection site (Passmore et al., 2006). The presence of HPV-specific memory T cells correlates to the clearance of HPV or regression of HPV-related diseases (Coleman et al., 1994; Woo et al., 2008).

Peripheral HPV E2-specific memory T cell responses are commonly detected in healthy individuals with no active HPV infection or diseases suggesting that they have successfully cleared the infection (de Jong et al., 2002), regression of HPV-induced lesions (Woo et al., 2008) or in children whose mothers have incidence of cervical intraepithelial neoplasia (CIN) (Koskimaa et al., 2014). Based on the functional role of E2 protein, HPV E2-specific memory T cells are predominantly associated with effective control of HPV infection because E2 protein plays a key role to initiate HPV life cycle. In contrast to E2 proteins, E6 and E7 proteins, the initiators of carcinogenesis and are expressed in large amounts in the later stage of diseases at which cellular morphology changes are usually observed. This is consistent with the observation that immune responses including lymphoproliferation, IFN_{γ}-secreting T cells and CTL against synthetic E6 and E7 peptides frequently detected in patients with low grade of intraepithelial lesion, regression of intraepithelial lesion and healthy individuals, as well as clearance of HPV (Chan et al., 2011; Kadish et al., 1997; K. H. Kim et al., 2012; Nakagawa et al., 1997; Nakagawa et al., 2000; Woo et al., 2008). However, there are inconsistencies in that several studies show that immune responses to E7 peptides are not commonly detected regardless of the infection and diseases status as compared to E2 and E6 peptides (Koskimaa et al., 2014; Woo et al., 2010). This may be due to the length of E7 amino acid (aa) which is shorter than E6 and thus decreasing the chances of exposure to T cells. Consistently, the collective detection of all HPV E2, E6 and E7-specific

immune cells are rarely found in patients with cancers, recurrence and progression of intraepithelial lesion, as well as persistence of HPV infection in all the studies.

The expansion of antigen-specific memory T cells and the diversity of T cell subsets with different functional roles are the unique properties found in adaptive immunity. Studies have shown that the capacity of lymphoproliferation in response to HPV16 E6 and E7 peptides correlates to the grades of CIN (de Jong et al., 2004; Kadish et al., 1997; Nakagawa et al., 1996). However, it has also been demonstrated that lymphocyte proliferation may be associated with a loss of effector function or imbalance of Th type 1 (Th 1) and Th type 2 (Th 2) cytokines (de Jong et al., 2004). The predominant secretion of IL-10, the Th 2 cytokine suppresses the Th1 promoting inflammatory microenvironment, promotes humoral immunity and tumour growth in patients with CIN III and cervical cancer (de Jong et al., 2004; Koskimaa et al., 2014). Regulatory T cells (Treg) is another T cell subset that has been shown to suppress or inhibit clearance of HPV infection, and is associated with progression of HPV caused diseases (Kojima et al., 2013; Russell et al., 2013). In addition, high levels of Th17 with Th17 cytokines are detected in patients with persistent HPV infection, high grade of CIN and cervical cancer compared to healthy individuals (Z. Chen et al., 2013; Gosmann, Mattarollo, Bridge, Frazer, & Blumenthal, 2014; Molling et al., 2007). Chen and colleagues suggested that the imbalance of Th17/Treg ratio can be a predictor for the development of cervical cancer (Z. Chen et al., 2013) when they demonstrated that level of IFN_{γ} to be negatively correlated to Treg cell ($CD4+CD25+CD127^{low/-}$) numbers whilst Th17 cells promoted tumour angiogenesis and development of tumour. However, the knowledge of TH17 is limited and more study on this line of T cells is required.

In the presence of Th2 cytokine, B cells develop to initiate humoral immunity required to opsonize and kill the viral particle (Sasagawa, Takagi, & Makinoda, 2012). Seroconversion (serum IgG) has been detected within 18 months of the detecting HPV DNA in only 50-70% of HPV-infected women while some with persistent HPV infection never serocenverted (Carter et al., 2000). Such weak humoral immune responses observed in natural HPV infection may be because transient HPV DNA leads to failure in seroconversion or limited access of the viral particle load to lymph node where specific immune responses are initiated (Carter et al., 2000; Stanley, 2012).

All these findings support the important role of cellular immunity against HPV. This has been observed particularly among untreated HIV+ patients with CD4+T cell count less than 200cells/mm^3 were found to be at highest risk of HPV infection compared to those with CD4+T cell count >200cells/ mm^3 (Ahdieh et al., 2000; Delmas et al., 2000; Rositch et al., 2013). Thus, it is expected that cART would reduce the incidence of HPV infection and related diseases after optimal CD4 recovery. However, the recovery of CD4+T cell count ($\geq$500cells/mm^3) by cART has not been shown to consistently reduce the incidence of HPV infection and related diseases. Therefore, the impact of cART remains inconsistent and inconclusive. These inconsistent outcomes may due to the diversity in the cohort studied, differences in length of follow-up time, lack of standard diagnostic assessment for HPV DNA from clinical specimen, definition of immune reconstitution based on CD4+T cell counts.

2.1.3 Global burden of HPV infection and related diseases in general and HIV populations

The global prevalence of HPV infection is about 11% (Bruni et al., 2010). The five most common HPV types detected worldwide include HPV16, 18, 52 and 58 whereby HPV52 and 58 are relatively higher in Asia (Chan et al., 1999; C. M. Ho, Chien, Huang, Lee, & Chang, 2006; Sasagawa, Basha, Yamazaki, & Inoue, 2001). There are molecular and epidemiologic evidence showing that about 100% of cervical cancers, 90-93% of anal cancers, 40-64% of vaginal cancer, 40-51% of vulvar cancer, 36-40% of penile cancers and 12-63% of oropharyngeal cancers are attributed to HPV infection (Gillison, Chaturvedi, & Lowy, 2008; Parkin & Bray, 2006). Moreover, oncogenic HPV16 and 18 contributes to the majority of these cancer cases. Taken together, approximately 5.2% of all cancers that occur worldwide are attributed to HPV infection (Piirsoo, Ustav, Mandel, Stenlund, & Ustav, 1996). In addition, the incidence rate of HPV associated cancers shows an increasing trend over the years (Figure 2.2).

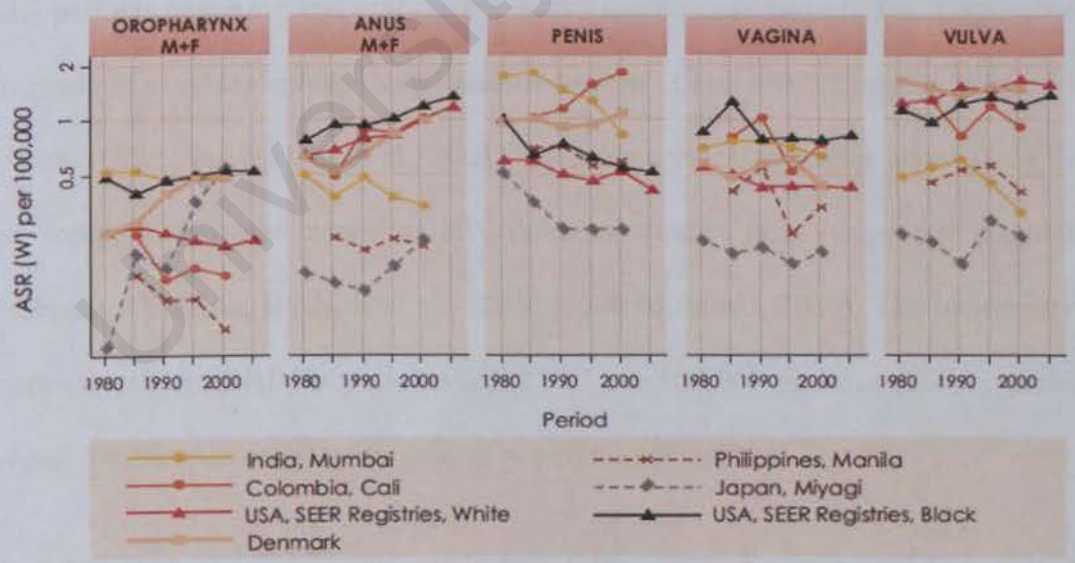

Figure 2.2: Incidence rate of HPV associated cancer
(Forman et al., 2012)

Among those living with HIV, the incidence of HPV associated diseases are increasing and higher than in the general population, particularly among those HIV-infected patients with CD4+ cell count <200cells/mm^3 (Delmas et al., 2000; Liu et al., 2015). It was hoped that the introduction of cART resulting in elevation of CD4+ level would lead to a reduction in the incidence of high grade cervical intraepithelial lesions and cervical cancer. Unfortunately, the effect of cART on HPV associated diseases remains uncertain.

Cervical cancer is the third most common cancer among women worldwide with an estimated incidence of new cases increasing from 493,000 to 530,000 new cases and 274,000 to 275,000 deaths between 2002 and 2008 (Ferlay et al., 2010; Parkin, Bray, Ferlay, & Pisani, 2005). About 70-76% of cervical cancer cases are contributed by HPV16 or 18 (Gillison et al., 2008; Parkin & Bray, 2006). HPV infection is common in sexually active young women with age of 18 to 30 years old. After 30 years of age, the prevalence of HPV infection decrease and cervical cancer is more common in women older than 35 years (Burd, 2003). In addition to age, other risk factors including multiple sexual partners, smoking and oral contraceptive is also contribute in the progression of high grade of cervical intraepithelial lesions (Adam et al., 2000; Lippman et al., 2010; Moscicki, Hills, Shiboski, & et al., 2001). Invasive cervical cancer is about three times more frequent and persistence in HIV-infected women than in general population (Ellerbrock, Chiasson, Bush, & et al., 2000; Jay & Moscicki, 2000). The incidence rate of cervical cancer in HIV population was 134.5 per 100,000 person-years compared to 11.4 per 100,000 person-years in general population (Patel et al., 2008).

In contrast, anal cancer is relatively rare in the general population. However, this disease is the second most common HPV associated cancer after cervical cancer with an estimated 27,000 new cases in 2008 (de Martel et al., 2012). About 93% of anal cancers are caused by HPV16 or 18 (Gillison et al., 2008; Parkin & Bray, 2006). Anal cancer is

more common in women compared to men in general population and is significantly higher among men who have sex with men (MSM) and in particular HIV-infected MSM (J. M. Palefsky et al., 1998; van Rijn et al., 2014). In the general population, the annual incidence of anal cancer is about 1 per 100,000 with an increasing rate of 2% per year (Johnson, Madeleine, Newcomer, Schwartz, & Daling, 2004). Inversely, the incidence of anal cancer among HIV-infected from Europe and the United States is about 14 to 78 cases per 100,000 person-years (D'Souza et al., 2008; Patel et al., 2008; Piketty et al., 2008). Furthermore, these studies show that the incidence for anal cancer among those living with HIV is increasing over the years. Unlike women where the prevalence declines after age of 30, anal HPV in HIV negative- MSM remains high and constant throughout life (Chin-Hong & Palefsky, 2005; Schiffman, 1992).

About 25% of oropharyngeal cancers is caused by HPV in general population (Kreimer, Clifford, Boyle, & Franceschi, 2005). HPV associated-oropharyngeal cancers are restricted to lingual and palatine tonsils, base of tongue and back of throat (Jemal et al., 2013) in both males and females. HPV16 is the most commonly associated HPV type even more than in cervical cancers (Syrjänen et al., 2011). The estimated new cases for this cancer associated with HPV is 22,000 in year 2008 (de Martel et al., 2012). The incidence rates of oropharyngeal cancers are highly dependent on geographical regions, for instance, cancers of mouth and of tongue generally predominate in developing countries whereas pharyngeal cancers are more common in developed countries. The risk factors for HPV associated-oropharyngeal cancers are smoking, alcohol consumption as well as oral sex (Cubie, 2013). Studies have observed that HIV population have a 2-4 fold higher odds of oral HPV infection compared to the general population (Elford, 2006; Shiels, Cole, Kirk, & Poole, 2009). Unlike anal cancer, HIV-infected heterosexual males have a higher incidence of oral HPV as compared to MSM and drug injection users (Aidala, Lee, Garbers, & Chiasson, 2006; Chaturvedi, Madeleine, Biggar, & Engels,

2009; Schneider et al., 2005). However, the incidence rates of oral HR HPV infection in HIV-infected MSM is still higher than HIV-uninfected MSM (1.3-3.5 per 1000 person months vs 0.2-1.1 per 1000 person months) (van Aar et al., 2014).

Penile cancer which is restricted to men, especially in less developed countries is a rare disease. Penile cancer is second lower incidence among HPV-associated cancer followed by vaginal cancer (de Martel et al., 2012). In contrast to cervical cancer, incidence of penile cancer increases after age of 50 years (Giuliano et al., 2008). Risk factors associated with penile HPV have been included multiple sexual partners (Svare et al., 2002), lack of circumcision (Castellsague et al., 2002; Lajous et al., 2005), having sexual partners with CIN (Bleeker et al., 2002), a history of other sexually transmitted infections (Lajous et al., 2005), and a history of smoking (Partridge et al., 2007).

Warts is the most common HPV-associated diseases. Generally, warts spread by direct contact from person to person or indirectly by contaminated surfaces or objects. Histologically, warts are benign lesions with hypertrophy of all layers of the dermis, resulting in thickening, folding and hyperkeratosis (increase in the horny layer) often with abnormal keratohyaline granules. Warts usually disappear spontaneously but sometimes may be resistant and treatment may be required (Cubie, 2013).

2.1.4 Anal squamous intraepithelial lesions (Anal SILs)

The dysplastic changes in the anal canal are collectively termed as anal squamous intraepithelial lesions Anal SILs. According to the Bethesda system of cytological classification, the anal dysplastic changes can be divided into atypical squamous epithelial cell of undetermined significance (ASCUS), high-grade dysplasia cannot be excluded (ASC-H), low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL) (Oon & Winter, 2010). A cytology specimen is interpreted as ASCUS if morphologic of cells is undetermined either normal or abnormal cells whereas ASC-H is identified if only a single abnormal cell show cytologic features of HSIL in a cytology specimen (Solomon, Davey, Kurman, & et al., 2002). LSILs demonstrates nuclear atypia and perinuclear cytoplasmic cavitation (koilocytosis), with a nucleus that is larger than those in a normal intermediate squamous cell on cytology (Apgar, Zoschnick, & Wright, 2003). Histologically, these lesions are classified as AIN I characterized by low nuclear/cytoplasmic ratios (koilocytes), atypical cells observed in the lower one third of the skin covering the anus (Longacre, Kong, & Welton, 2008). As HPV infection persists, the morphology of infected cells can progress from LSIL to HSIL with higher nuclear/cytoplasmic ratios on cytology, and cells sizes smaller than those with LSIL (Apgar et al., 2003). HSIL is characterized by high nuclear/cytoplasmic ratios and full-thickness atypia, which includes parabasal atypia, in the lower two thirds of the skin (Longacre et al., 2008). Furthermore, HSIL histologically divided into AIN II that characterized by moderate dysplasia that confined to lower two thirds of the epithelium and AIN III (or in situ carcinoma) which is severe dysplasia with full thickness (Martin & Bower, 2001).

2.1.5 HPV detection assays

2.1.5.1 Polymerase chain reaction (PCR) based assays

There are a variety of PCR based assays to detect HPV DNA in clinical specimens such as type-specific PCR, broad-spectrum PCR, real-time PCR, reverse-transcription PCR, PCR-restriction fragment length polymorphism and direct sequencing. The significant advantage of this assay is the ability to amplify a particular DNA sequence several million fold. However, the sensitivity and specificity of PCR based assays varies, depending on DNA extraction procedures, site and type of clinical specimen, type of primer, size of PCR product, reaction conditions, and ability to detect multiple types (Zaravinos, Mammas, Sourvinos, & Spandidos, 2009). In this subchapter, only a few assays which are frequently used by other studies are selected for review.

Type-specific PCR assay uses type-specific primers to amplify a particular single HPV genotype and detects the presence of HPV DNA in a single specimen. Multiple type-specific PCR reactions must be performed separately in order to know if the specimen has multiple HPV genotypes (Molijn, Kleter, Quint, & van Doorn, 2005). In contrast, consensus primers such as degenerated primer MY09/11, PGMY09/11 and GP5/6 pair primer can amplify a broad spectrum of HPV genotypes. The differences between these three sets of primer are the length of primers, number of primer in each primer set and target of nucleotide sequence in L1 gene. A study comparing the efficiency of PCR with GP5/6, MY09/11 and PGMY09/11 for the prevalence of HPV genotypes showed that the sensitivity of GP5/6 primer set was lower than the other two primer sets, especially in the detection of HPV52 (Chan et al., 2006).

Reverse-transcription polymerase chain reaction (RT-PCR) assay involves reverse transcriptase to reverse transcribe a targeted messenger ribonucleic acid (mRNA) into its complementary DNA (cDNA), amplified cDNA through PCR. Both E6 and E7 mRNA

are the favoured target for this assays as the expression of both oncoproteins increases in patients with persistent viral infection and severity of lesion (Wang-Johanning, Lu, Wang, Johnson, & Johanning, 2002). Therefore, this assay may be clinically more useful for clinical management.

2.1.5.2 Signal amplification-based detection method

Hybrid Capture II (Digene Corp., USA) is a nucleic acid amplification method based on the hybridization of the target HPV-DNA to labelled RNA probes in solution to generate semi-quantitative data. The RNA-DNA hybrids are captured by a specific monoclonal antibody coated on the surface of microplate well. Multiple alkaline phosphates- conjugated antibodies bind to each captured RNA-DNA hybrids to amplify the signal. Once the substrate is cleaved by the bound alkaline phosphatase, light is emitted and measured as relative light units (RLUs). The limitations identified including it does not identify specific HPV genotype, less sensitivity than PCR, and cross-reactivity of the RNA probes (for low and high risk) is observed (Castle et al., 2002; Poljak, Marin, Seme, & Vince, 2002).

2.1.5.3 Reverse dot-blot hybridization assay

This detection method involves amplification of DNA via PCR and hybridization of PCR product to multiple oligonucleotide probes. In general, a PCR product is generated with biotinylated primers. The PCR product is added and hybridized to correspond HPV-specific probes that are immobilized on a solid surface such as nitrocellulose (NC) membrance. After hybridization, a colourimetric reaction is performed. Each spot represent a HPV genotype. Linear array (Roche, USA) and HPV GenoArray Test kit (HybriBio Limited, HongKong) are examples of this detection method. HPV GenoArray Test kit is used in the present study.

2.1.6 Immunoassays

There are numerous assays such as intracellular cytokine staining (ICS) via flow cytometry, enzyme-linked immunosorbent assay (ELISA), enzyme-linked immunospot (ELISPOT), cytotoxic T lymphocyte (CTL) assay, lymphoproliferative assay, and peptide major histocompatibility complex (MHC) tetramers have been developed to quantify and qualify the immune responses to infection diseases. In this subchapter, only a few assays which are commonly use are discuss here.

2.1.6.1 ELISA and ELISPOT

Cytokine secretion by T cells in response to pathogens can be detected by measuring either cytokine at systemic level through enzyme-linked immunosorbent assay (ELISA) or enumerating the cytokine secreted by pathogen-specific T cell via enzyme-linked immunospot (ELISPOT) assay. Specimens for ELISA are serum and plasma whereas peripheral blood mononuclear cells (PBMCs) which is pre-stimulated by interest synthetic peptides is used for ELISPOT. The principle of both ELISA and ESLIPOT is similar where (i) cytokine specific-primary antibodies are coated on a solid surface such as NC and polyvinylidene fluoride (PVDF), (ii) specimen is added to primary antibodies and incubate for a period of time, (iii) secreted cytokines bind to primary antibodies, (iv) biotinylated cytokine specific- secondary antibodies are added and (v) detecting the antibody-cytokine complex after colorimetric reaction occurred between enzyme attaced to secondary antibody and subtrate (Clay, Hobeika, Mosca, Lyerly, & Morse, 2001). In ELISPOT, each spot represent one single cell secreting the interest cytokine. Therefore, this assay can provides a single cell information which is in contrast to ELISA that provide overall immunne responses of a given study subject based on the colour intensity of the tested solution.

2.1.6.2 Flow cytometry

Flow cytometry is a technology which can determine the phenotypes represented by the receptors expressed on the surface of immunity cells such as macrophrages, T lymphocytes. Additionally, flow cytometry can also quantify intracellular cytokine staining (ICS). Futhermore, ICS assay is able to measures multiple cytokines in a single reaction compared to ELISPOT. Similar to ELISPOT, ICS also provides single cell information.

2.1.6.3 Lymphoproliferative assay

Lymphoproliferative assay is to measure the ability of T cells to proliferate in response to antigen. PBMCs are stimulated with antigen in the presence of irradiated autologous antigen-presenting cell. [H^3]thymidine is added and cell proliferation is measured based on the synthesis of DNA where the radiolabeled thymidine incorporated into (Clay et al., 2001). The drawbacks of this assay are (i) the proliferation level of antigen stimulated T cell may not correlate to the number of antigen-specific T cells present such as high level of proliferation by a few cells or vice versa would give a similar proliferation level and (ii) it does not measure the effector function of T cells. This may overesimate the function of T cells. In the published data, T cell proliferation with poor cytokine secretion were observed among cancer patients (de Jong et al., 2004).

2.2 HIV infection

2.2.1 Global burden HIV infection

In 2013, 35 million people are estimated to be living with HIV with 2.1 million individuals newly infected with HIV globally (WHO 2014). Sub-Saharan Africa has the highest burden of HIV with 24.7 million persons infected with HIV and accounts for approximately 70% of the total global new cases of HIV infection. The number of acquired immune deficiency syndrome (AIDS)-related death declined from 2.3 million in 2005 to 1.5 million in 2013. This precipitous drop was attributed to the introduction of combination of antiretroviral therapy (cART). However, death due to non-AIDS defining illnesses such as non-AIDS-related cancers, cardiovascular disease and liver disease is still rising in the ART era (Palella et al., 2006; Sackoff, Hanna, Pfeiffer, & Torian, 2006). Sadly, not all the HIV-infected patients can access ART with only 12.9 million people living with HIV receiving ART (WHO 2014). Sexual activities, drug injection, blood transfusion, and mother-to-child transmission are reported to be the route of HIV transmission from infected individual to an uninfected individual.

Until 2014, 105,189 HIV infections cases were reported in Malaysia since reporting started in 1986 (Ministry of Health 2015). Malaysia was estimated to have 91, 848 people living with HIV by the end of 2014. About 3517 new HIV cases were reported, of which 697 cases were women. About 16.6% of 1445 injecting drug users, 7.3% of 839 female sex workers, 8.9% of 531 MSM, and 5.6% of 1247 transgender were diagnosed positive for HIV infection. The number of HIV cases in Malaysia decreased from 28.4 in 2002 to 11.7 cases per 100,000 populations in 2014. Similarly, a steady decline in the number of AIDS-related deaths have been reported after the introduction of ART.

2.2.2 The pathogenesis of HIV and its immune responses

HIV infection is transmitted through blood circulates to resting CD4+T cells in lymphoid tissues which act as the primary reservoir for the virus. Other CD4 receptor expressed-cells such as monocytes, macrophages, and dendritic cells are also targeted by HIV. There are three phases of HIV replication in an infected patients: Acute infection (or primary), chronic infection (or clinical latency) and onset of AIDS (Figure 2.3).

The phase of acute infection usually take about 2 to 4 weeks at which HIV replicates vigorously upon interaction with the target cells. This stage is characterized by severe viremia (about 10^7 or more copies of viral RNA per milliliter of blood) and with large numbers of circulating CD4+T cells as well as lymphoid tissues resulting in depletion of CD4+T cells. Within the first three weeks after presented to HIV, HIV-specific CD8+T cells against HIV antigens can be detected. At this stage, CTL controls the viral replication by exerting its cytotoxic effect. At the same time, anti-HIV antibodies appear indicating seroconversion in an infected individual after 2-3 months infection (Clark et al., 1991; Koup et al., 1994). At this stage, non-specific symptoms such as fever, diarrhea, generalized lymphadenopathy, pharyngitis and rash can be observed in infected patients (Palmisano & Vella, 2011).

The end of acute phase is characterized by dramatic decline of viremia in the presence of CTL to a set point that is maintained at a plateau or slowly increasing levels of viremia (McMichael & Dorrell, 2009). This slow progression or less active of viral replication is called chronic infection that usually take 1 to 20 years. At this stage, the number of CD4+T cells would recover to near normal concentration as a result of homeostasis response to the loss of CD4+T cells during acute infection. Gradually, the CD4+T cells decrease by about 50-100cells/mm^3 (Maartens, Celum, & Lewin, 2014) due to persistently activation of CD4+T cells by residual HIV infection and microbial

translocation (Hearps et al., 2011; Lane, 2010). An infected patient at this phase is clinically asymptomatic.

The reduction of CD4+T cell count in the blood indicates the onset of AIDS and patients are susceptible to opportunistic infections. This is followed by an increase in the plasma virus load, decrease in HIV-specific CD8+T cells and neutralizing antibodies and patients may die if remain untreated (Alimonti, Ball, & Fowke, 2003).

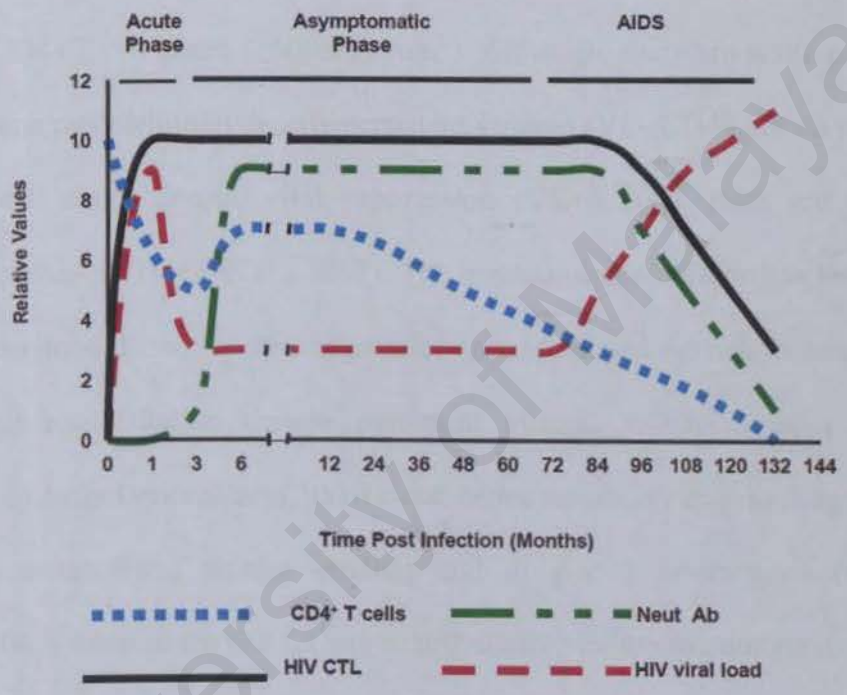

Figure 2.3: Kinetics of HIV infection
(Alimonti et al., 2003)

2.2.3 Alteration of CMI in HIV patients

cART was first introduce in late 1990s. A standard cART regimens combine two nucleoside reverse transcriptase inhibitors (NRTI) with a non-nucleoside reverse transcriptase inhibitor (NNRTI), protease inhibitor, or integrase inhibitor, fusion inhibitor (Maartens et al., 2014). Each of the antiretroviral drug is classified according to the HIV life cycle the drug inhibit (Figure 2.4).

Generally, the initiation of cART leads to reduction in HIV plasma viral load and increase in CD4+T cell count (≥500cells/mm^3). Although, there are some patients who exhibit immune reconstitution despite persistent viremia (VL-/CD4+) or do not increase in CD4+T cell count despite viral suppression (VL+/CD4-). Both are referred as discordant responses (Tuboi et al., 2007). The mechanisms of discordant responses are not fully understood. However, several studies have identified the risk factors associated with immune reconstitution despite persistent viremia which includes poor drug adherence, genetic polymorphisms, pharmacokinetics variability such as drug absorption, distribution, metabolism, protein binding and drug-drug interactions (Cressey & Lallemant, 2007) whereas the risk factors contributing to failure in immune reconstitution despite suppression viral include low baseline CD4+T cells and small thymic size (Fernandez, Price, McKinnon, Nolan, & French, 2006; Lawn, Myer, Bekker, & Wood, 2006; T. Li et al., 2011).

In the presence of cART, large amounts of gut associated CD4+T cell is depleted leading to increase in the mucosal permeability of gastrointestinal (GI) tract which in turn, allows the microorganisms residing in the GI tract and microbial products such as lipopolysaccharides (LPS) to translocate into blood vessel (Brenchley et al., 2006). In the presence of HIV, microbial translocation, and opportunistic infection, the immune cells in HIV patients are highly activated (CD38+HLA-DR+). As a result of immune

activation, pro-inflammatory cytokines such as IL-1β, TNFα and IL-6 are secreted to generate an inflammation environment to synergize the activation and proliferation of T cells against HIV infection (Decrion, Dichamp, Varin, & Herbein, 2005). Subsequently, the increasing numbers of activated memory T cells become the reservoir for HIV replication which leads to large amounts of T cells undergoing apoptosis and death while a stable of CD8+T cells pool is established during HIV infection (Ferreira, Barthlott, Garcia, Zamoyska, & Stockinger, 2000; Homann, Teyton, & Oldstone, 2001). Eventually, increasing expression of CD57 and lack of CD28 expression are observed in T cell population as a result of persistent T cells activation and expansion of memory T cells. The CD4+ and CD8+T cell activation drive the depletion of T cell, high level of inflammation and accelerating T cell senescence in this population (Hazenberg, Hamann, Schuitemaker, & Miedema, 2000; D. D. Ho et al., 1995; Mojumdar et al., 2011; B. E. Palmer, Blyveis, Fontenot, & Wilson, 2005; Papagno et al., 2004).

After implementation of cART, the incidence of AIDS-defining illnesses precipitously declined over years. Thus, the morbidity and mortality reduced while life expectancy extended (Michaels, Clark, & Kissinger, 1998; Schwarcz et al., 2013). Unfortunately, deaths resulting from non-AIDS defining illnesses such as liver, lung, anal, head and neck cancers, cardiovascular disease, bone disease, liver disease, kidney disease and neurocognitive disorder which usually observed in elderly healthy individuals have been increasing (Deeks, 2011; Wang, Silverberg, & Abrams, 2014). In healthy elderly individuals, immune activation and inflammation are found to promote an age-related decline of the functionality of the immune system, leading to immunosenescence (Franceschi et al., 1996). Similarly, cART-treated HIV patients exhibit high level of immune activation and senescence phenotypes compared to healthy controls but lower than untreated HIV patients (Hunt et al., 2003; Ruel et al., 2009). In the presence of persistent, immune activation activation-induced cell death with poor T cell restoration

(Kelley et al., 2009a) and, a progressive loss of the naïve and memory T cell pool are observed (Desai & Landay, 2010). Consequently, the onset of those pre-mature diseases are observed in cART-treated HIV patients (Deeks, 2011).

Clearly, cART successfully extends life expectancy of HIV-infected patients. Consequently, this provides sufficient lifespan to develop age-related diseases in the presence of persistence immune activation found in cART-treated patients despite viral suppression. Thus, cART fail to fully restore healthy of HIV-infected patients.

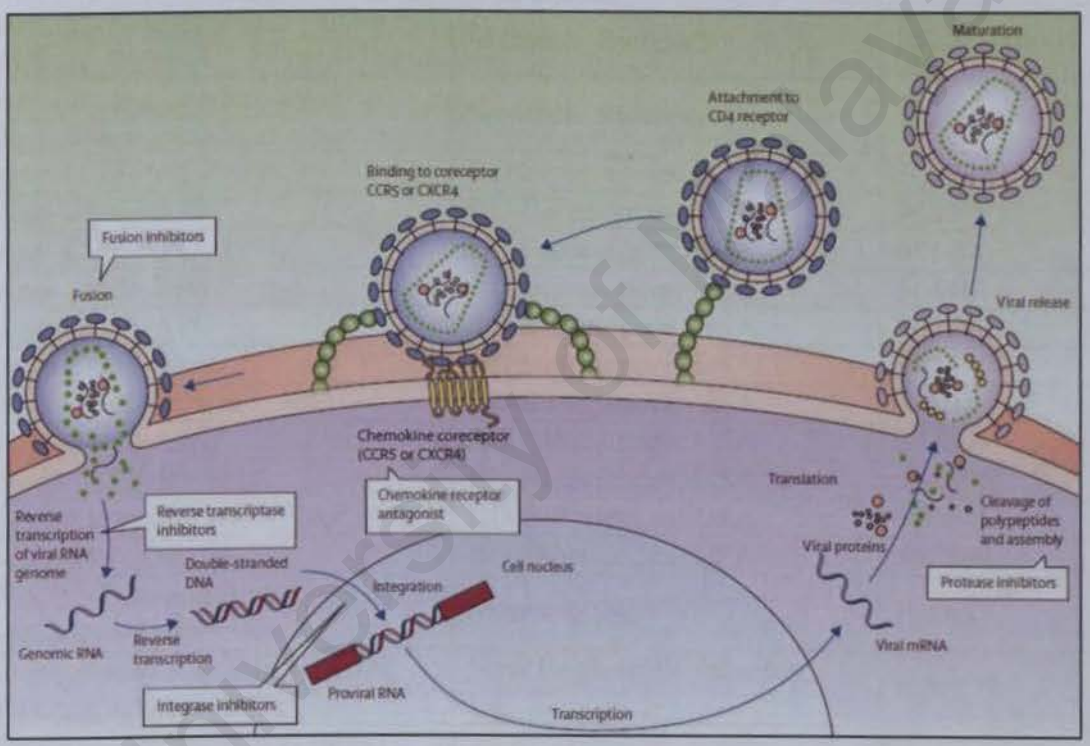

Figure 2.4: Action of each class of antiretroviral drug on correspond HIV life cycle (B. R. Walker, Colledge, Ralston, & Penman, 2013)

CHAPTER 3: MATERIALS AND METHODS

3.1 Materials

These are the reagents and materials utilized in the current study.

Table 3.1: Reagents and materials

Reagents/ Materials	Manufacturer	Catalogue no./ Product code
Agarose powder	1st Base Pte. Ltd, Singapore	BIO-1000-500g
Alexa Fluor647 Mouse IgG1 k Isotype control	BD Biosciences, USA	557714
Anti-mouse Ig, k/Negative control compensation particles set	BD Biosciences, USA	552843
Anti-human Interferon γ mAb 1-D1K	Mabtech, Sweeden	3420-3-1000
Anti-human interferon γ mAb 7-B6-1-Biotin	Mabtech, Sweeden	3420-6-1000
Anti-CD3-PerCP-Cy5.5	BD Biosciences, USA	340949
Anti-CD4-PE-Cy7	BD Biosciences, USA	348789
Anti-CD8-APC-H7	BD Biosciences, USA	641400
Anti-CD25-BB515	BD Biosciences, USA	564467
Anti-CD28-APC	BD Biosciences, USA	560683
Anti-CD38-PE	BD Biosciences, USA	347687
Anti-CD45RA-PE	BD Biosciences, USA	561883
Anti-CD57-FITC	BD Biosciences, USA	347393
Anti-CCR7-BV421	BD Biosciences, USA	562555
Anti-Alexa Fluor647-FoxP3	BD Biosciences, USA	560045
Anti-HLA-DR-BV421	BD Biosciences, USA	562804
BICP/NBT –Alkaline phosphatase substrate	Sigma, USA	B-5655
Deoxynucleotide solution mix, dNTP, 40uM per dNTP	New England Biolabs Inc., UK	N04471
Dimethyl Sulphoxide (DMSO) Hybri-Max	Sigma, USA	D2650
ExtrAvidin-Alkaline Phosphatase	Sigma, USA	E2636-.5ML
Female sample collection kit	Hybribio Limted, Hong Kong	N/A
Fetal Bovine Serum	Gibco, USA	16000-044
Fixable viability stain 510	BD Biosciences, USA	564406
HGH Primer I, 100uM	AITbiotech Pte. Ltd., Singapore	203881
HGH Primer II, 100uM	AITbiotech Pte. Ltd., Singapore	203882
HPV52 E6 peptides	Biobasic Inc., Canada	N/A
Human FoxP3 buffer set	BD Biosciences, USA	560098

Table 3.1: Reagents and materials (Continued)

Reagents/ Materials	Manufacturer	Catalogue no./ Product code
Hybribio Rapid HPV GenoArray test kit (DNA extraction part, PCR part, Hybridization part)	Hybribio Limted, Hong Kong	N/A
Iscove's Modified Dulbecco's medium	Lonza, USA	12-722Q
Lymphoprep (Ficoll)	Axis-Shield PoC AS, Norway	1114547
L-Glutamine solution, 200mM	Safcbiosciences, UK	59202C
Memory Recall Mix peptides, purity>95%, 9mg per peptide	Biobasic Inc., Canada	N/A
Multiscreen 96-wells filter plates	Milipore, Ireland	MSHAS4510
Penicillin/ Streptomycin antibiotic	Gibco, USA	15140
PepMix HPV16 (protein E6), 25ug per peptide	JPT, Gemany	PM-HPV16-E6
Lectin from *Phaseolus vulgaris*, PHA (red kidney bean), 25mg	Sigma, USA	L2646
Phosphate-buffered Saline (PBS) tablet	Calbiochem, Germany	524650
Quick-Load 2-log DNA ladder	New England Biolabs Inc., UK	N0469A
Sybersafer (SYBR) Safe DNA gel stain	Invitrogen, USA	S33102
Stabilizing fixative 3x	BD Biosciences, USA	338036
Taq polymerase, 5,000U/ml	New England Biolabs Inc., UK	M0320S
Thinprep preservcyt solution	Hologic Inc., USA	70097-005
Trypan Blue	Sigma, USA	T8154
Tween 20	Sigma, USA	P1379-500ML
2-Mercaptoethanol	Sigma, USA	M3148-250ML
6x loading dye bromophenol blue	Bioatlas, Estonia	BA01401
10x PCR buffer	New England Biolabs Inc., UK	B9014S
24-well plate with lid	Milipore, Ireland	PIMWS2450
50X TAE electrophoresis buffer	Thermo scientific, Lithuania	B49

3.2 Methods

3.2.1 Patients

This cross sectional study was approved by the Medical Ethics Committee of University of Malaya Medical Centre (MEC Ref. No.: 865.18) (Appendix A). The inclusion criteria for the cases were: male with age of 18 to 60 years old, receiving cART as their first antiretroviral therapy for a minimum of two years, CD4+T cell count persistently <350cells/mm^2 (sIR) or >500cells/mm^2 (oIR), sustained suppressive viral load RNA <50copies/ml over a year following cART. Patients were recruited from the Infectious Diseases clinic in UMMC (Appendix B). Age matched controls were recruited from the community clinics (Appendix C).

The sample size calculation for the study was based on the assumption that the immune responses to HPV16 E6 among oIR would be similar to that of healthy individuals while that of sIR were similar to patients with cervical cancer (de Jong et al., 2004). The proportion of HPV specific immune responses among oIR was 0.45 while that of sIR was 0.08. This translated to 22 patients per arm.

A detailed questionnaire on socio-demographic information (Appendix D) was administered. Specimens obtained from each participant included an anal swab collected with female collection kit (Hybribio Limited, Hong Kong), oral rinse collected with Listerine mouthwash, 30-40ml of venous blood and additional 10ml of blood from only HIV-infected participant was taken for CD4+T cell count assay that have been done in Central Diagnostic Laboratory in UMMC.

3.2.2 Anal swab and oral rinse processing

Anal specimen was preserved in 20ml of Thinprep PreservCyt solution (Hologic Inc. USA). Cell pellet was obtain from 5ml of the solution after centrifugation at 13,300rpm for 10minutes while the remaining preservative solution was use for cytology (Chapter

3.2.7). Cell pellet was washed with 1ml of 1x Phosphate-buffered saline (PBS) twice prior to DNA extraction.

About 5ml of oral rinse specimen was collected from each participant with Listerine mouthwash. Cell pellet was obtained after centrifuged at 3,000g for 10minutes. Cell pellet was washed with 1ml of 1xPBS twice before DNA extraction.

3.2.3 Anal swab and oral rinse DNA extraction

DNA extraction from anal swab and oral rinse cell pellet were performed with Hybribio Rapid HPV GenoArray test kit (Hybribio Limited, Hong Kong) as per manufacturer. Briefly, cell pellet was lysed with 400ul of solution I at 95°C for 20minutes followed by DNA precipitation with 400ul of solution II. The supernatant was discarded after being centrifuged at 13, 300rpm for 5minutes. Pellet was dried at room temperature for at least 25minutes. DNA was resuspended in 50ul of solution III and stored at 4°C overnight for PCR amplification or -20°C for long term storage.

3.2.4 Testing for DNA adequacy

3.2.4.1 Human growth hormone gene amplification by polymerase chain reaction (PCR)

The adequacy of DNA extracted from anal swab and oral rise was examined through PCR amplification of Human Growth Hormone (HGH) gene. The PCR conditions are described as below (Table 3.2 and 3.3).

Table 3.2: PCR mixture of HGH gene amplification

PCR reagents	stock concentration	Volume (ul)	Final concentration
PCR buffer	10x	1.00	1x
dNTP mix	10mM each dNTP	0.20	0.2mM
HGH primer I	10uM	0.50	0.5uM
HGH primer II	10uM	0.50	0.5uM
Taq polymerase	5000U/ml	0.05	25U/ml
dH_2O	--	6.75	--
DNA template	0.10-280ng/ul	1.00	0.01-28.00ng/ul

Table 3.3: PCR condition of HGH gene amplification

Condition	Temperature (°C)	Duration	Number of cycle
Pre-denaturation	95	30 sec	--
Denaturation	95	30 sec	35
Annealing	55	30 sec	
Extension	68	30 sec	
Post-extension	68	5 min	--
Cold	16	Hold	--

3.2.4.2 Agarose gel electrophoresis

The HGH gene amplification PCR products were examined on 1% agarose gel electrophoresis. Genome DNA was considered sufficient if HGH PCR product was visible and vice versa. 1xTAE buffer was used to prepare agarose gel and as an electrophoresis running buffer. About 1.2ul of Sybersafer was added into gel before it solidifies in order to visualize the band under UV transilluminator (UVP, USA). About 5ul of each PCR product was dye with 6ul Bromophenol Blue. About 1.5ul of 100bp DNA ladder was run in a well as a reference. Agarose gel electrophoresis was carried out with gel electrophoresis system (Hoefer, USA) at 110V for 22mins. The expected size of HGH gene PCR product was approximately 439bp (Appendix E).

3.2.5 HPV genotyping assay

3.2.5.1 HPV DNA amplification

PCR mixture was prepared (Table 3.4) and the PCR cycle was performed (Table 3.5) on PCR thermocycler (Applied Biosystem, USA). After PCR cycle finished, proceed the PCR products to HPV DNA genotyping.

Table 3.4: PCR mixture of HPV DNA amplification

Components	Volume (ul) per reaction
PCR master mix	23.25
DNA Taq polymerase	0.75
DNA template	1.00
Total	25.00

Table 3.5: PCR condition of HPV DNA amplification

Condition	Temperature (°C)	Duration	Number of cycle
Pre-denaturation	95	9 min	--
Denaturation	95	20 sec	40
Annealing	55	30 sec	
Extension	72	30 sec	
Post-extension	72	5 min	--
Cold	4	Hold	--

3.2.5.2 HPV DNA genotyping by flow-through hybridization

DNA extraction and HPV DNA genotyping was performed with Hybribio Rapid HPV GenoArray test kit (Hybribio Limited, Hong Kong). This kit can detect 21 specific HPV subtypes: (a) HR: HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, (b) LR: HPV6, 11, 42, 43, 44, CP8304 (81) and (c) Undetermined risk: HPV53. The tests were performed according to the manufacturer's protocol. Briefly, extracted DNA was proceeded to PCR amplification. Next, PCR product was added into a probed membrane followed by flow-through hybridization. The membrane was then subjected to colourimetric reaction for detection of amplified HPV DNA. Result was manually interpreted using the provided guide (Appendix F).

3.2.6 Anal swab microscope slide

Thin prep solution receiving the anal swabs were also aliquoted for slide preparation by Pathology Lab, UMMC. Anal swab microscope slide was prepared with ThinPrep 2000 Processor (Hologic, USA) and Papanicolaou's staining. All sides were sent to Douglass Hanly Moir pathology lab (Australia) and read by a single experienced pathologist (Dr. Jennifer Roberts), utilising the Bethesda System 2001 criteria and terminology (Nayar & Wilbur, 2015).

3.2.7 Isolation and cryopreservation of PBMC

About 40ml of whole blood in EDTA tubes was taken from each patient. The yellowish layer plasma and buffy coats were removed after centrifuge the whole blood. PBMC was harvested from the buffy coat through Ficoll gradient as described by Dyer and colleagues (Dyer et al., 2007). PBMC was freezed at a density of $10x10^6$ PBMC/vial) in freezing medium (10% Dimethyl sulfoxide (DMSO)/ Fetal bovine serum (FBS)). The cryovials was pre-cooled in Coolcell unit (BioCision, USA) at -80°C freezer for overnight before store at Liquid Nitrogen (LN_2) tank for long term storage.

3.2.8 Interferon-γ (IFNγ) ELISPOT

Thawed peripheral blood mononuclear cells (PBMCs) were seeded at a density of $2x10^6$ cells/well of a 24-well plate in 1ml of IMDM enriched with 10% FBS in the presence of combination of HPV16E6 peptides at 1ug/ml/peptide and HPV52E6 peptides at 10ug/ml/peptide (Appendix G). As a positive control, PBMC was cultured in the presence of memory recall mix (MRM) peptide pool at 3ug/ml/peptide pool containing peptides from human cytomegalovirus (CMV) and tetanus (Appendix H). Unstimulated PBMC in IMDM enriched with 10% FBS only was as negative control. After four days of incubation at 37°C, PBMC were harvested, washed, and seeded in three replicate wells at a density of $5x10^4$ cells per well in in a multiscreen 96-well plate (Millipore, USA) coated with IFN_γ catching antibody (Mabtech, Ireland). Phytohaemagglutinin (PHA) at 2ug/ml was added to PBMC as antigenic stimulation controls. Further incubation with secondary antibody and development of colour, spots were counted with a fully automated ELISPOT reader (CTL analyzers, USA) with ImmunoSpot software version5.1 (CTLAnalyzers, USA) (Appendix I).

3.2.9 T cell immunophenotyping by flow cytometry

Thawed PBMC were recovered for IFNγ ELISPOT and flow cytometry. Briefly, $1x10^6$ PBMC were stained in two separate panels: 1) CD3 (PerCP-Cy5.5, clone SK7), CD4 (PE-Cy7, clone SK3), CD8 (APC-H7, clone SK1), CD28 (APC, clone CD28.2), CD57 (FITC, clone HNK-1), CD45RA (PE, clone HI100), CCR7 (BV421, clone 150503) 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 dye (FVS510) (Figure 3.1) . All antibodies were purchased from BD Pharmigen. Cells were stained for surface markers for 20mins at room temperature and washed twice before acquisition. PBMCs stained with second panel were 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 30mins at RT. All samples were resuspended in stabilizing fixative. 150,000 events were acquired with FACSCanto II and analyzed using FACS Diva v6. For gating strategy, doublets were excluded based on forward- (FSC) and side- (SSC) scatter. Then, dead cells (FVS510+) were discriminated prior to gate T lymphocytes based on CD3+CD4+ and CD3+CD8+. Senescence (CD28-CD57+), activation (CD38+HLA-DR+) T cells subsets (Naïve T cell: CD45RA+CCR7+, Central memory T cell: CD45RA-CCR7+, Effector memory T cell: CD45RA-CCR7- and Terminal effector T cell: CD45RA+CCR7-) was gated on both CD4+ and CD8+ T cells whereas Foxp3+CD25+ was gated on CD4+ T cells. All the quantity of T cells were reported as percentage.

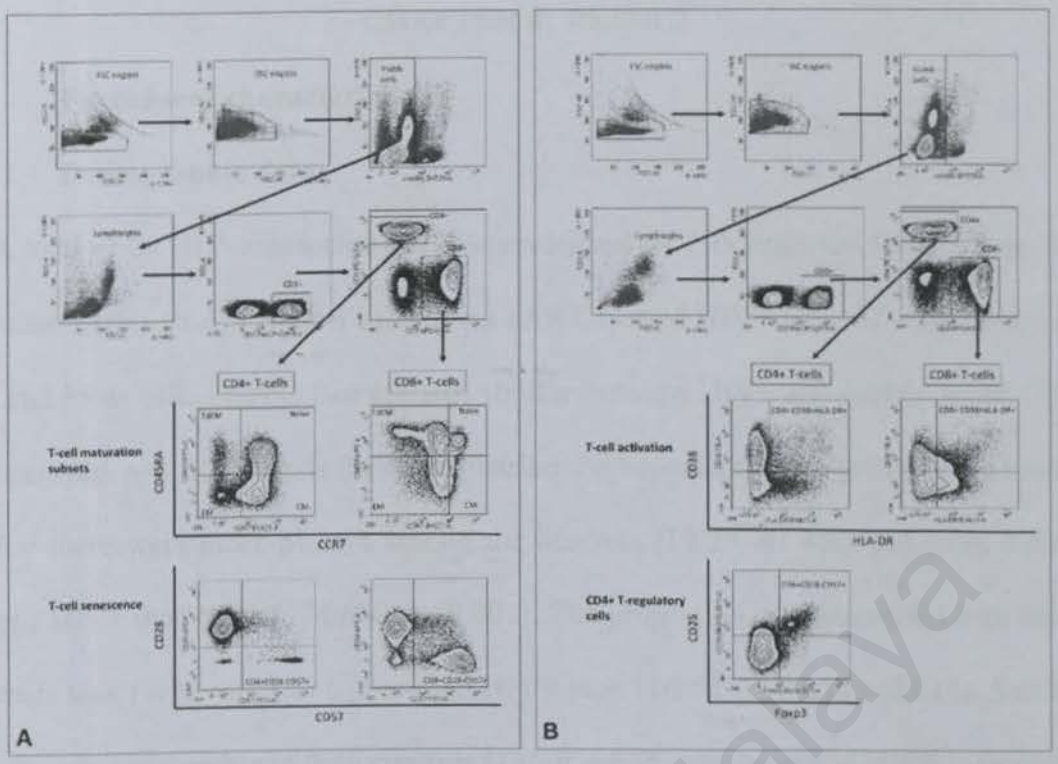

Figure 3.1: Gating strategy of immunophenotypes with marker panel A and B

3.2.10 Data analysis

All data was analyzed with SPSS version16 (IBM Inc. USA). Clinical and immunological characteristics were compared using. Chi-square test and Mann Whitney U test. Chi-square test was used for data express in categorical variables. Mann Whitney U test was performed for data express in continuous variables. Logistic regression analysis was used to assess clinical data and immunological risk factor associate with HPV carriage and HPV16/52 E6-specific CMI. Co-variates with $p<0.25$ and well established risk factors were included in the multivariate where a *p*-value of <0.05 was considered statistically difference.

CHAPTER 4: RESULT

4.1 Participant characteristics

4.1.1 Demographic data

A total of 29 HIV-uninfected (HIV- control) and 67 HIV-infected (HIV+) men were included in this study (Table 4.1). Among cART-treated HIV+ men, 32 were considered sIR and 35 as oIR. The median age was similar between HIV+ and control group (41 vs 39 years old, p=0.314), while the racial distribution between the two groups were unequal in that there were more Malays among the controls (12/29, 41.4%) and more Chinese among HIV+ men (50/67, 74.6%) (p<0.001). The proportion of circumcised men among controls was 17/28 (60.7%) higher than HIV+ men (16/50, 32.0%) (p=0.014). Smoking history was comparable in both controls (18/28, 64.3% smokers) and HIV+ men (40/63, 63.5% smokers) (p=0.942). Both HIV+ group and controls had similar median age of first sexual debut (19 vs 21 years old, p=0.069).For sexual behavior, 12/25 controls (48.0%) and 39/50 HIV+ men (78.0%) engaged in oral sex (p=0.009). On the other hand, receptive (50% vs 4%, p=0.001) and insertive (52.5% vs 4%, p=0.001) anal sex were more common among HIV+ men compared to controls. As anal sex was uncommon among controls, the usage of condom for anal sex was less likely found in this group. In contrast, the usage of condom for receptive (40% vs 0%, p=0.017) and insertive (40% vs 4%, p=0.005) anal sex among HIV+ men were significantly higher than controls. For oral sex, HIV+ men more likely to have receptive (72% vs 48%, p=0.041) and insertive (62% vs 40%, p=0.071) oral sex compared to controls. Accordingly, the usage of condoms for both receptive (32% vs 8%, p=0.133) and insertive (50% vs 40%, p=0.069) oral sex among HIV+ men was more prevalent than controls. In the last 12 months, 8/25 of controls and 23/40 of HIV+ men (p=0.246) were sexually active. In addition, a significant difference was observed in the sexual orientation between both groups (p<0.001). Controls were

predominantly heterosexual (24/25, 96.0%) whilst 27/64 (42.4%) HIV+ men were men who have sex with men (MSM).

4.1.2 HIV-specific clinical parameters

Before receiving cART, sIR group had higher median baseline HIV viral load than oIR group (194245.5 vs 92461.0 copies/mL, p=0.018) with lower baseline CD4+ T cell counts (28 vs 190cells/mm^3, p<0.001), CD8+ T cell counts (473 vs 711cells/mm^3, p=0.011) and CD4/CD8 ratio (0.07 vs 0.22, p<0.001). In addition, sIR group had more AIDS-defining illnesses compared to oIR group (84.4% vs 54.3%, p=0.008).

In this cohort, all HIV+ men received NNRTI-based cART with suppressive viral load (<50 copies/mL). Both sIR and oIR groups had similar median of 5 years duration of cART (p=0.904). Group of oIR initiated cART at a younger age (35 vs 38 years old, p=0.037), and had higher CD4+T cell count (733 vs 285cells/mm^3, p<0.001), CD8+T cell count (950 vs 799cells/mm^3, p=0.049), and CD4/CD8 ratio (0.78 vs 0.37, p<0.001) than sIR group at study entry. After receiving cART, sIR group displayed a significantly lower CD4+T cell count gain compared to oIR group (253 vs 607cells/mm^3; p<0.001). Additionally, the inflammation marker hsCRP was measured in this study. There was no significant difference between median hsCRP levels of HIV+ men and controls (0.26 vs 0.21mg/dL, p=0.518) as well as between sIR and oIR (0.16 vs 0.24mg/dL, p=0.079).

4.1.3 Immunophenotype of CD4+ and CD8+ T cells subsets

As previously reported, skewed memory T cell subsets were observed among HIV+ individuals particularly in sIR cohorts despite having suppressive viral load (Marchetti et al., 2010). Similarly, sIR group in this study showed higher proportion of CD4+ central memory T cell (T_{CM}) (46.2% vs 38.1%, p<0.05) and effector memory T cell (T_{EM}) in both CD4+ (26.8% vs 19.0%, p=0.001) and CD8+ (56.1% vs 43.6%, p<0.05) subsets, with lower naïve CD4+ (24.9% vs 36.4%, p<0.05) and CD8+ (8.1% vs 15.7%, p<0.05) T cells

when compared to oIR group (Table 4.2 & Figure 4.1). Control and oIR groups had similar percentage of CD4+ and CD8+ memory T cell subsets (T_{CM}, T_{EM}, T_{dEM}) with a greater tendency toward decline in naïve CD4+ and CD8+ T cells. Overall, HIV+ men had more memory T cell subsets and lower naïve T cells compared to controls.

In this study, the median percentage of activated CD4+ (7.9% vs 4.5%, p<0.001) and CD8+ (22.2% vs 14.1%, p=0.003) T cells in sIR group was higher than oIR group. Group of oIR showing higher percentage of activated CD4+ (4.5% vs 3.2%, p=0.016) and similar percentage of activated CD8+ (14.1% vs 14%, p=0.684) T cells when compared to controls (Table 4.1 and Figure 4.2). T cell activation has been shown to be the key factor that driving memory T cells to immunosenescence (Papagno et al., 2004). As expected, the sIR group who possessed high levels of activated T cells showed higher levels of senescent T cells compared to oIR. Similarly, senescent CD8+T cell levels of sIR group in this study was higher than oIR group (44.3% vs 29.8%, p=0.005), but no difference in CD4+T cells (3.4% vs 3.7%, p=0.906) have been observed between both groups.

Many studies have showed that regulatory T cells (Treg) associated with disease progression in both HIV and HPV infections (Kojima et al., 2013; Saison et al., 2015). Thus, the level of CD4+CD25+Foxp3+ Treg was measured in this study. As expected, significantly different level of regulatory Treg was observed between sIR and oIR groups (7.3% vs 5.7%, p=0.006). In response to cART, oIR exhibited a similar level of Treg as observed in controls (5.7% vs 5.8%, p=0.766).

In summary, although cART was able to suppress HIV viral replication effectively, sIR group failed to recover their T cell immunophenotypes to a normal level as observed in controls. The failure of immune recovery is associated with cART initiation at older age, low baseline CD4, higher viral load as observed in sIR.

when compared to oIR group (Table 4.2 & Figure 4.1). Control and oIR groups had similar percentage of CD4+ and CD8+ memory T cell subsets (T_{CM}, T_{EM}, T_{dEM}) with a greater tendency toward decline in naïve CD4+ and CD8+ T cells. Overall, HIV+ men had more memory T cell subsets and lower naïve T cells compared to controls.

In this study, the median percentage of activated CD4+ (7.9% vs 4.5%, $p<0.001$) and CD8+ (22.2% vs 14.1%, $p=0.003$) T cells in sIR group was higher than oIR group. Group of oIR showing higher percentage of activated CD4+ (4.5% vs 3.2%, $p=0.016$) and similar percentage of activated CD8+ (14.1% vs 14%, $p=0.684$) T cells when compared to controls (Table 4.1 and Figure 4.2). T cell activation has been shown to be the key factor that driving memory T cells to immunosenescence (Papagno et al., 2004). As expected, the sIR group who possessed high levels of activated T cells showed higher levels of senescent T cells compared to oIR. Similarly, senescent CD8+T cell levels of sIR group in this study was higher than oIR group (44.3% vs 29.8%, $p=0.005$), but no difference in CD4+T cells (3.4% vs 3.7%, $p=0.906$) have been observed between both groups.

Many studies have showed that regulatory T cells (Treg) associated with disease progression in both HIV and HPV infections (Kojima et al., 2013; Saison et al., 2015). Thus, the level of CD4+CD25+Foxp3+ Treg was measured in this study. As expected, significantly different level of regulatory Treg was observed between sIR and oIR groups (7.3% vs 5.7%, $p=0.006$). In response to cART, oIR exhibited a similar level of Treg as observed in controls (5.7% vs 5.8%, $p=0.766$).

In summary, although cART was able to suppress HIV viral replication effectively, sIR group failed to recover their T cell immunophenotypes to a normal level as observed in controls. The failure of immune recovery is associated with cART initiation at older age, low baseline CD4, higher viral load as observed in sIR.

Table 4.1: Epidemiological, virological and immunological characteristics of HIV- and HIV+ men

Parameter	HIV- group (n=29)	HIV+ group		P-value
		sIR (n=32)	oIR (n=35)	
Age (years)	39 (29-53)	42 (39-50)	40 (38-46)	0.314*
Ethnicity				
Malay	12/29 (41.4)	2/32 (6.3)	7/35 (20.0)	<0.001
Chinese	9 /29 (31.0)	29/32 (90.6)	21/35 (60.0)	*
Indian	8/29 (27.6)	1/32 (3.1)	7/35 (20.0)	
Circumcised (yes)	17/28 (60.7)	6/24 (25.0)	10/26 (38.5)	0.014*
Smoking history (yes)	18/28 (64.3)	20/30 (66.7)	20/33 (60.6)	0.942*
Age of first sex (years)[a]	19 (18-21)	20 (19-25)	21 (18-25)	0.069
Receptive anal sex[b]	1/25 (4.0)	8/24 (33.3)	12/26 (46.2)	0.001
Insertive anal sex[b]	1/25 (4.0)	10/24 (41.7)	11/26 (42.3)	0.001
Condom usage for receptive anal sex[b]				
All the time	0/25 (0)	1/24 (4.2)	3/26 (11.5)	
Most of the time	0/25 (0)	2/24 (8.3)	3/26 (11.5)	0.017
Sometimes	0/25 (0)	5/24 (20.8)	6/26 (23.1)	
Never use	1/25 (4.0)	0/24 (0)	0/26 (0)	
Condom usage for insertive anal sex[b]				
All the time	0/25 (0)	1/24 (4.2)	3/26 (11.5)	
Most of the time	1/25 (4.0)	3/24 (12.5)	3/26 (11.5)	0.005
Sometimes	0/25 (0)	5/24 (20.8)	5/26 (19.2)	
Never use	0/25 (0)	1/24 (4.2)	0/26 (0)	
Receptive oral sex[b]	12/25 (48.0)	16/24 (66.7)	20/26 (76.9)	0.041
Insertive oral sex[b]	10/25 (40)	13/24 (54.2)	18/26 (69.2)	0.071
Condom usage for receptive oral sex[b]				
All the time	0/25 (0)	1/24 (4.2)	0/26 (0)	
Most of the time	0/25 (0)	1/24 (4.2)	3/26 (11.5)	0.133
Sometimes	2/25 (8.0)	3/24 (12.5)	8/26 (30.8)	
Never use	10/25 (40.0)	11/24 (45.8)	9/26 (34.6)	
Condom usage for insertive oral sex[b]				
All the time	0/25 (0)	2/24 (8.3)	2/26 (7.7)	
Most of the time	1/25 (4.0)	3/24 (12.5)	4/26 (15.4)	0.069
Sometimes	9/25 (36.0)	5/24 (20.8)	9/26 (34.6)	
Never use	0/25 (0)	3/24 (12.5)	3/26 (11.5)	
Sexually active in the last 12months[b]	8/25 (32.0)	9/24 (37.5)	14/26 (53.8)	0.246
Oral sex	12/25 (48.0)	17/24 (70.8)	22/26 (84.6)	0.009*
Sexual orientation				
Homosexual	0/25 (0)	11/30 (36.7)	16/34 (47.1)	<0.001
Heterosexual	24/25 (96.0)	18/30 (60)	16/34 (47.1)	*
Bisexual	1/25 (4.0)	1/30 (3.3)	2/34 (5.9)	
No. lifetime sexual partners				
1	6/25 (24.0)	2/24 (8.3)	4/26 (15.4)	0.148
≥2	17/25 (68.0)	15/24 (62.5)	17/26 (65.4)	
Do not remember	2/25 (8.0)	7/24 (29.2)	5/26 (19.2)	

Table 4.2: Epidemiological, virological and immunological characteristics of HIV- and HIV+ men (Continued)

Parameter	HIV- group (n=29)	HIV+ group		P-value
		sIR (n=32)	oIR (n=35)	
cART regimen				
NNRTI-based	NA	32/32 (100.0)	35/35 (100.0)	0.008
PI-based		0	0	
Duration on cART (years)	NA	5 (3-9)	5 (3-9)	0.904
Age of cART initiation (years)	NA	38 (36-44)	35 (30-39)	0.037
History of AIDS-defining illnesses	NA	27/32 (84.4)	19/35 (54.3)	0.008
HIV viral load (copies/mL)				
Baseline	NA	194245.5 (97525-405805)	92461.0 (37599-233000)	0.018
At study entry	NA	Below limit of detection (<50copies/mL)	Below limit of detection (<50copies/mL)	
CD4+T cell count (cells/mm^3)[c]				
Baseline	NA	28 (10-59)	190 (66-314)	<0.001
At study entry		285 (224-332)	733 (671-945)	<0.001
CD8+T cell count, (cells/mm^3)				
Baseline[d]	NA	473 (237-876)	711 (492.3-1391)	0.011
At study entry[e]		799 (560-982)	950 (723-1421)	0.049
CD4:CD8 ratio				
Baseline[d]	NA	0.07 (0.03-0.12)	0.22 (0.09-0.31)	<0.001
At study entry[e]		0.37(0.31-0.45)	0.78 (0.57-1.09)	<0.001
CD4+ T cell count gain, (cells/mm^3)	NA	253 (156-311)	607 (500-790)	<0.001
hsCRP at study entry (mg/dL)	0.26 (0.045-0.68)	0.16 (0.043-0.30)	0.24 (0.09-0.55)	0.216

Continuous variable are expressed as median (IQR) and categorical variables are expressed as number of cases (%).
[a]HIV- group, n=24; sIR group, n=23; oIR group, n=23
[b]HIV- group, n=25; sIR group, n=24 oIR group, n=26
[c]sIR group, n=30; oIR group, n=35
[d]sIR group, n=31; oIR group, n=34
[d]sIR group, n=30; oIR group, n=34
*Statistical analysis of significance between HIV- and HIV+ (sIR and oIR combined) groups was calculated using chi-square test, while others were calculated using Mann-Whitney U test.

Table 4.3: Immunophenotypes of CD4+ and CD8+ T cells among HIV- and HIV+ men

T cell immunophenotypes	HIV- group (n=29)	HIV+ group	
		sIR (n=32)	oIR (n=35)
CD4+ T cell subsets			
Naïve (%)[d] CD4+CD45RA+CCR7+	43.0 (35.4-47.2)	24.9 (14.5-31.6)	36.4 (23-51.2)
Central memory, T_{CM} (%)[a] CD4+CD45RA-CCR7+	36.8 (30.7-43.1)	46.2 (39-50.5)	38.1 (34.-48.4)
Effector memory, T_{EM} (%)[a] CD4+CD45RA-CCR7-	15.1 (13.4-20.1)	26.8 (19.7-36.4)	19.0 (10.5-26.1)
Terminal effector, T_{dE} (%)[a] CD4+CD45RA+CCR7-	1.3 (0.8-3.8)	1.2 (0.7-2.7)	1.9 (0.6-5.5)
CD4 activation (%)[b] CD4+CD38+HLADR+	3.2 (2.6-4.8)	7.9 (5.9-11)	4.5 (3.9-6.7)
CD4 senescence (%)[a] CD4+CD28-CD57+	0.8 (0.2-3.2)	3.4 (0.9-7.5)	3.7 (0.45-10. 8)
Regulatory, Trge (%)[b] CD4+CD25+Foxp3+	5.8 (4.5-6.1)	7.3 (5.4-8.5)	5.7 (4.63-6.65)
CD8+ T cell subsets			
Naïve (%)[a] CD8+CD45RA+CCR7+	21.0 (8.8-27.8)	8.1 (3.9-15.5)	15.7 (10.0-25.1)
Central memory, T_{CM} (%)[a] CD8+CD45RA-CCR7+	5.5 (4.0-8.6)	4.5 (2.2-8.3)	7.0 (4.3-9.4)
Effector memory, T_{EM} (%)[a] CD8+CD45RA-CCR7-	45.5 (34.1-50.6)	56.1 (42.8-62.5)	43.6 (36.0-53.8)
Terminal effector, T_{dE} (%)[a] CD8+CD45RA+CCR7-	31.4 (22.8-40.4)	28.2 (19.0-35.1)	29.8 (19.0-38.4)
CD8 activation (%)[b] CD8+CD38+HLA-DR+	14 (7.9-20.8)	22.2 (13.1-30.9)	14.1 (10.5-19.4)
CD8 senescence (%)[a] CD8+CD28-CD57+	33.1 (26.2-42.9)	44.3 (32-55.8)	29.8 (21.1-43.6)

[a]HIV- group, n=27; sIR group, n=31; oIR group, n=34

[b]HIV- group, n=27; sIR group, n=32; oIR group, n=34

Statistical analysis of significance was calculated using Mann-Whitney U test as shown in figure 4.1 and 4.2. P-value <0.05 was considered statistically significant.

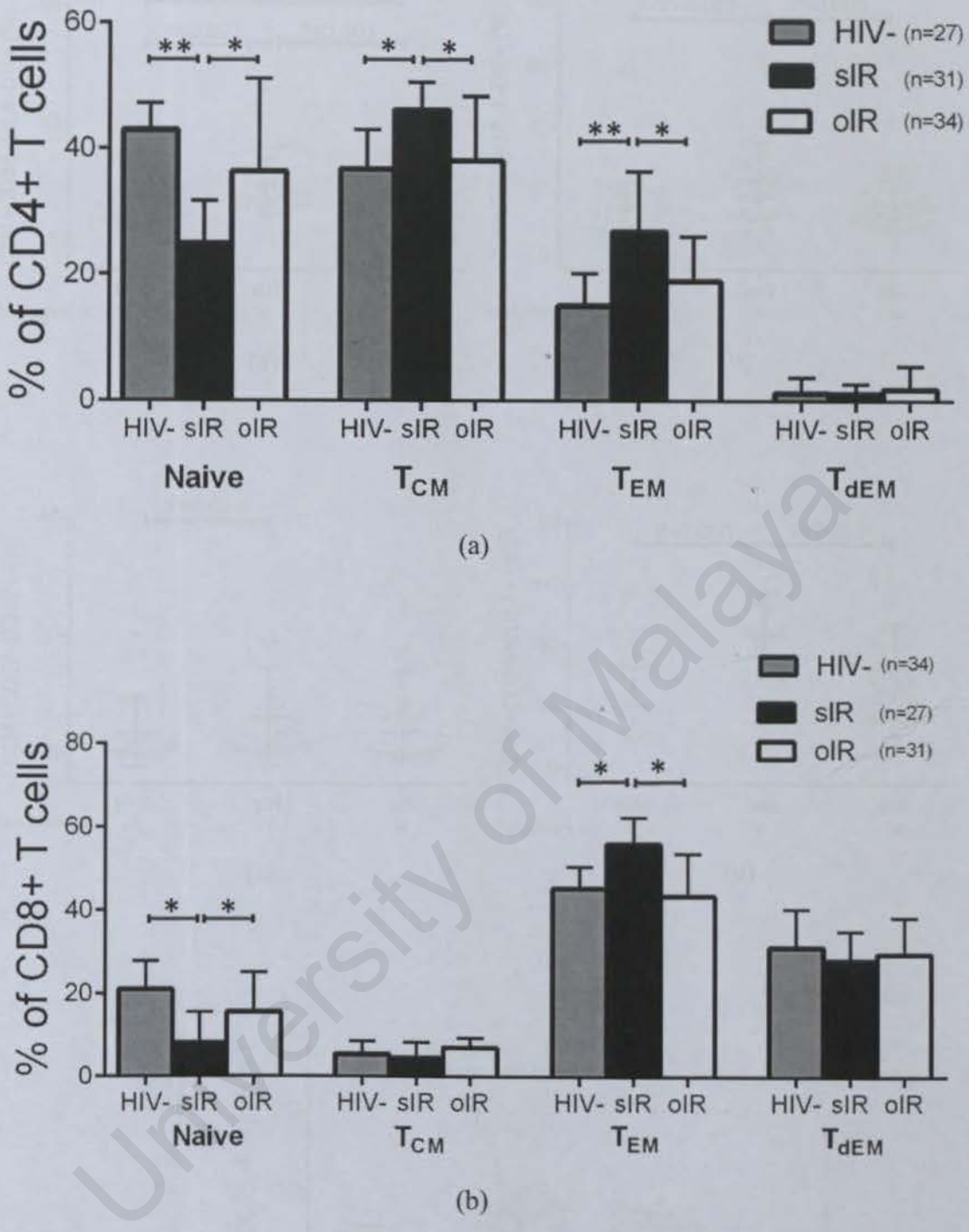

Figure 4.1: Comparison of T cell subset between HIV- and HIV+ groups
(a) Percentage of CD4+ naïve and memory T cell subsets. (b) CD8+ naïve and memory T cell subsets. Statistical analysis of significance was calculated using Mann Whitney U test. P-value <0.05 was considered as statistically significant. *p-value<0.05 and **p-value<0.001

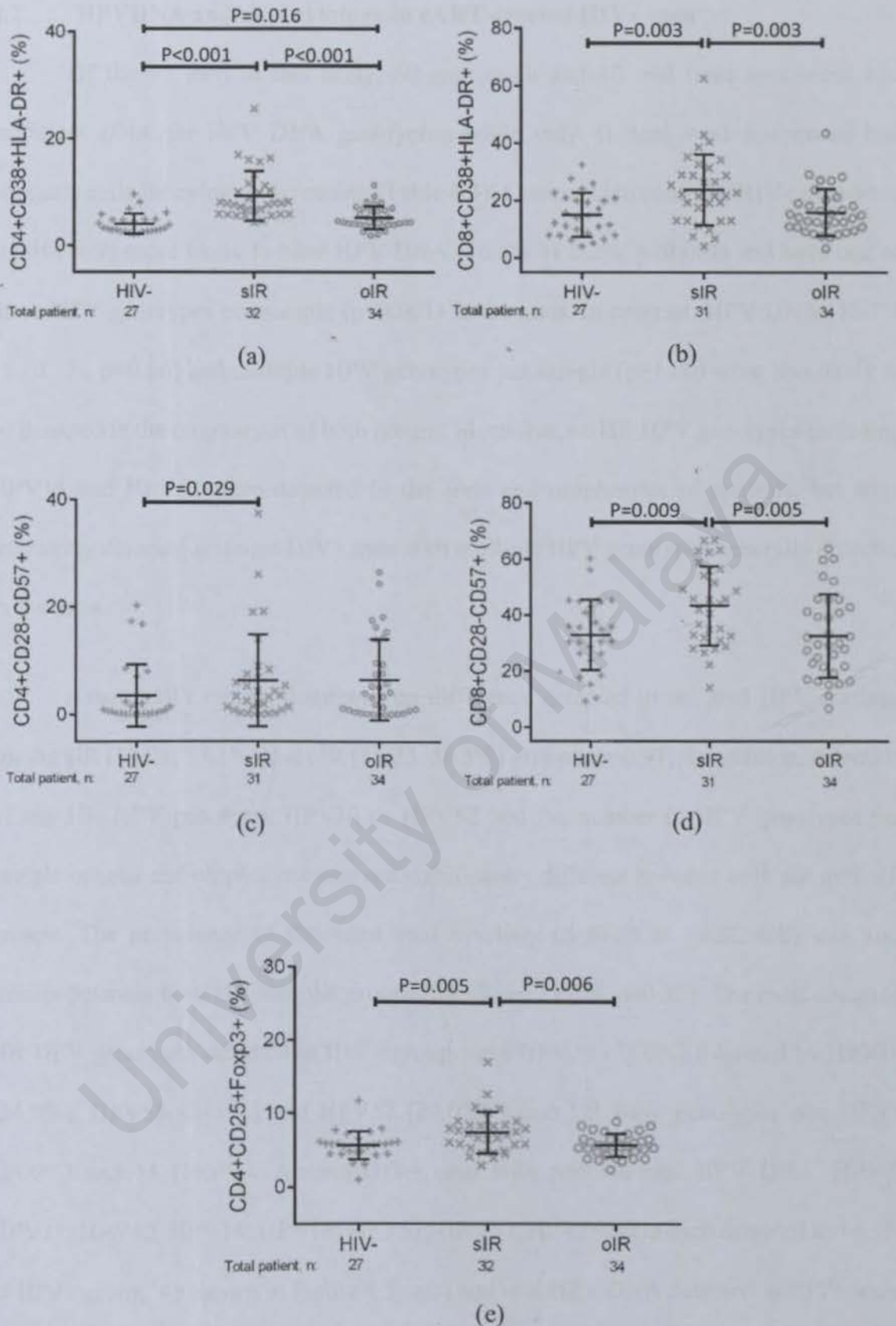

Figure 4.2: Comparison of immunological phenotypes between HIV- and HIV+ groups

(a) and (b) Percentage of activated CD4+ and CD8+ T cells, respectively. (c) and (d) Percentage of senescent CD4+ and CD8+ T cells, respectively. (e) Percentage of regulatory T cells. Statistical analysis of significance was calculated using Mann-Whitney U test. P-value <0.05 was considered as statistically significant.

4.2 HPVDNA and anal cytology in cART-treated HIV+ men

Of the 96 men in this study, 60 anal swab and 80 oral rinse specimens had sufficient DNA for HPV DNA genotyping while only 41 anal swab specimens had adequate cells for cytology screening (Table 4.3). Compared to controls, HIV+ men were significantly more likely to have HPV DNA (56.8% vs 6.2%, $p<0.001$) and have one or more HPV genotypes per sample ($p<0.001$) in the anus. In contrast, HPV DNA (13.7% vs 10.3%, $p=0.66$) and multiple HPV genotypes per sample ($p=1.00$) were less likely to be detected in the oropharynx of both groups. Moreover, no HR HPV genotypes including HPV16 and HPV52 were detected in the anus and oropharynx of controls, but were frequently detected amongst HIV+ men with multiple HPV genotypes generally detected in the anus.

Among HIV+ men, there was no difference detected in the anal HPV carriage among sIR (12/21, 57.1%) and oIR (13/23, 56.5%) groups ($p=0.97$). In addition, detection of any HR HPV genotype, HPV16 or HPV52 and the number of HPV genotypes per sample in anus and oropharynx was not significantly different between both sIR and oIR groups. The prevalence of abnormal anal cytology (defined as ≥ASC-US) was also similar between both sIR and oIR groups (38.9% vs 25.0%, $p=0.36$). The most common HR HPV genotypes detected in HIV+ group were HPV16 (28.0%) followed by HPV18 (24.0%), HPV58 (24.0%) and HPV52 (20.0%) while LR HPV genotypes was HPV6 (24.0%) and 11 (16.0%). Among HIV+ men with positive oral HPV DNA, HPV6, HPV11, HPV42, HPV16, HPV18, HPV51, HPV53, HPV59 were each detected in 14.3% of HIV+ group. As shown in Figure 4.3, anal and oral HPV DNA detected in HIV+ were independent of the patients' CD4+ T cell count, whereas abnormal anal cytology (≥ASCUS) were more likely to be detected in HIV+ with low CD4+T cell count ($<1000 cells/mm^3$).

In HIV+ men, anal HPV DNA was detected in all individuals with abnormal anal cytology (100%) and 42.3% of individuals with normal anal cytology (p=0.001) (Table 4.4). The prevalence of HR HPV as well as HPV16 and HPV52 detected in anus was not different between individuals with normal and abnormal anal cytology. However, abnormal anal cytology were more likely to have multiple anal HPV genotypes detected in anus per sample compared to those with normal anal cytology (p<0.001).

Table 4.4: HPV DNA genotyping and anal cytology of HIV- and HIV+ men

	HIV- group	HIV+ group		p-value	
		sIR	oIR	HIV- vs HIV+	sIR vs oIR
Anal HPV DNA, n	16	21	23		
Any HPV positive, (%)	1 (6.3)	12 (57.1)	13 (56.5)	<0.001	0.97
Any HR HPV positive, (%)	1/1 (100)	10/12 (83.3)	11/13 (84.6)	0.66	0.93
HPV 16 positive, (%)	0/1 (0)	3/12 (25.0)	4/13 (30.8)	0.54	0.75
HPV 52 positive, (%)	0/1 (0)	4/12 (33.3)	1/13 (7.7)	0.62	0.11
No. of genotypes detected per sample, median (IQR)	0 (0-0)	1 (0-3)	1 (0-2)	<0.001	0.41
Oral HPV DNA, n	29	24	27		
Any HPV positive, (%)	3 (10.3)	4 (16.7)	3 (11.1)	0.66	0.57
Any HR HPV positive, (%)	3/3 (100)	2/4 (50.0)	2/3 (66.7)	0.18	0.66
HPV 16 positive, (%)	0/3 (0)	1/4 (25.0)	0/3 (0)	0.49	0.35
HPV 52 positive, (%)	0/3 (0)	0/4 (0)	0/3 (0)	--	--
No. of genotypes detected per sample, median (IQR)	1 (1-1)	1 (1-1)	1 (1-2)	1.00	0.25
Anal cytology, n	3	18	20		
NILM	3 (100)	11 (61.1)	15 (75.0)	0.86	0.28
ASC-US	0 (0)	2 (11.1)	1 (5.0)		
LSIL	0 (0)	2 (11.1)	3 (15.0)		
ASC-H	0 (0)	3 (16.7)	0 (0)		
HSIL	0 (0)	0 (0)	1 (5.0)		

Statistical analysis of significance was calculated using chi-square test. P-value <0.05 was considered as statistically significant.

Table 4.5: Anal HPV DNA carriage of HIV+ men in relation to anal cytology

Anal HPV DNA carriage	Anal cytology		p-value
	Normal, n=26	≥ASCUS[a], n=12	
Any HPV positive, n (%)	11 (42.3)	12 (100)	0.001
Any HR HPV positive, n (%)	8/11 (72.7)	11/12 (91.7)	0.23
HPV 16 positive, n (%)	4/11 (36.4)	3/12 (25.0)	0.55
HPV 52 positive, n (%)	1/11 (9.1)	2/12 (16.7)	0.59
No. of genotypes detected, median (IQR)	0 (0-1)	3 (2-5)	<0.001

[a]Included ASC-US, LSIL, ASC-H and HSIL.

Statistical analysis of significance was calculated using chi-square test. P-value <0.05 was considered as statistically significant.

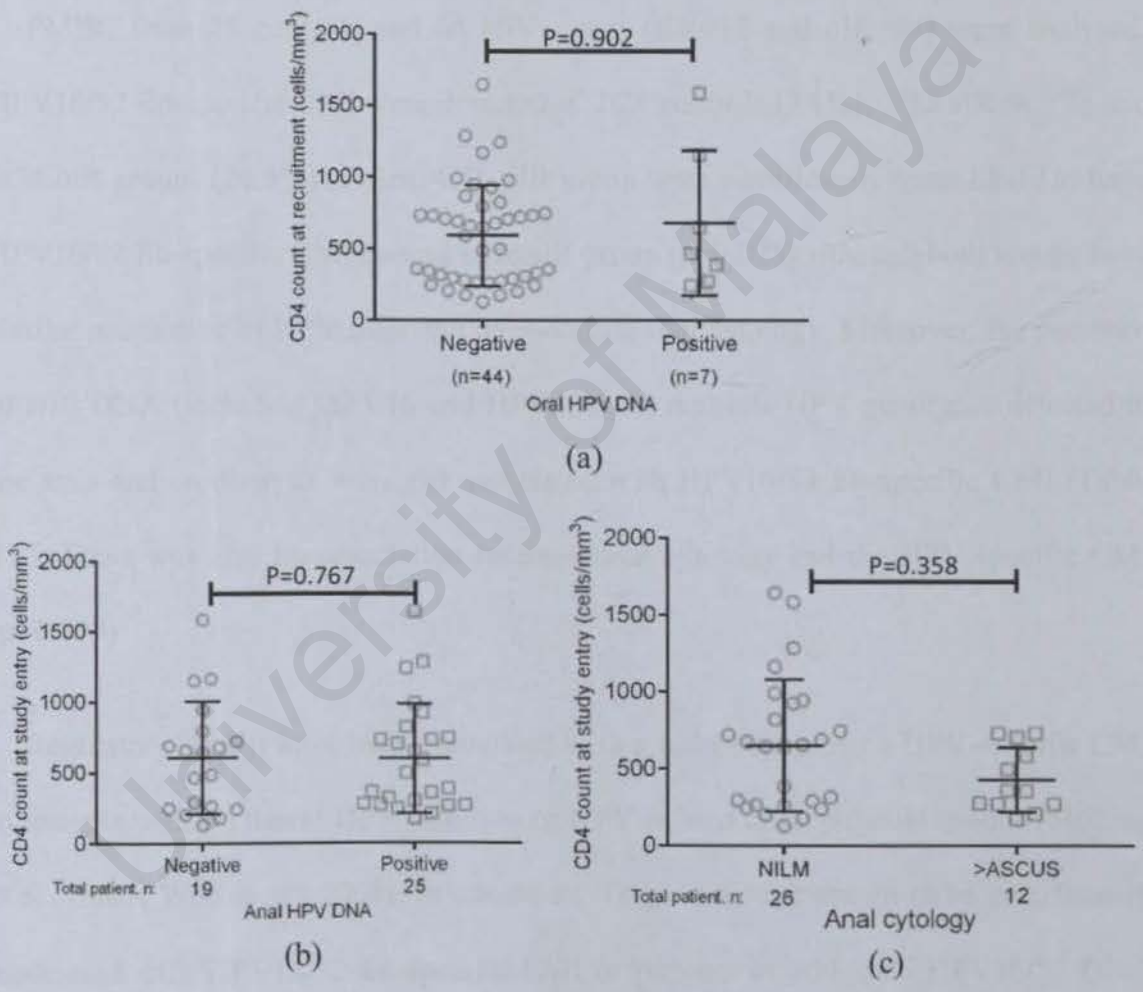

Figure 4.3: Detection of oral, anal HPV DNA, and anal cytology related to CD4+ T cell counts of HIV+ men at study entry

Statistical analysis of significance was calculated using Mann-Whitney U test. P-value <0.05 was considered as statistically significant.

4.3 HPV16 and HPV52 E6-specific CMI

As reviewed earlier, E6 and E7 are HPV oncogenes that contribute to oncogenic effects of the HPV. E6 peptides were selected to stimulate PBMC in this study because E6-specific memory T cells have been frequently correlated with protective immunity (de Jong et al., 2004; Woo et al., 2010). In this study, HPV16 and HPV52 were among the most common HR (oncogenic) HPV genotypes detected. Therefore, a mixture of HPV16 and HPV52 E6 peptides were used in this study.

PMBC from 28 controls and 64 HIV+ men (sIR=32 and oIR=34) were analysed. HPV16/52 E6-specific CMI were detected in 2/28 controls (7.1%), 2/32 sIR (6.3%) and 9/34 oIR groups (26.5%) (Figure 4.4). oIR group were significantly more likely to have HPV16/52 E6-specific CMI compared to sIR group (p=0.028) although both groups have similar prevalence of HPV infection or abnormal anal cytology. Moreover, the presence of HPVDNA (including HPV16 and HPV52) and multiple HPV genotypes detected in the anus and oropharynx were not associated with HPV16/52 E6-specific CMI (Table 4.5). There was also no association between anal cytology and the HPV-specific CMI (p=0.899).

Regulatory T cells have been associated with a failure to mount a HPV-specific CMI in patients with persistent HPV infection or HPV-related intraepithelial lesions (Molling et al., 2007; Woo et al., 2008). In our study, Tregs were not shown to be significantly associated with HPV16/52 E6-specific CMI in patients or with anal HPV16/52 DNA (p=0.918) (Figure 4.5).

Overall, HPV16/52 E6-specific CMI was frequently detected in HIV+ men with complete immune recovery or oIR regardless of HPV DNA or HPV genotypes detected in the anus and oropharynx.

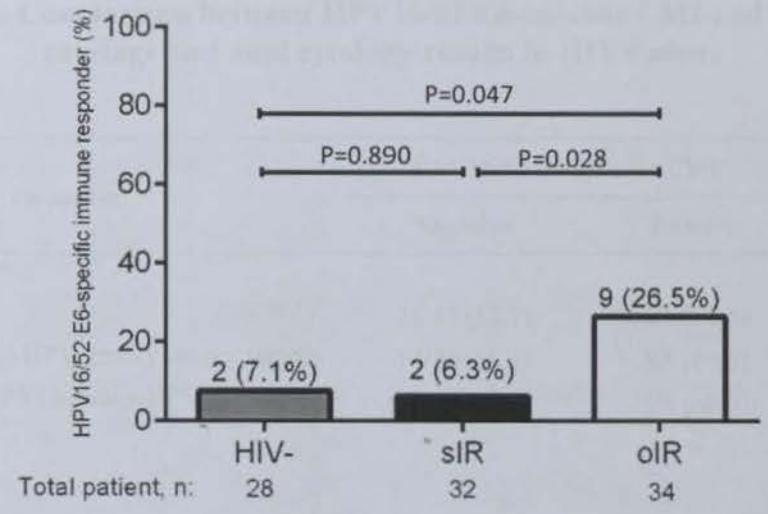

Figure 4.4: HPV16 and 52 E6-specific immune responders in HIV- and HIV+ men

Statistical analysis of significance was calculated using Chi-square test. P-value <0.05 was considered as statistically significant.

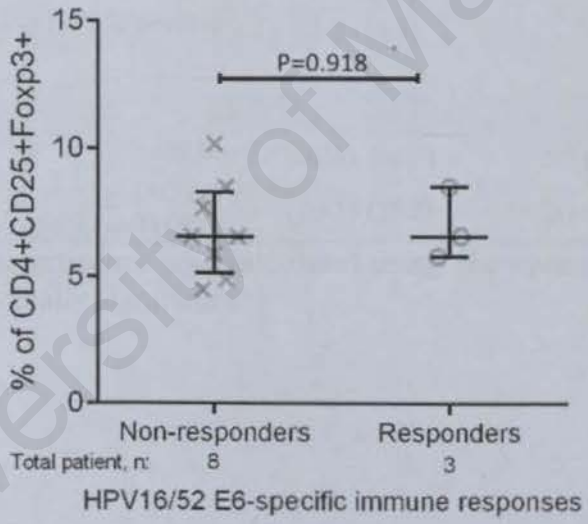

Figure 4.5: Percentage of Treg (CD4+CD25+Foxp3+) of anal HPV16/52 DNA positive-HIV+ men without (non-responders) and with (responders) HPV16/52E6-specific CMI.

Statistical analysis of significance was calculated using Mann-Whitney U test. P-value <0.05 was considered as statistically significant.

Table 4.6: Comparison between HPV16/52 E6-specific CMI and HPV DNA carriage and anal cytology results in HIV+ men

Parameter	HPV16/52 E6-specific CMI[a]		P
	Negative	Positive	
Oral and/or anal (%)			
Any HPV	24/42 (57.1)	5/8 (62.5)	0.78
Multiple (≥2) HPV genotypes per sample	14/24 (58.3)	3/5 (60.0)	0.945
HPV16 and/or HPV52 positive	8/24 (33.3)	3/5 (60.0)	0.264
Anal HPV (%)			
Any HPV	19/36 (52.8)	5/7 (71.4)	0.363
Multiple (≥2) HPV genotypes per sample	13/19 (68.4)	3/5 (60.0)	0.722
HPV16 and/or HPV52 positive	8/19 (42.1)	3/5 (60.0)	0.475
Oral HPV, n (%)			
Any HPV	7/42 (16.7)	0/8 (0)	0.213
Multiple HPV (≥2 HPV genotype)	1/7 (14.3)	0	--
HPV16 and/or HPV52 positive	1/7 (14.3)	0	--
Anal cytology, n (%)			
NILM	20/30 (66.7)	5/7 (71.4)	0.717
≥ ASC-US (included ASC-US, LSIL, ASC-H and HSIL)	10/30 (33.3)	2/7 (28.6)	

Statistical analysis of significance was calculated using chi-square test. P-value <0.05 was considered as statistically significant.

4.4 Clinical correlates of HPV carriage and HPV16/52 E6-specific CMI

Risk factors associated with anal HPV DNA carriage as well as HPV16/52 E6-specific immune responses were determined. Due to the low prevalence of oral HPV carriage in this study, further statistical analysis was not performed.

In HIV+ cohort, parameters such as age, circumcision, history of anal sex, use of condoms, history of sexual activity in the last 12 months and sexual orientation were not associated with the anal HPV carriage (Table 4.6). Analysis had showed that the age of cART initiation (OR=1.13 [95% CI 1.01-1.27], p=0.03), baseline CD8+T cell count (OR=1.00 [95% CI 0.99-1.00], p=0.04) correlated to anal HPV carriage whilst baseline CD4/CD8 ratio at study entry showed a tendency towards an increase the risk of HPV infection, although the correlation was weak (OR=114.27 [95% CI 0.81-16080], p=0.06). Furthermore, multivariate analysis showed that the age of cART initiation (OR=1.21 [95% CI 1.04-1.40], p=0.01) and serum hsCRP level (OR=908 [95% CI 4.3-191720], p=0.01) were correlated with anal HPV carriage.

In the assessment of HPV16/52 E6-specific immune responses, age, circumcision, smoking history, receptive anal intercourse, and sexual orientation were no associated with the presence of HPV16/52 E6-specific CMI (Table 4.7). However, CD4+T cell count at the study entry (OR=1.00 [95% CI 1.00-1.004], P=0.03) and CD4 gain (OR=1.00 [95% CI 1.00-1.004], P=0.04) were positively correlate to HPV16/52 E6-specific CMI. All immunophenotypes including activation and senescence level of CD4+ and CD8+T cells as well as Treg were not correlated to HPV16/52 specific-CMI.

Overall, age of cART initiation and baseline CD8+ T cell counts were independent risk factors to positive anal HPV carriage whereas hsCRP level can be a potential predictor for the risk of anal HPV carriage. HPV16/52 E6-specific CMI responses were

positively correlated to CD4+T cell count but no specific T cell immunophenotypes correlated to HPV16/52 E6-specific CMI responses.

Table 4.7: Univariate analysis of clinical associations with anal HPV carriage in HIV+ men

Parameter	Univariate analysis		P	Multivariate analysis		P
	Coefficient	OR (95% CI)		Coefficient	OR (95% CI)	
Age (years)	0.08	1.08 (0.98-1.20)	0.12			
Circumcised (yes)	0.99	2.68 (0.68-10.53)	0.16			
Age of first sex (years)	0.057	1.06 (0.93-1.2)	0.37			
Receptive anal sex (yes)	0.54	1.71 (0.50-5.86)	0.39			
Insertive anal sex (yes)	0.71	2.03 (0.59-6.93)	0.26			
Condom usage for receptive anal sex						
All the time		1				
Most of the time	-0.41	0.67 (0.05-9.47)	0.77			
Sometimes	1.39	4.00 (0.33-48.66)	0.28			
Condom usage for insertive anal sex						
All the time		12.00 (0.11-35.81)				
Most of the time	0.69	8.00 (0.46-139.29)	0.64			
Sometimes	2.08		0.15			
Sexually active in the last 12 months (yes)	0.11	1.11 (0.33-3.71)	0.86			
Receptive anal sex in the last 12 months (yes)	1.18	3.27 (0.55-19.25)	0.19			
Insertive anal sex in the last 12 months (yes)	0.69	2 (0.28-14.20)	0.49			
Sexual orientation						
Non-homosexual[a]		1	0.39			
Homosexual	0.54	1.71 (0.50-5.86)				
No. of lifetime sexual partners						
1		1	0.84			
≥2	-0.20	0.82 (0.12-5.67)				
Age of initiation cART (years)	0.13	1.13 (1.01-1.27)	0.03	0.19	1.21 (1.04-1.40)	0.01

Table 4.8: Univariate analysis of clinical associations with anal HPV carriage in HIV+ men (Continued)

Parameter	Univariate analysis		P	Multivariate analysis		P
	Coefficient	OR (95% CI)		Coefficient	OR (95% CI)	
Duration on cART (years)	-0.14	0.87 (0.69-1.10)	0.25			
Baseline CD4+T cell count (cells/mm^3)	0.001	1.00 (1.00-1.01)	0.81			
Baseline CD8+T cell count (cells/mm^3)	-0.001	1.00 (0.99-1.00)	0.04			
CD4+T cell count at study entry (cells/mm^3)	0.00	1.00 (0.99-1.00)	0.95			
CD8+T cell count at study entry (cells/mm^3)	0.00	1.00 (0.99-1.00)	0.20			
Baseline CD4/CD8 ratio	4.74	114.27 (0.81-16080)	0.06	8.55	5143 (2.08-12689897)	0.32
CD4/CD8 ratio at study entry	1.03	2.81 (0.46-17.11)	0.26			
CD4+T cell count gain (cells/mm^3)	0.00	1.00 (0.99-1.00)	0.86			
HsCRP (mg/dL)	1.55	4.73 (0.47-47.44)	0.19	6.81	908 (4.3-191720)	0.01
HPV16/52 E6-specific immune responses						
Not detected		1				
Detected	0.81	2.24 (0.38-13.07)	0.37			

[a]Included heterosexual and bisexual men.

Univariate analysis was tested with logistic regression. Variable with p-value <0.25 in univariate analysis included for multivariate analysis. P-value <0.05 was considered as statistically significant.

Table 4.9: Univariate analysis of clinical and immunophenotypes associated with HPV16/52 E6-specific CMI responders

Parameter	HPV16/52 E6-specific CMI responders		P
	Coefficient	OR (95% CI)	
Age (years)	-0.02	0.98 (0.88-1.08)	0.63
Smoking history (yes)	0.51	1.67 (0.40-7.03)	0.49
Age of cART initiation (years)	-0.04	0.97 (0.87-1.07)	0.51
Duration on cART (years)	0.01	1.01 (0.81-1.25)	0.96
Baseline CD4+T cell count (cells/mm^3)	0.002	1.002 (0.99-1.01)	0.30
Baseline CD8+T cell count (cells/mm^3)	0.00	1.00 (0.99-1.00)	0.48
Baseline CD4/CD8 ratio	1.52	4.55 (0.13-155.94)	0.40
CD4+T cell at study entry (cells/mm^3)	0.002	1.00 (1.00-1.004)	0.03
CD8+T cell at study entry (cells/mm^3)	0.001	1.00 (1.00-1.00)	0.17
CD4/CD8 ratio at study entry	0.64	1.89 (0.49-7.30)	0.36
CD4 gain (cells/mm^3)	0.002	1.00 (1.00-1.004)	0.04
HsCRP (mg/dL)	0.12	1.12 (0.13-9.37)	0.92
Naïve CD4+T cells CD4+CD45RA+CCR7+	0.01	1.01 (0.97-1.05)	0.71
Central memory CD4+T cells, T_{CM} CD4+CD45RA-CCR7+	-0.01	0.99 (0.92-1.06)	0.70
Effector memory CD4+T cells, T_{EM} CD4+CD45RA-CCR7-	0.00	1.00 (0.94-1.06)	0.97
Terminal effector CD4+T cells, T_{dEM} CD4+CD45RA+CCR7-	-0.03	0.97 (0.81-1.16)	0.71
Naïve CD8+T cells CD8+CD45RA+CCR7+	0.03	1.00 (0.95-1.07)	0.92
Central memory CD8+ T cells, T_{CM} CD8+CD45RA-CCR7+	-0.01	1.00 (0.84-1.17)	0.96
Effector memory CD8+T cells, T_{EM} CD8+CD45RA-CCR7-	-0.03	0.97 (0.92-1.03)	0.34
Terminal effector CD8+T cells, T_{dEM} CD8+CD45RA+CCR7-	0.03	1.03 (0.97-1.09)	0.32
Activated CD4+T cells CD4+CD38+HLA-DR+	-0.18	0.83 (0.64-1.08)	0.17
Activated CD8+T cells CD8+CD38+HLA-DR+	-0.04	0.96 (0.89-1.03)	0.27
Senescent CD4+T cells CD4+CD28-CD57+	-0.02	0.98 (0.89-1.08)	0.65
Senescent CD8+T cells CD8+CD28-CD57+	-0.004	1.00 (0.95-1.04)	0.86
Regulatory T cells, Treg CD4+CD25+Foxp3+	-0.03	0.98 (0.74-1.29)	0.86

Univariate analysis was tested with logistic regression. Variable with p-value <0.25 in univariate analysis included for multivariate analysis. P-value <0.05 was considered as statistically significant.

CHAPTER 5: DISCUSSION

5.1 Immunological characteristic of cART-treated HIV+ men

The immune reconstitution trends among cART-treated HIV positive individuals generally fall into two distinct groups despite achieving viral suppression: 1) sIR with CD4+T cell count less than 350cells/mm^3 and 2) oIR as defined by an increase in CD4+T cell count to or above 500cells/mm^3 (Hirnschall, Harries, Easterbrook, Doherty, & Ball, 2013; Kelley et al., 2009b; Torti et al., 2012).

Factors associated with sIR include older age of cART initiation (Khanna et al., 2008; A. S. Walker, Doerholt, Sharland, Gibb, & Committee, 2004; Yin et al., 2014), lower baseline CD4+T cell count prior to cART (Cohen et al., 2011; Florence et al., 2003; Kaufmann et al., 2005), skewed maturation of memory T cells (Marchetti et al., 2010), types of antiretroviral drug regimens (Abadi et al., 2006; Dronda et al., 2002; Palma et al., 2007) and low baseline viral load. Similarly, in our cohort, older age of cART initiation, and lower baseline CD4+T cell count were observed in sIR compared to oIR groups (Table 4.1). There have been conflicting reports on the associations between baseline viral load and immune recovery and the reasons remain unclear (Corbeau & Reynes, 2011). Some studies have shown that lower baseline viral load was associated with sIR group (Dronda et al., 2002; Gandhi et al., 2006); while others shown an opposite trend (Falster et al., 2009; Goicoechea et al., 2006; Phillips, Staszewski, Weber, & et al., 2001). In our study, sIR group had significantly higher baseline viral load compared to oIR group (Table 4.1).

Despite viral suppression, sIR have been shown higher levels of Tregs (CD4+CD25+Foxp3+) (Horta et al., 2013; Méndez-Lagares et al., 2012), activation markers (CD38+HLA-DR+) (Albuquerque et al., 2007; Saison et al., 2014) and senescence markers (CD28-CD57+ or CD57+) (Molina-Pinelo et al., 2009; Serrano-

Villar et al., 2014) on CD4+ and CD8+T cells when compared to oIR group. In our study, compared to oIR group, sIR group exhibited significantly higher levels of Tregs, activated CD4+ and CD8+T cells as well as senescent CD8+T cells (Table 4.2 and Figure 4.2). As expected, the HIV+ groups displayed a higher level of senescent CD4+T cells compared to controls. oIR group also exhibited higher levels of activated and senescent CD4+T cells compared to control (not statistically significant), while the proportion of Treg, and levels of activated and senescent CD8+T cells were nearly normalized when compared to controls. These observations are consistent with published data (Albuquerque et al., 2007; Bordoni et al., 2012; Lederman et al., 2011; Méndez-Lagares et al., 2012).

On the other hand, sIR group had lower percentages of naïve CD4+ and CD8+ T cells with higher percentage of memory T cells (T_{CM} and T_{EM}) compared to oIR group (Table 4.2 and Figure 4.1), which is in agreement with published data (Marchetti et al., 2010). This likely due to the differentiation of naïve T cells into mature T cells during persistent and chronic HIV infection in the sIR group (Geldmacher et al., 2010; Riou et al., 2015; Riou et al., 2012).

In this study, we have not demonstrated a significant difference in the HPV carriage rates among sIR and oIR. This is most likely due to the high HPV prevalence among the HIV-infected population. The observation that HPV DNA detection itself may not commensurate the detection of HPV specific immunity have also been described in other studies (de Jong et al., 2002; Woo et al., 2010). Ideally, a longer prospective study that follows sIR and oIR groups for a longer length of time will confirm if the presence of HPV immunity confers long term protection against disease among oIR.

5.2 Prevalence of HPV carriage in cART-treated HIV+ men

Oral HPV can be detected in 2.3-20% of healthy individuals (Antonsson et al., 2014; Colon-López et al., 2014; Gillison, Broutian, Pickard, & et al., 2012; Rosen et al., 2015; Vacharotayangul et al., 2015) which was similar to the controls in our study (Table 4.3). The prevalence of anal HPV among controls (majority were heterosexual men with no history of anal intercourse) was similar to published prevalence of 5-8% among HIV-uninfected heterosexual men (P. V. Chin-Hong et al., 2004; Nicolau et al., 2005; Nyitray et al., 2010) but was lower than the prevalence of anal HPV among HIV-uninfected MSM (33-57%) (P. V. Chin-Hong et al., 2004; Goldstone et al., 2011; Phanuphak et al., 2013; D. Y. Zhang et al., 2014).

Regardless of cART treatment or CD4+T cell count, the prevalence of anal HPV infection in HIV+ population is 71-85% in HIV+ MSM and 40-68% HIV+ heterosexual men (Phanuphak et al., 2013; Piketty et al., 2003; Sirera et al., 2006; Videla et al., 2013) while abnormal anal intraepithelial lesions including ASC-US, LSIL and HSIL were detected in 7-12%, 8-42%, and 0.2-5% of HIV+ men, respectively (Gonzalez et al., 2013; A. H. Li et al., 2009; Melo et al., 2014). In addition, the prevalence of oral HPV is high among HIV+ men at 16-20% (Darwich et al., 2014; Parisi et al., 2011; Vacharotayangul et al., 2015; Videla et al., 2013). Our study was consistent with published data (Table 4.3).

In our study, HPV16 was the most prevalent anal HPV genotype among HIV+ men regardless of CD4+T cell count which was similar to previous studies (Darwich et al., 2014; Sirera et al., 2008; Videla et al., 2013). In addition, our observation were in line with the prevalence of HPV types detected in Asia. A study conducted among Chinese MSM documented that four most common HR anal HPV to be HPV16, followed by HPV18, HPV58 and HPV52 (D. Y. Zhang et al., 2014). Noteworthy, both HPV58 and

HPV52 (anogenital genotypes) are HPV genotypes that were commonly detected in Asian populations (Chan et al., 2014; H. C. Chen et al., 2011; Takehara et al., 2011). We also found that HR HPV genotypes and multiple genotypes of HPV per sample were common among HIV+ individuals (Beachler, D'Souza, Sugar, Xiao, & Gillison, 2013; Sirera et al., 2008) compared to controls, again consistent with previous studies (D.H. Adler et al., 2015; Beachler et al., 2015; Hu et al., 2013; Latini et al., 2014; Palefsky, Holly, Ralston, & Jay, 1998).

Among the HIV+ men in this study, HPV DNA was detected in nearly half of men with normal anal cytology and in all abnormal anal cytology (Table 4.4). We also found that the HR HPV was detected in up to 73% of men with normal cytology and 90% of men with abnormal anal cytology, which was again consistent with published findings (Firnhaber et al., 2009; Gonzalez et al., 2013). In addition, multiple HPV genotypes per sample were also more likely to be associated with abnormal cytology as reported by previous published data (D. H. Adler et al., 2014; Blossom et al., 2007; Jing et al., 2014). However, we were not able to compare anal cytology between HIV+ men and controls as we had only 3 out of 29 control anal cytology that were satisfactory for anal cytology screening.

Taken together, the prevalence of HPV infection in anus and oropharynx observed in current study was comparable to previous studies. However, the prevalence of HPV carriage was not lower among oIR patients who achieved CD4+T cell count more than 500cells/mm^3. The may be due to impaired functionality of CD4+T cell or shift of Th1 and Th2 cytokines by CD4+T cell although the ability of CD4+T cell proliferation is preserved. This has been observed in a study on HPV-specific CMI on patients with cervical cancer (de Jong et al., 2004).

5.3 Correlates of HPV carriage in cART-treated HIV+ men

The prevalence of anal HPV DNA among HIV+ men was very high compared to controls. In this study, there are several risk factors showed correlation to anal HPV.

Nadir/baseline CD4+T cell count (CD4+T cell count prior to cART, hereafter called baseline) is a strong predictor for immune recovery as a lower baseline CD4+T cells count is associated with poor CD4+T cell recovery (Castilho et al., 2015; Kaufmann et al., 2005; Massanella et al., 2010). This was also observed in our cohort where sIR group who had initiated cART at a lower baseline CD4+T cell count hardly reached CD4+T cell count of more than 500cells/mm^3 at entry study, which was at a median of 5 years after cART initiation. Poor immune recovery reflects the impairment of T cell homeostasis, likely caused by high turnover of antigen-experienced T cell from naïve T cell in response to HIV infection (Schacker et al., 2002). Thus, lower baseline CD4+T cell count (Darwich et al., 2014; Menezes et al., 2015; Wilkin, Palmer, Brudney, Chiasson, & Wright, 2004) and lower CD4+T cell count at study entry (Beachler et al., 2013; Castilho et al., 2015; Melo et al., 2014; Parisi et al., 2011) have been reported as risk factors for HPV infection. However, in our study both baseline CD4+T cell count and CD4+T cell count at study entry did not correlate to HPV infection (Table4.6) as observed by other studies (Hidalgo-Tenorio et al., 2014; Piketty et al., 2013). This suggests that the improvement of HIV-caused immunosuppression by cART was insufficient to reduce the risk of HPV infection in this cohort. Other cofactors such as active sexual intercourse (for newly acquired HPV) or latent phase of HPV infection (escape host immunesurveillence) contributes to the higher prevalence of HPV DNA detection although patients have optimal immunological status. Interestingly, HIV+ men in our study with CD4+T cell count >900cells/mm^3 did not show abnormal anal cytology (Figure 4.3c). This may be attributed to the better immune recovery in these men as they are less likely to develop AIDS-defining diseases (Okulicz, Le, Agan, & et al., 2015).

In this study, age did not correlate to anal HPV infection. This finding was in agreement with previous studies that showed the prevalence of anal HPV DNA did not decline with age for both female and male which was in contrast to prevalence of cervical HPV DNA (Peter V. Chin-Hong et al., 2004; Donà et al., 2014; Poynten et al., 2015). This disparate observation (between both prevalence of cervical and anal HPV) may be due to the sexual behaviour among MSMs regardless of age (Peter V. Chin-Hong et al., 2004; Donà et al., 2014) and hormonal changes in transformation zone of cevix (which is not found in anus) increasing susceptibility to HPV infection during the different reproductive phases of life (Peter V. Chin-Hong et al., 2004). On the other hand, age of cART initiation showed correlation to anal HPV carriage. This parameter reflects the degree of immune recovery's reliance on age. As we know, the elderly were more likely to experience impaired immune function due to a decline in generation of new naïve T and B lymphocytes and less competence in the expansion capacity of memory cells (Hakim & Gress, 2007). This immune aging process further accelerated in chronic HIV infection. As a result, HIV+ individuals show similar immunological changes as observed in elderly HIV- uninfected individuals (Ikezu, 2009; Teichmann et al., 2009). Therefore, older age may limit the capacity of immune recovery and thus unable to generate an efficient protective immunity against HPV. Taken together, age alone may not be informative to evaluate the risk of anal HPV but age of cART initiation does.

Baseline CD8+T cell count is one of the risk factors to HPV infection in our cohort (Table 4.5). The effective cytotoxic CD8+T cells respond against HPV infection in healthy individuals have been widely reported (Nakagawa et al., 1997; Nakagawa et al., 2000). In cART-untreated HIV+ population, CD8+T cells are susceptible to bystander apoptosis (Vivar et al., 2011) and have impaired proliferative ability (Effros et al., 1996) due to loss CD28 receptor expression on CD4+ and CD8+T cells that were induced by active HIV replication (Tassiopoulos et al., 2012; Vivar et al., 2011), as well as reduced

cytotoxicity and cytokine production (Shankar et al., 2000). Eventually, the reduction of CD8+T cells would lead to ineffective immune responses against diseases including HPV (Gamberg, Pardoe, Bowmer, Howley, & Grant, 2004). However, the implementation of cART can delay the disease progression by enhancing the expression of CD28 (Vivar et al., 2011). Therefore, HIV+ individuals who had initiated cART before large amounts of their CD8+T cells affected by HIV can reduces the risk of HPV infection and HPV-related diseases.

Lower baseline CD4/ CD8 ratio prior to cART in current study showed a tendency towards higher risk of HPV carriage (Table 4.6). In cART-untreated individuals, high depletion of CD4+T cell count with elevation of CD8+T cell count in response to HIV evasion is often observed. In cART era, some patients still fail to achieve CD4+T cell count >500cells/mm^3 despite successful viral suppression on long term cART. Factors that correlate to this clinical outcome include low baseline CD4+T cell count and baseline CD4:CD8 ratio (Torti et al., 2012). These two factors were also observed in our sIR group when compared to the oIR group. On the other hand, low CD4/CD8 ratio was also observed in cART-treated HIV+ individuals despite CD4 count >500cells/mm^3. This indicates that those individuals did not truly achieve optimal immune restoration, as high level of T cell activation (HLA+CD38+) and senescence (CD57+CD28-/CD57-) are often observed (Sainz et al., 2013).

In multivariate analysis, the level of serum hsCRP showed a weak correlation to anal HPV infection due to wide confidence interval of odd ratio although it was statistically significant. This is most likely due to the sample size of HIV+ adults in this study was not powered to evaluate the correlation between hsCRP level and anal HPV carriage (du Prel, Hommel, Rohrig, & Blettner, 2009) (Table 4.6). CRP is a protein synthesized by hepatocytes as part of the inflammation response to tissue damage caused by infection

and malignant diseases. According to previous studies, the elevation of plasma hsCRP is associated with clinical disease stage such as oral cancer (Jablonska, Piotrowski, & Grabowska, 1997) and, HIV disease progression (Lau, Sharrett, Kingsley, & et al., 2006; MahdadNoursadeghi & Miller, 2005). However, the use of hsCRP as a prognostic marker remains controversial as there are also studies that show no associations between serum hsCRP levels and diseases outcomes (Kruse, Luebbers, & Grätz, 2010; S. M. Zhang, Buring, Lee, Cook, & Ridker, 2005).

5.4 HPV16/52 E6-specific CMI in cART-treated HIV+ men

In this study, the prevalence of anal HPV was not significantly different between sIR and oIR group even though immunologically those with oIR better than former. After initiation of cART, some patients failed to normalize CD4+T cell count to more than 500cells/mm^3 despite viral suppression. The failure to restore CD4+T cell count during cART may be caused by patients initiating cART with lower baseline CD4+T cell count (Falster et al., 2009) and impaired thymus activity (Benveniste et al., 2005) compared to those with complete immune recovery (CD4+T cell count > 500cells/mm^3). Lower baseline CD4+T cell count prior to cART suggests excessive CD4+T cell depletion caused by persistent T cell activation which accelerates immunesenescence (Brenchley et al., 2003; W. Cao et al., 2009), whereas impaired thymus activity is caused by over regeneration of naïve T cell to replenish the depletion of memory T cells. All these clinical features can limit the restoration of immune system. Thus, only a small proportion of sIR group exhibited HPV16/52 E6-specific CMI which was significantly lower than oIR group.

Many studies have demonstrated that failure of HPV-specific CMI detected in patients associated with HPV DNA and abnormal cervical cytology (de Jong et al., 2004; Molling et al., 2007; Woo et al., 2008; Woo et al., 2010). This may due to immunosuppression by Treg, impaired proliferation of T cells or imbalance of Th1 and Th2 cytokines. Based on our data, we did not find an association between Tregs and HPV16/52 E6-specific CMI in the presence of HPV16/52 DNA (Figure 4.5). This lack of clinical correlation may be due to the lack of histological specimens (biopsy and cytology) correlates as reported by previous studies (Molling et al., 2007; Woo et al., 2010).

5.5 Correlates of HPV16/52 E6-specific CMI in cART treated HIV+ men

5.5.1 T cell activation causes depletion of pathogen-specific memory T cells

The depletion of T cells is a hallmark of HIV infection. Direct lysis of HIV-infected cells by HIV (J. Cao, Park, Cooper, & Sodroski, 1996) is one of the factors that contribute to T cell depletion. Indeed, activated pathogen-specific memory CD4+T cells which are actively expanded clonally due to reactivation of a given pathogen such as *Mycobacterium tuberculosis* (MBT) often becomes the target of HIV infection (Biancotto et al., 2008; Geldmacher et al., 2010). However, this is not the main cause leading massive T cell depletion in HIV-infected patients. Studies revealed that most of the dead cells are HIV-uninfected CD4+ and CD8+T cells (Finkel et al., 1995; Muro-Cacho, Pantaleo, & Fauci, 1995) which are largely attributed to activation-induced cell death (Katsikis, Wunderlich, Smith, Herzenberg, & Herzenberg, 1995; Meyaard, Otto, Keet, Roos, & Miedema, 1994). One possible mechanism to explain the death of HIV-uninfected CD4+ and CD8+T cells is the bystander activation effect.

As immune response, HIV or co-infections such as CMV or MBT activates memory T-cells which secrete plenty of inflammatory cytokines such as Tumour necrosis factor α (TNFα) resulting in a chronic inflammation microenvironment (Decrion et al., 2005; Geldmacher et al., 2010). This could result in bystander activation of other pathogen-specific memory T cells (Both CD4+ and CD8+T cells) without T cell receptor (TCR) stimulation (De et al., 2005; Holm & Gabuzda, 2005). For example, Epstein–Barr virus (EBV), CMV and Influenza (FLU)-specific CD8+T cells are activated (CD38+ and HLA-DR+, respectively) in patients with primary HIV-infection despite absent reactivity of EBV, CMV and FLU infection (Doisne et al., 2004). Furthermore, this bystander activation declines after one year on cART. Although cART suppresses viral replication effectively, systemic T cell activation in cART-treated patients with poor immune recovery (CD4<500cells/mm^3) remains high due to residual viral production (Yukl et al.,

2010) and microbial translocation (Jiang et al., 2009). Under such a chronic inflammation, bystander activation may occur in the same manner in untreated- HIV+ individuals.

Based on above published data, chronic immune activation of both HIV-infected and uninfected (bystander) T cells contributed to the depletion of T cell including HPV-specific CMI. However, this correlation was not established in this study.

5.5.2 Immunesenescence as a consequence of persistent T cell activation

Patients with persistent viral infections such as HIV, CMV or Hepatitis B virus (HBV) are shown to have shortened telomere length, and also lack CD28 expression which indicates premature immunesenescence (Effros et al., 1996; Kitay-Cohen, Goldberg-Bittman, Hadary, Fejgin, & Amiel, 2008; Molina-Pinelo et al., 2009; Pourgheysari et al., 2007). Although no correlation has been observed between immunesenescence and HPV-specific CMI in current study, we hypothesized that the depletion of T cells caused by HIV potentially induces the reactivation of HPV infection in cART-treated HIV+ men. As a result, HPV infection may be persistent due to poor immunological status and causes HPV-specific CMI undergoes immunesenescence. A possible explanation as to why the correlation was not observed is most like due to insufficient length of time to observe the immunesenescence.

5.5.3 Skewed maturation of memory T cell subsets

Many studies show an association between chronic viral infections and disturbed T cell differentiation and maturation leading to heterogeneity of T cell profile. For example, CMV-specific CD4+ and CD8+T cells are mainly T_{EM} and terminal differentiated effector T cells (T_{dEM}), while HIV and MTB-specific T cells are T_{CM} or T_{EM} (Geldmacher et al., 2010; Riou et al., 2015; Riou et al., 2012). Such heterogeneity of T cell profile depends on the duration and intensity of antigenic exposure (Streeck et al., 2008). Similarly, skewed T cell maturation is also observed in HPV-induced CIN I (Pita-Lopez,

Ortiz-Lazareno, Navarro-Meza, Santoyo-Telles, & Peralta-Zaragoza, 2014). Patients with CIN I exhibited high percentage of T_{dEM} at systemic level compared to patients with positive HPV DNA and healthy controls although not significantly different. However, a skewed T cell maturation did not correlated to HPV16/52 E6 CMI in current study.

Furthermore, skewed maturation of T cells due to persistent and chronic viral infections also alter polyfunctionality of memory T cell subsets (Riou et al., 2012). The alteration of polyfunctionality of memory T cell leads an individual fail to mount an effective immune response in controlling viral infection. In addition, most of the pathogen-specific T cells were less differentiated (T_{CM} and T_{EM}). According to a study, CD57- memory CD4 T cells (T_{CM} and part of the T_{EM}) were preferentially infected by HIV (Brenchley et al., 2004) compared to CD57+ memory CD4+T cells (T_{dEM}) which are susceptible to apoptosis after re-exposured to a given antigen (B. E. Palmer et al., 2005). This preference can assist HIV to replicate competently. Taken together, HIV infection significantly affect the ability of HIV+ patients in mounting an effective pathogen-specific CMI. In this experiment, we used only IFNγ ELISPOT to enumerate the frequency of responding cells and thus we were unable to measure the polyfunctionality of each memory subsets. We speculate that the decrease in polyfunctional HPV-specific memory T cell subset may be one of the risk factors to HPV infection even though patients have complete immune recovery.

5.5.4 The role of Treg in co-infection HPV and HIV

Tregs are known to play role in immunosuppression. Despite the presence of HPV-specific CMI, disease progression can be observed particularly in the presence of Tregs (Kojima et al., 2013; Molling et al., 2007). Similarly, HIV disease progression, particularly among patients with low CD4+T cell count has also been found to be associated with higher Tregs (Saison et al., 2015). Based on these findings, Tregs were

expected to be one of the factors suppress the function of HPV16/52 E6-specific CMI which may alter HPV reactivity. In this study, we did not demonstrated any association between Tregs and HPV-specific immunity. It is possible that the regulatory effects may be less pronounced in the presence of chronic immune activation in our HIV infected population. Hence, the unobservable differences.

CHAPTER 6: CONCLUSION

6.1 Conclusion

The present study is the first study to investigate the presence of HPV-specific CMI among cART-treated HIV patients with optimal and suboptimal CD4 recovery. The information obtained from this study can add to our understanding of the epidemiology and immunology factors associated with the dynamic of HPV-specific CMI. In this study, we have demonstrated that

1) The prevalence of anal (57.1% vs 56.5%) and oral (16.7% vs 11.1%) HPV DNA in suboptimal immune reconstitution (sIR) group was not higher than optimal immune reconstitution (oIR).
2) The presence of HPV16/52 E6-specific CMI among oIR was higher than sIR.
3) Patient characteristics such as sexual orientation, number of sexual partners and history of anal intercourse was not associated with HPV carriage among those who were HIV+. However, clinic factors such as age of initiate cART, and baseline CD8 count were associated with HPV carriage.
4) Apart from CD4+T cell count, T cell immunophenotypes which are activation (CD38+HLA-DR+), senescence (CD2-CD57+), Treg (Foxp3+CD25+) and T cell subsets did not correlate with the presence of HPV16/52 E6-specific CMI.

6.2 Limitation of the study

There are several limitations that have been identified in our study. First, HPV induced disease in the anus was assessed by anal cytology with no anoscopy and biopsy. The use of cytology examination may underestimate the prevalence of abnormal anal intraepithelial. Secondly, HPV16/52 E6-specific immune measured in this study are at systemic level, which may not truly reflect the localized HPV-specific CMI. This is because HPV infection is localized infection instead of systemic infection. Information on local responses may provide us with more information. Thirdly, we explored systemic immunophenotypes instead of immunophenotypes of HPV16/52 E6– stimulated PBMC. This may not truly reflect the immunophenotypes of HPV-specific T cells. In addition, we detect HPV-specific CMI against only E6 peptides. In fact, HPV-specific CMI response to HPV E2, E7 as well as L1 peptides have been reported previously. Thus, we may underestimate HPV-specific immune responses against other HPV antigen. In addition, the polyfunctionality across memory T cell subsets is important to ensure the quality of T cells against pathogen. However, current study is unable to measure this since IFNγ ELISPOT was used. Perhaps, intracellular cytokine with flow cytometry can help to measure the polyfunctionality of HPV-specific memory T cell subsets. This study has been powered to assess the differences in HPV E6-specific immunity among oIR and sIR. The cohorts were underpowered to assess the differences in HPV carriage or HPV-associated diseases such as AIN or warts. To study this, a larger prospective cohort observed over a longer period of time would be required. Lastly, sexual orientation was not comparable between healthy control and HIV+ men group as this factor has been found to be associated with inflammation (C. D. Palmer et al., 2014)

6.3 Future work

In order to have a better understanding of the dynamic of HPV antigen-specific CMI, several suggestions may help improve the current research. To assess HPV-induced intraepithelial lesion precisely, histology examination on anal tissue is strongly recommended. Secondly, tissue resident-memory T cell specific to HPV antigen is recommended as HPV antigen-specific CMI at systemic level may not truly reflect the localized immune responses. Additionally, HPV antigen-specific CMI against peptides E2 and E7 should be measured as both genes are the key role to initiate the replication of HPV and expression of oncogene, respectively. As per discussion, the polyfunctionality of memory T cell is important to understand the efficiency of pathogen-specific CMI. An intracellular cytokine flow cytometry to measure Th1 and Th2 cytokines secreted by HPV antigen-specific CMI is recommended. The expression of PD-1 expression on both CD4+ and CD8+T cells is suggested to measure. This is because upregulation of PD-1is correlate to diseases progression with decreased proliferation and production of cytokines of functional T cells (C. L. Day et al., 2006; Trautmann et al., 2006).

REFERENCES

Abadi, J., Sprecher, E., Rosenberg, M. G., Dobroszycki, J., Sansary, J., Fennelly, G., & Wiznia, A. (2006). Partial Treatment Interruption of Protease Inhibitor-Based Highly Active Antiretroviral Therapy Regimens in HIV-Infected Children. *JAIDS Journal of Acquired Immune Deficiency Syndromes, 41*(3), 298-303.

Adam, E., Berkova, Z., Daxnerova, Z., Icenogle, J., Reeves, W. C., & Kaufman, R. H. (2000). Papillomavirus detection: demographic and behavioral characteristics influencing the identification of cervical disease. *American Journal of Obstetrics and Gynecology, 182*(2), 257-264.

Adler, D. H., Wallace, M., Bennie, T., Abar, B., Sadeghi, R., Meiring, T., . . . Bekker, L. G. (2015). High risk human papillomavirus persistence among HIV-infected young women in South Africa. *Int J Infect Dis, 33*, 219-221.

Adler, D. H., Wallace, M., Bennie, T., Mrubata, M., Abar, B., Meiring, T. L., . . . Bekker, L. G. (2014). Cervical dysplasia and high-risk human papillomavirus infections among HIV-infected and HIV-uninfected adolescent females in South Africa. *Infect Dis Obstet Gynecol, 2014*, 498048.

Ahdieh, L., Gange, S. J., Greenblatt, R., Minkoff, H., Anastos, K., Young, M., . . . Munoz, A. (2000). Selection by indication of potent antiretroviral therapy use in a large cohort of women infected with human immunodeficiency virus. *American Journal of Epidemiology, 152*(10), 923-933.

Aidala, A. A., Lee, G., Garbers, S., & Chiasson, M. A. (2006). Sexual Behaviors and Sexual Risk in a Prospective Cohort of HIV-Positive Men and Women in New York City, 1994–2002: Implications for Prevention. *AIDS Education and Prevention, 18*(1), 12-32.

Albuquerque, A. S., Foxall, R. B., Cortesão, C. S., Soares, R. S., Doroana, M., Ribeiro, A., . . . Sousa, A. E. (2007). Low CD4 T-cell counts despite low levels of circulating HIV: Insights from the comparison of HIV-1 infected patients with a discordant response to antiretroviral therapy to patients with untreated advanced HIV-2 disease. *Clinical Immunology, 125*(1), 67-75.

Alimonti, J. B., Ball, T. B., & Fowke, K. R. (2003). Mechanisms of CD4+ T lymphocyte cell death in human immunodeficiency virus infection and AIDS. *J Gen Virol, 84*(Pt 7), 1649-1661.

Ambarus, C. A., Krausz, S., van Eijk, M., Hamann, J., Radstake, T. R. D. J., Reedquist, K. A., . . . Baeten, D. L. P. (2012). Systematic validation of specific phenotypic markers for in vitro polarized human macrophages. *Journal of Immunological Methods, 375*(1–2), 196-206.

Antonsson, A., Cornford, M., Perry, S., Davis, M., Dunne, M. P., & Whiteman, D. C. (2014). Prevalence and Risk Factors for Oral HPV Infection in Young Australians. *Plos One, 9*(3), e91761.

Apgar, B. S., Zoschnick, L., & Wright, T. C., Jr. (2003). The 2001 Bethesda System terminology. *Am Fam Physician, 68*(10), 1992-1998.

Arreygue-Garcia, N. A., Daneri-Navarro, A., del Toro-Arreola, A., Cid-Arregui, A., Gonzalez-Ramella, O., Jave-Suarez, L. F., . . . Del Toro-Arreola, S. (2008). Augmented serum level of major histocompatibility complex class I-related chain A (MICA) protein and reduced NKG2D expression on NK and T cells in patients with cervical cancer and precursor lesions. *BMC Cancer, 8*, 16.

Ashrafi, G. H., Haghshenas, M. R., Marchetti, B., O'Brien, P. M., & Campo, M. S. (2005). E5 protein of human papillomavirus type 16 selectively downregulates surface HLA class I. *International Journal of Cancer, 113*(2), 276-283.

Beachler, D. C., D'Souza, G., Sugar, E. A., Xiao, W., & Gillison, M. L. (2013). Natural History of Anal vs Oral HPV Infection in HIV-Infected Men and Women. *The Journal of Infectious Diseases, 208*(2), 330-339.

Beachler, D. C., Sugar, E. A., Margolick, J. B., Weber, K. M., Strickler, H. D., Wiley, D. J., . . . D'Souza, G. (2015). Risk Factors for Acquisition and Clearance of Oral Human Papillomavirus Infection Among HIV-Infected and HIV-Uninfected Adults. *American Journal of Epidemiology, 181*(1), 40-53.

Benveniste, O., Flahault, A., Rollot, F., Elbim, C., Estaquier, J., Pédron, B., . . . Leport, C. (2005). Mechanisms Involved in the Low-Level Regeneration of CD4+ Cells in HIV-1—Infected Patients Receiving Highly Active Antiretroviral Therapy Who Have Prolonged Undetectable Plasma Viral Loads. *Journal of Infectious Diseases, 191*(10), 1670-1679.

Bernard, B. A., Bailly, C., Lenoir, M. C., Darmon, M., Thierry, F., & Yaniv, M. (1989). The Human Papillomavirus Type-18 (Hpv18) E2 Gene-Product Is a Repressor of the Hpv18 Regulatory Region in Human Keratinocytes. *Journal of Virology, 63*(10), 4317-4324.

Biancotto, A., Iglehart, S. J., Vanpouille, C., Condack, C. E., Lisco, A., Ruecker, E., . . . Grivel, J.-C. (2008). HIV-1–induced activation of CD4(+) T cells creates new targets for HIV-1 infection in human lymphoid tissue ex vivo. *Blood, 111*(2), 699-704.

Bleeker, M. C. G. M. D. a., Hogewoning, C. J. A. M. D. b., van den Brule, A. J. C. P. a., Voorhorst, F. J. M. D. P. c., van Andel, R. E. a., Risse, E. K. J. M. D. P. a., . . . Meijer, C. J. L. M. M. D. P. a. (2002). Penile lesions and human papillomavirus in male sexual partners of women with cervical intraepithelial neoplasia. *Journal of the American Academy of Dermatology, 47*(3), 351-357.

Blossom, D. B., Beigi, R. H., Farrell, J. J., Mackay, W., Qadadri, B., Brown, D. R., . . . Salata, R. A. (2007). Human Papillomavirus Genotypes Associated With Cervical Cytologic Abnormalities and HIV Infection in Ugandan Women. *J Med Virol, 79*(6), 758-765.

Bordoni, V., Agrati, C., Rinaldi, A., Viola, D., De Simone, G., Gioia, C., & Martini, F. (2012). In HIV-infected patients, some differential alterations of CD4 and CD8 T cell homeostasis may not be restored by ⩾7 years of highly active antiretroviral therapy, in spite of good CD4 T cell repopulation. *Journal of Antimicrobial Chemotherapy*.

Brenchley, J. M., Hill, B. J., Ambrozak, D. R., Price, D. A., Guenaga, F. J., Casazza, J. P., . . . Koup, R. A. (2004). T-cell subsets that harbor human immunodeficiency virus (HIV) in vivo: implications for HIV pathogenesis. *J Virol, 78*(3), 1160-1168.

Brenchley, J. M., Karandikar, N. J., Betts, M. R., Ambrozak, D. R., Hill, B. J., Crotty, L. E., . . . Koup, R. A. (2003). Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8+ T cells. *Blood, 101*(7), 2711-2720.

Brenchley, J. M., Price, D. A., Schacker, T. W., Asher, T. E., Silvestri, G., Rao, S., . . . Douek, D. C. (2006). Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med, 12*(12), 1365-1371.

Bruni, L., Diaz, M., Castellsague, X., Ferrer, E., Bosch, F. X., & de Sanjose, S. (2010). Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. *J Infect Dis, 202*(12), 1789-1799.

Buck, C. B., Day, P. M., & Trus, B. L. (2013). The Papillomavirus Major Capsid Protein L1. *Virology, 445*(0), 169-174.

Burd, E. M. (2003). Human papillomavirus and cervical cancer. *Clin Microbiol Rev, 16*(1), 1-17.

Cao, J., Park, I. W., Cooper, A., & Sodroski, J. (1996). Molecular determinants of acute single-cell lysis by human immunodeficiency virus type 1. *Journal of Virology, 70*(3), 1340-1354.

Cao, W., Jamieson, B. D., Hultin, L. E., Hultin, P. M., Effros, R. B., & Detels, R. (2009). Premature Aging of T cells Is Associated With Faster HIV-1 Disease Progression. *Journal of acquired immune deficiency syndromes (1999), 50*(2), 137-147.

Carter, J. J., Koutsky, L. A., Hughes, J. P., Lee, S. K., Kuypers, J., Kiviat, N., & Galloway, D. A. (2000). Comparison of human papillomavirus types 16, 18, and 6 capsid antibody responses following incident infection. *J Infect Dis, 181*(6), 1911-1919.

Castellsague, X., Bosch, F. X., Munoz, N., Meijer, C. J., Shah, K. V., de Sanjose, S., . . . International Agency for Research on Cancer Multicenter Cervical Cancer Study, G. (2002). Male circumcision, penile human papillomavirus infection, and cervical cancer in female partners. *N Engl J Med, 346*(15), 1105-1112.

Castilho, J. L., Levi, J. E., Luz, P. M., Cambou, M. C., Vanni, T., de Andrade, A., . . . Friedman, R. K. (2015). A cross-sectional study of high-risk human papillomavirus clustering and cervical outcomes in HIV-infected women in Rio de Janeiro, Brazil. *BMC Cancer, 15*, 478.

Castle, P. E., Schiffman, M., Burk, R. D., Wacholder, S., Hildesheim, A., Herrero, R., . . . Lorincz, A. (2002). Restricted Cross-Reactivity of Hybrid Capture 2 with Nononcogenic Human Papillomavirus Types. *Cancer Epidemiology Biomarkers & Prevention, 11*(11), 1394-1399.

Chan, P. K. S., Cheung, T. H., Tam, A. O. Y., Lo, K. W. K., Yim, S. F., Yu, M. M. Y., . . . Margaret, I. P. (2006). Biases in human papillomavirus genotype prevalence assessment associated with commonly used consensus primers. *International Journal of Cancer, 118*(1), 243-245.

Chan, P. K. S., Ho, W. C. S., Chan, M. C. W., Wong, M. C. S., Yeung, A. C. M., Chor, J. S. Y., & Hui, M. (2014). Meta-Analysis on Prevalence and Attribution of Human Papillomavirus Types 52 and 58 in Cervical Neoplasia Worldwide. *Plos One, 9*(9), e107573.

Chan, P. K. S., Li, W. H., Chan, M. Y. M., Ma, W. L., Cheung, J. L. K., & Cheng, A. F. (1999). High prevalence of human papillomavirus type 58 in Chinese women with cervical cancer and precancerous lesions. *J Med Virol, 59*(2), 232-238.

Chan, P. K. S., Liu, S. J., Cheung, J. L., Cheung, T. H., Yeo, W., Chong, P., & Man, S. (2011). T-cell response to human papillomavirus type 52 L1, E6, and E7 peptides in women with transient infection, cervical intraepithelial neoplasia, and invasive cancer. *J Med Virol, 83*(6), 1023-1030.

Chaturvedi, A. K., Madeleine, M. M., Biggar, R. J., & Engels, E. A. (2009). Risk of Human Papillomavirus-Associated Cancers Among Persons With AIDS. *J Natl Cancer Inst, 101*(16), 1120-1130.

Chen, H. C., You, S. L., Hsieh, C. Y., Schiffman, M., Lin, C. Y., Pan, M. H., . . . Group, C.-H. S. (2011). Prevalence of genotype-specific human papillomavirus infection and cervical neoplasia in Taiwan: a community-based survey of 10,602 women. *International Journal of Cancer, 128*(5), 1192-1203.

Chen, Z., Ding, J., Pang, N., Du, R., Meng, W., Zhu, Y., . . . Ding, Y. (2013). The Th17/Treg balance and the expression of related cytokines in Uygur cervical cancer patients. *Diagnostic Pathology, 8*, 61-61.

Chiang, C. M., Ustav, M., Stenlund, A., Ho, T. F., Broker, T. R., & Chow, L. T. (1992). Viral-E1 and Viral-E2 Proteins Support Replication of Homologous and Heterologous Papillomaviral Origins. *Proceedings of the National Academy of Sciences of the United States of America, 89*(13), 5799-5803.

Chin-Hong, P. V., & Palefsky, J. M. (2005). Human papillomavirus anogenital disease in HIV-infected individuals. *Dermatol Ther, 18*(1), 67-76.

Chin-Hong, P. V., Vittinghoff, E., Cranston, R. D., Buchbinder, S., Cohen, D., Colfax, G., . . . Palefsky, J. M. (2004). Age-Specific Prevalence of Anal Human Papillomavirus Infection in HIV-Negative Sexually Active Men Who Have Sex with Men: The EXPLORE Study. *Journal of Infectious Diseases, 190*(12), 2070-2076.

Chin-Hong, P. V., Vittinghoff, E., Cranston, R. D., Buchbinder, S., Cohen, D., Colfax, G., . . . Palefsky, J. M. (2004). Age-Specific prevalence of anal human papillomavirus infection in HIV-negative sexually active men who have sex with men: the EXPLORE study. *J Infect Dis, 190*(12), 2070-2076.

Clark, S. J., Saag, M. S., Decker, W. D., Campbell-Hill, S., Roberson, J. L., Veldkamp, P. J., . . . Shaw, G. M. (1991). High titers of cytopathic virus in plasma of patients with symptomatic primary HIV-1 infection. *N Engl J Med, 324*(14), 954-960.

Clay, T. M., Hobeika, A. C., Mosca, P. J., Lyerly, H. K., & Morse, M. A. (2001). Assays for Monitoring Cellular Immune Responses to Active Immunotherapy of Cancer. *Clinical Cancer Research, 7*(5), 1127-1135.

Cohen, M. S., Chen, Y. Q., McCauley, M., Gamble, T., Hosseinipour, M. C., Kumarasamy, N., . . . Fleming, T. R. (2011). Prevention of HIV-1 Infection with Early Antiretroviral Therapy. *New England Journal of Medicine, 365*(6), 493-505.

Coleman, N., Birley, H. D., Renton, A. M., Hanna, N. F., Ryait, B. K., Byrne, M., . . . Stanley, M. A. (1994). Immunological events in regressing genital warts. *Am J Clin Pathol, 102*(6), 768-774.

Colon-López, V., Quiñones-Avila, V., Del Toro-Mejías, L. M., Reyes, K., Rivera, M. E., Nieves, K., . . . Ortiz, A. P. (2014). Oral HPV infection in a clinic-based sample of Hispanic men. *BMC Oral Health, 14*, 7-7.

Corbeau, P., & Reynes, J. (2011). Immune reconstitution under antiretroviral therapy: the new challenge in HIV-1 infection. *Blood, 117*(21), 5582-5590.

Cressey, T. R., & Lallemant, M. (2007). Pharmacogenetics of antiretroviral drugs for the treatment of HIV-infected patients: An update. *Infection, Genetics and Evolution, 7*(2), 333-342.

Cubie, H. A. (2013). Diseases associated with human papillomavirus infection. *Virology, 445*(1-2), 21-34.

D'Souza, G., Wiley, D. J., Li, X., Chmiel, J. S., Margolick, J. B., Cranston, R. D., & Jacobson, L. P. (2008). Incidence and epidemiology of anal cancer in the Multicenter AIDS Cohort Study (MACS). *Journal of acquired immune deficiency syndromes (1999), 48*(4), 491-499.

Darwich, L., Cañadas, M. P., Videla, S., Coll, J., Molina-López, R. A., Cobarsi, P., . . . Clotet, B. (2014). Oral human papillomavirus type-specific infection in HIV-infected men: a prospective cohort study among men who have sex with men and heterosexual men. *Clinical Microbiology and Infection, 20*(9), O585-O589.

Daud, I. I., Scott, M. E., Ma, Y., Shiboski, S., Farhat, S., & Moscicki, A.-B. (2011). Association between Toll-like Receptors expression and Human Papillomavirus Type 16 Persistence. *International journal of cancer. Journal international du cancer, 128*(4), 879-886.

Day, C. L., Kaufmann, D. E., Kiepiela, P., Brown, J. A., Moodley, E. S., Reddy, S., . . . Walker, B. D. (2006). PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature, 443*(7109), 350-354.

Day, P. M., Lowy, D. R., & Schiller, J. T. (2003). Papillomaviruses infect cells via a clathrin-dependent pathway. *Virology, 307*(1), 1-11.

De, A. K., Miller-Graziano, C. L., Calvano, S. E., Laudanski, K., Lowry, S. F., Moldawer, L. L., . . . Tompkins, R. G. (2005). Selective Activation of Peripheral Blood T Cell Subsets by Endotoxin Infusion in Healthy Human Subjects Corresponds to Differential Chemokine Activation. *The Journal of Immunology, 175*(9), 6155-6162.

de Jong, A., van der Burg, S. H., Kwappenberg, K. M., van der Hulst, J. M., Franken, K. L., Geluk, A., . . . Offringa, R. (2002). Frequent detection of human papillomavirus 16 E2-specific T-helper immunity in healthy subjects. *Cancer Research, 62*(2), 472-479.

de Jong, A., van Poelgeest, M. I., van der Hulst, J. M., Drijfhout, J. W., Fleuren, G. J., Melief, C. J., . . . van der Burg, S. H. (2004). Human papillomavirus type 16-positive cervical cancer is associated with impaired CD4+ T-cell immunity against early antigens E2 and E6. *Cancer Research, 64*(15), 5449-5455.

de Martel, C., Ferlay, J., Franceschi, S., Vignat, J., Bray, F., Forman, D., & Plummer, M. (2012). Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. *The Lancet Oncology, 13*(6), 607-615.

de Villiers, E.-M., Fauquet, C., Broker, T. R., Bernard, H.-U., & zur Hausen, H. (2004). Classification of papillomaviruses. *Virology, 324*(1), 17-27.

Decrion, A. Z., Dichamp, I., Varin, A., & Herbein, G. (2005). HIV and inflammation. *Curr HIV Res, 3*(3), 243-259.

Deeks, S. G. (2011). HIV Infection, Inflammation, Immunosenescence, and Aging. *Annual review of medicine, 62*, 141-155.

Delmas, M. C., Larsen, C., van Benthem, B., Hamers, F. F., Bergeron, C., Poveda, J. D., . . . Brunet, J. B. (2000). Cervical squamous intraepithelial lesions in HIV-infected women: prevalence, incidence and regression. European Study Group on Natural History of HIV Infection in Women. *Aids, 14*(12), 1775-1784.

Desai, S., & Landay, A. (2010). Early Immune Senescence in HIV Disease. *Curr HIV/AIDS Rep, 7*(1), 4-10.

Doisne, J.-M., Urrutia, A., Lacabaratz-Porret, C., Goujard, C., Meyer, L., Chaix, M.-L., . . . Venet, A. (2004). CD8+ T Cells Specific for EBV, Cytomegalovirus, and Influenza Virus Are Activated during Primary HIV Infection. *The Journal of Immunology, 173*(4), 2410-2418.

Donà, M. G., Latini, A., Benevolo, M., Moretto, D., Cristaudo, A., & Giuliani, M. (2014). Anal human papillomavirus infection prevalence in men who have sex with men is age-independent: a role for recent sexual behavior? *Future Microbiol, 9*(7), 837-844.

Doorbar, J. (2005). The papillomavirus life cycle. *Journal of Clinical Virology, 32*, S7-S15.

Doorbar, J., Ely, S., Sterling, J., Mclean, C., & Crawford, L. (1991). Specific Interaction between Hpv-16 E1-E4 and Cytokeratins Results in Collapse of the Epithelial-Cell Intermediate Filament Network. *Nature, 352*(6338), 824-827.

Doorbar, J., Quint, W., Banks, L., Bravo, I. G., Stoler, M., Broker, T. R., & Stanley, M. A. (2012). The Biology and Life-Cycle of Human Papillomaviruses. *Vaccine, 30*, F55-F70.

Dronda, F., Moreno, S., Moreno, A., José, L. C., Pérez-Elías, M. J., & Antela, A. (2002). Long-Term Outcomes among Antiretroviral-Naive Human Immunodeficiency Virus–Infected Patients with Small Increases in CD4+ Cell Counts after Successful Virologic Suppression. *Clinical Infectious Diseases, 35*(8), 1005-1009.

du Prel, J. B., Hommel, G., Rohrig, B., & Blettner, M. (2009). Confidence interval or p-value?: part 4 of a series on evaluation of scientific publications. *Dtsch Arztebl Int, 106*(19), 335-339.

Dyer, W. B., Pett, S. L., Sullivan, J. S., Emery, S., Cooper, D. A., Kelleher, A. D., . . . Lewin, S. R. (2007). Substantial Improvements in Performance Indicators Achieved in a Peripheral Blood Mononuclear Cell Cryopreservation Quality Assurance Program Using Single Donor Samples. *Clinical and Vaccine Immunology, 14*(1), 52-59.

Dyson, N., Howley, P. M., Munger, K., & Harlow, E. (1989). The Human Papilloma Virus-16 E7-Oncoprotein Is Able to Bind to the Retinoblastoma Gene-Product. *Science, 243*(4893), 934-937.

Effros, R. B., Allsopp, R., Chiu, C. P., Hausner, M. A., Hirji, K., Wang, L., . . . Giorgi, J. V. (1996). Shortened telomeres in the expanded CD28-CD8+ cell subset in HIV disease implicate replicative senescence in HIV pathogenesis. *Aids, 10*(8), F17-22.

Elford, J. (2006). Changing patterns of sexual behaviour in the era of highly active antiretroviral therapy. *Current Opinion in Infectious Diseases, 19*(1), 26-32.

Ellerbrock, T. V., Chiasson, M., Bush, T. J., & et al. (2000). INcidence of cervical squamous intraepithelial lesions in hiv-infected women. *JAMA, 283*(8), 1031-1037.

Falster, K., Petoumenos, K., Chuah, J., Mijch, A., Mulhall, B., Kelly, M., & Cooper, D. A. (2009). Poor baseline immune function predicts an incomplete immune response to combination antiretroviral treatment despite sustained viral suppression. *Journal of acquired immune deficiency syndromes (1999), 50*(3), 307-313.

Ferlay, J., Shin, H. R., Bray, F., Forman, D., Mathers, C., & Parkin, D. M. (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *International Journal of Cancer, 127*(12), 2893-2917.

Fernandez, S., Price, P., McKinnon, E. J., Nolan, R. C., & French, M. A. (2006). Low CD4+ T-cell counts in HIV patients receiving effective antiretroviral therapy are associated with CD4+ T-cell activation and senescence but not with lower effector memory T-cell function. *Clinical Immunology, 120*(2), 163-170.

Ferreira, C., Barthlott, T., Garcia, S., Zamoyska, R., & Stockinger, B. (2000). Differential Survival of Naive CD4 and CD8 T Cells. *The Journal of Immunology, 165*(7), 3689-3694.

Finkel, T. H., Tudor-Williams, G., Banda, N. K., Cotton, M. F., Curiel, T., Monks, C., . . . Kupfer, A. (1995). Apoptosis occurs predominantly in bystander cells and not in productively infected cells of HIV- and SIV-infected lymph nodes. *Nat Med, 1*(2), 129-134.

Firnhaber, C., Zungu, K., Levin, S., Michelow, P., Montaner, L. J., McPhail, P., . . . Sanne, I. (2009). Diverse and High Prevalence of Human Papillomavirus Associated with a Significant High Rate of Cervical Dysplasia in Human Immunodeficiency Virus–Infected Women in Johannesburg, South Africa. *Acta cytologica, 53*(1), 10-17.

Florence, E., Lundgren, J., Dreezen, C., Fisher, M., Kirk, O., Blaxhult, A., . . . Euro, S. S. G. (2003). Factors associated with a reduced CD4 lymphocyte count response to HAART despite full viral suppression in the EuroSIDA study. *Hiv Medicine, 4*(3), 255-262.

Forman, D., de Martel, C., Lacey, C. J., Soerjomataram, I., Lortet-Tieulent, J., Bruni, L., . . . Franceschi, S. (2012). Global Burden of Human Papillomavirus and Related Diseases. *Vaccine, 30*, F12-F23.

Franceschi, C., Monti, D., Barbier, D., Salvioli, S., Grassilli, E., Capri, M., . . . Cossarizza, A. (1996). Successful immunosenescence and the remodelling of immune responses with ageing. *Nephrology Dialysis Transplantation, 11*(supp9), 18-25.

Frazer, I. H. (2004). Prevention of cervical cancer through papillomavirus vaccination. *Nat Rev Immunol, 4*(1), 46-55.

Frisch, M., Biggar, I. J., & Goedert, J. J. (2000). Human papillomavirus-associated cancers in patients with human immunodeficiency virus infection and acquired immunodeficiency syndrome. *J Natl Cancer Inst, 92*(18), 1500-1510.

Funk, J. O., Waga, S., Harry, J. B., Espling, E., Stillman, B., & Galloway, D. A. (1997). Inhibition of CDK activity and PCNA-dependent DNA replication by p21 is blocked by interaction with the HPV-16 E7 oncoprotein. *Genes & Development, 11*(16), 2090-2100.

Gadducci, A., Barsotti, C., Cosio, S., Domenici, L., & Riccardo Genazzani, A. (2011). Smoking habit, immune suppression, oral contraceptive use, and hormone replacement therapy use and cervical carcinogenesis: a review of the literature. *Gynecological Endocrinology, 27*(8), 597-604.

Gamberg, J., Pardoe, I., Bowmer, M. I., Howley, C., & Grant, M. (2004). Lack of CD28 expression on HIV-specific cytotoxic T lymphocytes is associated with disease progression. *Immunol Cell Biol, 82*(1), 38-46.

Gandhi, R. T., Spritzler, J., Chan, E., Asmuth, D. M., Rodriguez, B., Merigan, T. C., . . . Team, A. (2006). Effect of Baseline- and Treatment-Related Factors on Immunologic Recovery After Initiation of Antiretroviral Therapy in HIV-1-Positive Subjects: Results From ACTG 384. *JAIDS Journal of Acquired Immune Deficiency Syndromes, 42*(4), 426-434.

Garcia-Iglesias, T., Del Toro-Arreola, A., Albarran-Somoza, B., Del Toro-Arreola, S., Sanchez-Hernandez, P. E., Ramirez-Duenas, M. G., . . . Daneri-Navarro, A. (2009). Low NKp30, NKp46 and NKG2D expression and reduced cytotoxic activity on NK cells in cervical cancer and precursor lesions. *BMC Cancer, 9*, 186.

Geldmacher, C., Ngwenyama, N., Schuetz, A., Petrovas, C., Reither, K., Heeregrave, E. J., . . . Koup, R. A. (2010). Preferential infection and depletion of Mycobacterium tuberculosis–specific CD4 T cells after HIV-1 infection. *The Journal of Experimental Medicine, 207*(13), 2869-2881.

Gillison, M. L., Broutian, T., Pickard, R. L., & et al. (2012). PRevalence of oral hpv infection in the united states, 2009-2010. *JAMA, 307*(7), 693-703.

Gillison, M. L., Chaturvedi, A. K., & Lowy, D. R. (2008). HPV Prophylactic Vaccines and the Potential Prevention of Noncervical Cancers in Both Men and Women. *Cancer, 113*(10), 3036-3046.

Giroglou, T., Florin, L., Schäfer, F., Streeck, R. E., & Sapp, M. (2001). Human Papillomavirus Infection Requires Cell Surface Heparan Sulfate. *Journal of Virology, 75*(3), 1565-1570.

Giuliano, A. R., Lazcano-Ponce, E., Villa, L. L., Flores, R., Salmeron, J., Lee, J. H., . . . Quiterio, M. (2008). The human papillomavirus infection in men study: Human papillomavirus prevalence and type distribution among men residing in Brazil, Mexico, and the United States. *Cancer Epidemiology Biomarkers & Prevention, 17*(8), 2036-2043.

Goicoechea, M., Smith, D. M., Liu, L., May, S., Tenorio, A. R., Ignacio, C. C., . . . Haubrich, R. (2006). Determinants of CD4+ T Cell Recovery during Suppressive Antiretroviral Therapy: Association of Immune Activation, T Cell Maturation Markers, and Cellular HIV-1 DNA. *Journal of Infectious Diseases, 194*(1), 29-37.

Goldstone, S., Palefsky, J. M., Giuliano, A. R., Moreira, E. D., Aranda, C., Jessen, H., . . . Garner, E. I. O. (2011). Prevalence of and Risk Factors for Human Papillomavirus (HPV) Infection Among HIV-Seronegative Men Who Have Sex With Men. *Journal of Infectious Diseases, 203*(1), 66-74.

Gonzalez, C., Torres, M., Benito, A., del Romero, J., Rodriguez, C., Fontillon, M., . . . Grp, C.-H. S. (2013). Anal squamous intraepithelial lesions are frequent among young HIV-infected men who have sex with men followed up at the Spanish AIDS Research Network Cohort (CoRIS-HPV). *International Journal of Cancer, 133*(5), 1164-1172.

Gosmann, C., Mattarollo, S. R., Bridge, J. A., Frazer, I. H., & Blumenthal, A. (2014). IL-17 Suppresses Immune Effector Functions in Human Papillomavirus-Associated Epithelial Hyperplasia. *The Journal of Immunology, 193*(5), 2248-2257.

Hakim, F. T., & Gress, R. E. (2007). Immunosenescence: deficits in adaptive immunity in the elderly. *Tissue Antigens, 70*(3), 179-189.

Havre, P. A., Yuan, J. L., Hedrick, L., Cho, K. R., & Glazer, P. M. (1995). P53 Inactivation by Hpv16 E6 Results in Increased Mutagenesis in Human-Cells. *Cancer Research, 55*(19), 4420-4424.

Hazenberg, M. D., Hamann, D., Schuitemaker, H., & Miedema, F. (2000). T cell depletion in HIV-1 infection: how CD4+ T cells go out of stock. *Nat Immunol, 1*(4), 285-289.

Hearps, A. C., Angelovich, T. A., Jaworowski, A., Mills, J., Landay, A. L., & Crowe, S. M. (2011). HIV infection and aging of the innate immune system. *Sexual Health, 8*(4), 453-464.

Hidalgo-Tenorio, C., Rivero-Rodriguez, M., Gil-Anguita, C., Lopez De Hierro, M., Palma, P., Ramírez-Taboada, J., . . . Pasquau-Liaño, J. (2014). Antiretroviral Therapy as a Factor Protective against Anal Dysplasia in HIV-Infected Males Who Have Sex with Males. *Plos One, 9*(3), e92376.

Hirnschall, G., Harries, A. D., Easterbrook, P. J., Doherty, M. C., & Ball, A. (2013). *The next generation of the World Health Organization's global antiretroviral guidance*.

Ho, C. M., Chien, T. Y., Huang, S. H., Lee, B. H., & Chang, S. F. (2006). Integrated human papillomavirus types 52 and 58 are infrequently found in cervical cancer, and high viral loads predict risk of cervical cancer. *Gynecologic Oncology, 102*(1), 54-60.

Ho, D. D., Neumann, A. U., Perelson, A. S., Chen, W., Leonard, J. M., & Markowitz, M. (1995). Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature, 373*(6510), 123-126.

Holm, G. H., & Gabuzda, D. (2005). Distinct Mechanisms of CD4(+) and CD8(+) T-Cell Activation and Bystander Apoptosis Induced by Human Immunodeficiency Virus Type 1 Virions. *Journal of Virology, 79*(10), 6299-6311.

Holmgren, S. C., Patterson, N. A., Ozbun, M. A., & Lambert, P. F. (2005). The minor capsid protein L2 contributes to two steps in the human papillomavirus type 31 life cycle. *Journal of Virology, 79*(7), 3938-3948.

Homann, D., Teyton, L., & Oldstone, M. B. A. (2001). Differential regulation of antiviral T-cell immunity results in stable CD8+ but declining CD4+ T-cell memory. *Nat Med, 7*(8), 913-919.

Horta, A., Nobrega, C., Amorim-Machado, P., Coutinho-Teixeira, V., Barreira-Silva, P., Boavida, S., . . . Correia-Neves, M. (2013). Poor Immune Reconstitution in HIV-Infected Patients Associates with High Percentage of Regulatory CD4(+) T Cells. *Plos One, 8*(2), e57336.

Hu, Y., Qian, H.-Z., Sun, J., Gao, L., Yin, L., Li, X., . . . Vermund, S. H. (2013). Anal human papillomavirus infection among HIV-infected and uninfected men who have sex with men in Beijing, China. *Journal of acquired immune deficiency syndromes (1999), 64*(1), 103-114.

Hughes, F. J., & Romanos, M. A. (1993). E1 Protein of Human Papillomavirus Is a DNA Helicase/Atpase. *Nucleic Acids Research, 21*(25), 5817-5823.

Hunt, P. W., Martin, J. N., Sinclair, E., Bredt, B., Hagos, E., Lampiris, H., & Deeks, S. G. (2003). T Cell Activation Is Associated with Lower CD4+ T Cell Gains in Human Immunodeficiency Virus-Infected Patients with Sustained Viral Suppression during Antiretroviral Therapy. *Journal of Infectious Diseases, 187*(10), 1534-1543.

Ikezu, T. (2009). The Aging of Human-Immunodeficiency-Virus-Associated Neurocognitive Disorders. *Journal of Neuroimmune Pharmacology, 4*(2), 161-162.

Ishii, Y., Ozaki, S., Tanaka, K., & Kanda, T. (2005). Human papillomavirus 16 minor capsid protein L2 helps capsomeres assemble independently of intercapsomeric disulfide bonding. *Virus Genes, 31*(3), 321-328.

Jablonska, E., Piotrowski, L., & Grabowska, Z. (1997). Serum Levels of IL-1b, IL-6, TNF-a, sTNF-RI and CRP in Patients with Oral Cavity Cancer. *Pathol Oncol Res, 3*(2), 126-129.

Jackson, S., Harwood, C., Thomas, M., Banks, L., & Storey, A. (2000). Role of Bak in UV-induced apoptosis in skin cancer and abrogation by HPV E6 proteins. *Genes & Development, 14*(23), 3065-3073.

Jay, N., & Moscicki, A. B. (2000). Human papillomavirus infections in women with HIV disease: prevalence, risk, and management. *AIDS Read, 10*(11), 659-668.

Jemal, A., Simard, E. P., Dorell, C., Noone, A. M., Markowitz, L. E., Kohler, B., . . . Edwards, B. K. (2013). Annual Report to the Nation on the Status of Cancer, 1975-2009, featuring the burden and trends in human papillomavirus(HPV)-associated cancers and HPV vaccination coverage levels. *J Natl Cancer Inst, 105*(3), 175-201.

Jiang, W., Lederman, M. M., Hunt, P., Sieg, S. F., Haley, K., Rodriguez, B., . . . Brenchley, J. M. (2009). Plasma Levels of Bacterial DNA Correlate with Immune Activation and the Magnitude of Immune Restoration in Persons with Antiretroviral-Treated HIV Infection. *The Journal of Infectious Diseases, 199*(8), 1177-1185.

Jing, L., Zhong, X., Huang, W., Liu, Y., Wang, M., Miao, Z., . . . Wei, X. (2014). HPV genotypes and associated cervical cytological abnormalities in women from the Pearl River Delta region of Guangdong province, China: a cross-sectional study. *Bmc Infectious Diseases, 14*(1), 1-9.

Johnson, L. G., Madeleine, M. M., Newcomer, L. M., Schwartz, S. M., & Daling, J. R. (2004). Anal cancer incidence and survival: The Surveillance, Epidemiology, and End Results experience, 1973–2000. *Cancer, 101*(2), 281-288.

Jones, D. L., Alani, R. M., & Münger, K. (1997). The human papillomavirus E7 oncoprotein can uncouple cellular differentiation and proliferation in human keratinocytes by abrogating p21Cip1-mediated inhibition of cdk2. *Genes & Development, 11*(16), 2101-2111.

Kabsch, K., & Alonso, A. (2002). The human papillomavirus type 16 (HPV-16) E5 protein sensitizes human keratinocytes to apoptosis induced by osmotic stress. *Oncogene, 21*(6), 947-953.

Kadaja, M., Silla, T., Ustav, E., & Ustav, M. (2009). Papillomavirus DNA replication — From initiation to genomic instability. *Virology, 384*(2), 360-368.

Kadish, A. S., Ho, G. Y. F., Burk, R. D., Romney, S. L., Angeletti, R. H., Wang, Y., & Ledwidge, R. (1997). Lymphoproliferative Responses to Human Papillomavirus (HPV) Type 16 Proteins E6 and E7: Outcome of HPV Infection and Associated Neoplasia. *J Natl Cancer Inst, 89*(17), 1285-1293.

Katsikis, P. D., Wunderlich, E. S., Smith, C. A., Herzenberg, L. A., & Herzenberg, L. A. (1995). Fas antigen stimulation induces marked apoptosis of T lymphocytes in human immunodeficiency virus-infected individuals. *The Journal of Experimental Medicine, 181*(6), 2029-2036.

Kaufmann, G. R., Furrer, H., Ledergerber, B., Perrin, L., Opravil, M., Vernazza, P., . . . Swiss, H. I. V. C. S. (2005). Characteristics, determinants, and clinical relevance of CD4 T cell recovery to <500 cells/microL in HIV type 1-infected individuals receiving potent antiretroviral therapy. *Clinical Infectious Diseases, 41*(3), 361-372.

Kelley, C. F., Kitchen, C. M. R., Hunt, P. W., Rodriguez, B., Hecht, F. M., Kitahata, M., . . . Deeks, S. G. (2009a). Incomplete Peripheral CD4(+) Cell Count Restoration in HIV-Infected Patients Receiving Long-Term Antiretroviral Treatment. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 48*(6), 787-794.

Kelley, C. F., Kitchen, C. M. R., Hunt, P. W., Rodriguez, B., Hecht, F. M., Kitahata, M., . . . Deeks, S. G. (2009b). Incomplete Peripheral CD4+ Cell Count Restoration in HIV-Infected Patients Receiving Long-Term Antiretroviral Treatment. *Clinical Infectious Diseases, 48*(6), 787-794.

Khanna, N., Opravil, M., Furrer, H., Cavassini, M., Vernazza, P., Bernasconi, E., . . . Study, S. H. C. (2008). CD4+ T Cell Count Recovery in HIV Type 1–Infected Patients Is Independent of Class of Antiretroviral Therapy. *Clinical Infectious Diseases, 47*(8), 1093-1101.

Kim, K., & Lambert, P. F. (2002). E1 Protein of Bovine Papillomavirus 1 Is Not Required for the Maintenance of Viral Plasmid DNA Replication. *Virology, 293*(1), 10-14.

Kim, K. H., Greenfield, W. W., Cannon, M. J., Coleman, H. N., Spencer, H. J., & Nakagawa, M. (2012). CD4+ T-Cell Response Against Human Papillomavirus Type 16 E6 Protein Is Associated with A Favorable Clinical Trend. *Cancer Immunology, Immunotherapy, 61*(1), 63-70.

Kines, R. C., Thompson, C. D., Lowy, D. R., Schiller, J. T., & Day, P. M. (2009). The initial steps leading to papillomavirus infection occur on the basement membrane prior to cell surface binding. *Proceedings of the National Academy of Sciences, 106*(48), 20458-20463.

Kitay-Cohen, Y., Goldberg-Bittman, L., Hadary, R., Fejgin, M. D., & Amiel, A. (2008). Telomere length in Hepatitis C. *Cancer Genetics and Cytogenetics, 187*(1), 34-38.

Klingelhutz, A. J., & Roman, A. (2012). Cellular transformation by human papillomaviruses: Lessons learned by comparing high- and low-risk viruses. *Virology, 424*(2), 77-98.

Kojima, S., Kawana, K., Tomio, K., Yamashita, A., Taguchi, A., Miura, S., . . . Kozuma, S. (2013). The prevalence of cervical regulatory T cells in HPV-related cervical intraepithelial neoplasia (CIN) correlates inversely with spontaneous regression of CIN. *Am J Reprod Immunol, 69*(2), 134-141.

Koskimaa, H.-M., Paaso, A. E., Welters, M. J. P., Grénman, S. E., Syrjänen, K. J., van der Burg, S. H., & Syrjänen, S. M. (2014). Human papillomavirus 16 E2-, E6- and E7-specific T-cell responses in children and their mothers who developed incident cervical intraepithelial neoplasia during a 14-year follow-up of the Finnish Family HPV cohort. *Journal of Translational Medicine, 12*, 44-44.

Koup, R. A., Safrit, J. T., Cao, Y., Andrews, C. A., McLeod, G., Borkowsky, W., . . . Ho, D. D. (1994). Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. *J Virol, 68*(7), 4650-4655.

Kreimer, A. R., Clifford, G. M., Boyle, P., & Franceschi, S. (2005). Human Papillomavirus Types in Head and Neck Squamous Cell Carcinomas Worldwide: A Systematic Review. *Cancer Epidemiology Biomarkers & Prevention, 14*(2), 467-475.

Kruse, A. L., Luebbers, H. T., & Grätz, K. W. (2010). C-reactive protein levels: a prognostic marker for patients with head and neck cancer? *Head Neck Oncol, 2*, 21-21.

Lajous, M., Mueller, N., Cruz-Valdez, A., Aguilar, L. V., Franceschi, S., Hernandez-Avila, M., & Lazcano-Ponce, E. (2005). Determinants of prevalence, acquisition, and persistence of human papillomavirus in healthy Mexican military men. *Cancer Epidemiol Biomarkers Prev, 14*(7), 1710-1716.

Lane, H. C. (2010). Pathogenesis of HIV infection: total CD4+ T-cell pool, immune activation, and inflammation. *Top HIV Med, 18*(1), 2-6.

Latini, A., Gabriella Dona, M., Ronchetti, L., Giglio, A., Moretto, D., Colafigli, M., . . . Giuliani, M. (2014). Prevalence of anal human papillomavirus infection and cytologic abnormalities among HIV-infected and HIV-uninfected men who have sex with men. *J Int AIDS Soc, 17*(4Suppl 3), 19662.

Lau, B., Sharrett, A., Kingsley, L. A., & et al. (2006). C-reactive protein is a marker for human immunodeficiency virus disease progression. *Archives of Internal Medicine, 166*(1), 64-70.

Lawn, S. D., Myer, L., Bekker, L.-G., & Wood, R. (2006). CD4 cell count recovery among HIV-infected patients with very advanced immunodeficiency commencing antiretroviral treatment in sub-Saharan Africa. *Bmc Infectious Diseases, 6*, 59-59.

Lederman, M. M., Calabrese, L., Funderburg, N. T., Clagett, B., Medvik, K., Bonilla, H., . . . Rodriguez, B. (2011). Immunologic Failure Despite Suppressive Antiretroviral Therapy Is Related to Activation and Turnover of Memory CD4 Cells. *Journal of Infectious Diseases, 204*(8), 1217-1226.

Li, A. H., Phanuphak, N., Sahasrabuddhe, V. V., Chaithongwongwatthana, S., Vermund, S. H., Jenkins, C. A., . . . Ananworanich, J. (2009). Anal squamous intraepithelial lesions among HIV positive and HIV negative men who have sex with men in Thailand. *Sex Transm Infect, 85*(7), 10.1136/sti.2009.036707.

Li, T., Wu, N., Dai, Y., Qiu, Z., Han, Y., Xie, J., . . . Li, Y. (2011). Reduced Thymic Output Is a Major Mechanism of Immune Reconstitution Failure in HIV-Infected Patients After Long-term Antiretroviral Therapy. *Clinical Infectious Diseases, 53*(9), 944-951.

Lippman, S. A., Sucupira, M. C. A., Jones, H. E., Luppi, C. G., Palefsky, J., van de Wijgert, J. H. H. M., . . . Diaz, R. S. (2010). Prevalence, distribution and correlates of endocervical human papillomavirus types in Brazilian women. *Int J STD AIDS, 21*(2), 105-109.

Liu, E., McCree, R., Mtisi, E., Fawzi, W. W., Aris, E., Lema, I. A., . . . Spiegelman, D. (2015). Prevalence and risk factors of cervical squamous intraepithelial lesions among HIV-infected women in Dar es Salaam, Tanzania. *Int J STD AIDS*.

Longacre, T. A., Kong, C. S., & Welton, M. L. (2008). Diagnostic problems in anal pathology. *Adv Anat Pathol, 15*(5), 263-278.

Luxton, J. C., Nath, R., Derias, N., Herbert, A., & Shepherd, P. S. (2003). Human papillomavirus type 16-specific T cell responses and their association with recurrence of cervical disease following treatment. *Journal of General Virology, 84*, 1063-1070.

Maartens, G., Celum, C., & Lewin, S. R. (2014). HIV infection: epidemiology, pathogenesis, treatment, and prevention. *Lancet, 384*(9939), 258-271.

MahdadNoursadeghi, & Miller, R. F. (2005). Clinical value of C-reactive protein measurements in HIV-positive patients. *Int J STD AIDS, 16*(6), 438-441.

Majewski, S., Malejczyk, J., Jablonska, S., Misiewicz, J., Rudnicka, L., Obalek, S., & Orth, G. (1990). Natural Cell-Mediated Cytotoxicity against Various Target-Cells in Patients with Epidermodysplasia Verruciformis. *Journal of the American Academy of Dermatology, 22*(3), 423-427.

Malejczyk, J., Majewski, S., Jablonska, S., Rogozinski, T. T., & Orth, G. (1989). Abrogated Nk-Cell Lysis of Human Papillomavirus (Hpv)-16-Bearing Keratinocytes in Patients with Pre-Cancerous and Cancerous Hpv-Induced Anogenital Lesions. *International Journal of Cancer, 43*(2), 209-214.

Marchetti, G., Gazzola, L., Trabattoni, D., Bai, F., Ancona, G., Ferraris, L., . . . d'Arminio Monforte, A. (2010). Skewed T-cell maturation and function in HIV-infected patients failing CD4+ recovery upon long-term virologically suppressive HAART. *Aids, 24*(10), 1455-1460.

Martin, F., & Bower, M. (2001). Anal intraepithelial neoplasia in HIV positive people. *Sex Transm Infect, 77*(5), 327-331.

Massanella, M., Negredo, E., Pérez-Álvarez, N., Puig, J., Ruiz-Hernández, R., Bofill, M., . . . Blanco, J. (2010). CD4 T-cell hyperactivation and susceptibility to cell death determine poor CD4 T-cell recovery during suppressive HAART. *Aids, 24*(7), 959-968.

McCredie, M. R. E., Sharples, K. J., Paul, C., Baranyai, J., Medley, G., Jones, R. W., & Skegg, D. C. G. (2008). Natural history of cervical neoplasia and risk of invasive cancer in women with cervical intraepithelial neoplasia 3: a retrospective cohort study. *Lancet Oncology, 9*(5), 425-434.

McMichael, A., & Dorrell, L. (2009). The immune response to HIV. *Medicine, 37*(7), 321-325.

McPhillips, M. G., Oliveira, J. G., Spindler, J. E., Mitra, R., & McBride, A. A. (2006). Brd4 is required for E2-mediated transcriptional activation but not genome partitioning of all papillomaviruses. *Journal of Virology, 80*(19), 9530-9543.

Mellin, H., Friesland, S., Lewensohn, R., Dalianis, T., & Munck-Wikland, E. (2000). Human papillomavirus (HPV) DNA in tonsillar cancer: Clinical correlates, risk of relapse, and survival. *International Journal of Cancer, 89*(3), 300-304.

Melo, V. H., Guimaraes, M. D. C., Rocha, G. M., Araujo, A. C. L., Carmo, R. A., Grinsztejn, B., . . . Palefsky, J. M. (2014). Prevalence and Risk Factors Associated With Anal Intraepithelial Neoplasia Among HIV-Positive Men in Brazil. *Journal of Lower Genital Tract Disease, 18*(2), 128-135.

Méndez-Lagares, G., Pozo-Balado, M. M., Genebat, M., García-Pergañeda, A., Leal, M., & Pacheco, Y. M. (2012). Severe Immune Dysregulation Affects CD4+CD25hiFoxP3+ Regulatory T Cells in HIV-Infected Patients With Low-level CD4 T-Cell Repopulation Despite Suppressive Highly Active Antiretroviral Therapy. *Journal of Infectious Diseases, 205*(10), 1501-1509.

Menezes, L. J., Poongulali, S., Tommasino, M., Lin, H.-Y., Kumarasamy, N., Fisher, K. J., . . . Giuliano, A. R. (2015). Prevalence and concordance of human papillomavirus infection at multiple anatomic sites among HIV-infected women from Chennai, India. *Int J STD AIDS.*

Meyaard, L., Otto, S. A., Keet, I. P., Roos, M. T., & Miedema, F. (1994). Programmed death of T cells in human immunodeficiency virus infection. No correlation with progression to disease. *Journal of Clinical Investigation, 93*(3), 982-988.

Michaels, S. H., Clark, R., & Kissinger, P. (1998). Declining Morbidity and Mortality among Patients with Advanced Human Immunodeficiency Virus Infection. *New England Journal of Medicine, 339*(6), 405-406.

Ministry of Health Malaysia, HIV/STI section. (2015). *Malaysia 2015 country responses to HIV/AIDS.* Kuala Lumpur, Malaysia.

Mojumdar, K., Vajpayee, M., Chauhan, N. K., Singh, A., Singh, R., & Kurapati, S. (2011). Loss of CD127 & increased immunosenescence of T cell subsets in HIV infected individuals. *The Indian Journal of Medical Research, 134*(6), 972-981.

Molijn, A., Kleter, B., Quint, W., & van Doorn, L. J. (2005). Molecular diagnosis of human papillomavirus (HPV) infections. *Journal of Clinical Virology, 32 Suppl 1*, S43-51.

Molina-Pinelo, S., Vallejo, A., Díaz, L., Soriano-Sarabia, N., Ferrando-Martínez, S., Resino, S., . . . Leal, M. (2009). Premature immunosenescence in HIV-infected patients on highly active antiretroviral therapy with low-level CD4 T cell repopulation. *Journal of Antimicrobial Chemotherapy.*

Molling, J. W., de Gruijl, T. D., Glim, J., Moreno, M., Rozendaal, L., Meijer, C. J., . . . Bontkes, H. J. (2007). CD4(+)CD25hi regulatory T-cell frequency correlates with persistence of human papillomavirus type 16 and T helper cell responses in patients with cervical intraepithelial neoplasia. *International Journal of Cancer, 121*(8), 1749-1755.

Moscicki, A., Hills, N., Shiboski, S., & et al. (2001). RIsks for incident human papillomavirus infection and low-grade squamous intraepithelial lesion development in young females. *JAMA, 285*(23), 2995-3002.

Mota, F., Rayment, N., Chong, S., Singer, A., & Chain, B. (1999). The antigen-presenting environment in normal and human papillomavirus (HPV)-related premalignant cervical epithelium. *Clinical and Experimental Immunology, 116*(1), 33-40.

Munoz, N., Bosch, F. X., de Sanjose, S., Herrero, R., Castellsague, X., Shah, K. V., . . . International Agency for Research on Cancer Multicenter Cervical Cancer Study, G. (2003). Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med, 348*(6), 518-527.

Muro-Cacho, C. A., Pantaleo, G., & Fauci, A. S. (1995). Analysis of apoptosis in lymph nodes of HIV-infected persons. Intensity of apoptosis correlates with the general state of activation of the lymphoid tissue and not with stage of disease or viral burden. *The Journal of Immunology, 154*(10), 5555-5566.

Nakagawa, M., Stites, D. P., Farhat, S., Judd, A., Moscicki, A. B., Canchola, A. J., . . . Palefsky, J. M. (1996). T-cell proliferative response to human papillomavirus type 16 peptides: relationship to cervical intraepithelial neoplasia. *Clinical and Diagnostic Laboratory Immunology, 3*(2), 205-210.

Nakagawa, M., Stites, D. P., Farhat, S., Sisler, J. R., Moss, B., Kong, F., . . . Palefsky, J. M. (1997). Cytotoxic T lymphocyte responses to E6 and E7 proteins of human papillomavirus type 16: relationship to cervical intraepithelial neoplasia. *J Infect Dis, 175*(4), 927-931.

Nakagawa, M., Stites, D. P., Patel, S., Farhat, S., Scott, M., Hills, N. K., . . . Moscicki, A. B. (2000). Persistence of human papillomavirus type 16 infection is associated with lack of cytotoxic T lymphocyte response to the E6 antigens. *J Infect Dis, 182*(2), 595-598.

Nayar, R., & Wilbur, D. (2015). *The Bethesda System for Reporting Cervical Cytology: Definitions, Criteria, and Explanatory Notes*: Springer International Publishing.

Nicolau, S. M., Camargo, C. G. C., Stávale, J. N., Castelo, A., Dôres, G. B., Lörincz, A., & de Lima, G. R. (2005). Human papillomavirus DNA detection in male sexual partners of women with genital human papillomavirus infection. *Urology, 65*(2), 251-255.

Nyitray, A., Smith, D., Villa, L., Lazcano-Ponce, E., Abrahamsen, M., Papenfuss, M., & Giuliano, A. R. (2010). Prevalence of and risk factors for anal human papillomavirus in men having sex with women: A cross–national study. *The Journal of Infectious Diseases, 201*(10), 1498-1508.

Okulicz, J. F., Le, T. D., Agan, B. K., & et al. (2015). INfluence of the timing of antiretroviral therapy on the potential for normalization of immune status in human immunodeficiency virus 1–infected individuals. *Jama Internal Medicine, 175*(1), 88-99.

Oon, S. F., & Winter, D. C. (2010). Perianal condylomas, anal squamous intraepithelial neoplasms and screening: a review of the literature. *J Med Screen, 17*(1), 44-49.

Palefsky, J. M., Holly, E. A., Ralston, M. L., Arthur, S. P., Jay, N., Berry, J. M., . . . Darragh, T. M. (1998). Anal squamous intraepithelial lesions in HIV-positive and HIV-negative homosexual and bisexual men: prevalence and risk factors. *J Acquir Immune Defic Syndr Hum Retrovirol, 17*(4), 320-326.

Palefsky, J. M., Holly, E. A., Ralston, M. L., & Jay, N. (1998). Prevalence and Risk Factors for Human Papillomavirus Infection of the Anal Canal in Human Immunodeficiency Virus (HIV)-Positive and HIV-Negative Homosexual Men. *Journal of Infectious Diseases, 177*(2), 361-367.

Palella, F. J., Jr., Baker, R. K., Moorman, A. C., Chmiel, J. S., Wood, K. C., Brooks, J. T., . . . Investigators, H. I. V. O. S. (2006). Mortality in the highly active antiretroviral therapy era: changing causes of death and disease in the HIV outpatient study. *J Acquir Immune Defic Syndr, 43*(1), 27-34.

Palma, P., Romiti, M. L., Cancrini, C., Pensieroso, S., Montesano, C., Santucci, M. B., . . . Castelli-Gattinara, G. (2007). Successful simplification of protease inhibitor-based HAART with triple nucleoside regimens in children vertically infected with HIV. *Aids, 21*(18), 2465-2472.

Palmer, B. E., Blyveis, N., Fontenot, A. P., & Wilson, C. C. (2005). Functional and Phenotypic Characterization of CD57+CD4+ T Cells and Their Association with HIV-1-Induced T Cell Dysfunction. *The Journal of Immunology, 175*(12), 8415-8423.

Palmer, C. D., Tomassilli, J., Sirignano, M., Tejeda, M. R., Arnold, K. B., Che, D., . . . Altfeld, M. (2014). Enhanced Immune Activation Linked to Endotoxemia in HIV-1 Seronegative Men who have Sex with Men. *AIDS (London, England), 28*(14), 2162-2166.

Palmisano, L., & Vella, S. (2011). A brief history of antiretroviral therapy of HIV infection: success and challenges. *Ann Ist Super Sanita, 47*(1), 44-48.

Papagno, L., Spina, C. A., Marchant, A., Salio, M., Rufer, N., Little, S., . . . Appay, V. (2004). Immune activation and CD8+ T-cell differentiation towards senescence in HIV-1 infection. *PLoS Biol, 2*(2), E20.

Parisi, S. G., Cruciani, M., Scaggiante, R., Boldrin, C., Andreis, S., Bello, F. D., . . . Palù, G. (2011). 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, 11*, 150-150.

Parkin, D. M., & Bray, F. (2006). Chapter 2: The burden of HPV-related cancers. *Vaccine, 24 Suppl 3*, S3/11-25.

Parkin, D. M., Bray, F., Ferlay, J., & Pisani, P. (2005). Global Cancer Statistics, 2002. *CA: A Cancer Journal for Clinicians, 55*(2), 74-108.

Partridge, J. M., Hughes, J. P., Feng, Q., Winer, R. L., Weaver, B. A., Xi, L.-F., . . . Koutsky, L. A. (2007). Genital Human Papillomavirus Infection in Men: Incidence and Risk Factors in a Cohort of University Students. *Journal of Infectious Diseases, 196*(8), 1128-1136.

Passmore, J.-A. S., Milner, M., Denny, L., Sampson, C., Marais, D. J., Allan, B., . . . Williamson, A.-L. (2006). Comparison of cervical and blood T-cell responses to human papillomavirus-16 in women with human papillomavirus-associated cervical intraepithelial neoplasia. *Immunology, 119*(4), 507-514.

Patel, P., Hanson, D. L., Sullivan, P. S., Novak, R. M., Moorman, A. C., Tong, T. C., . . . Brooks, J. T. (2008). Incidence of Types of Cancer among HIV-Infected Persons Compared with the General Population in the United States, 1992–2003. *Annals of Internal Medicine, 148*(10), 728-736.

Phanuphak, N., Teeratakulpisarn, N., Pankam, T., Kerr, S. J., Barisri, J., Deesua, A., . . . Palefsky, J. M. (2013). 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 (1999), 63*(4), 472-479.

Phillips, A. N., Staszewski, S., Weber, R., & et al. (2001). HIv viral load response to antiretroviral therapy according to the baseline cd4 cell count and viral load. *JAMA, 286*(20), 2560-2567.

Piirsoo, M., Ustav, E., Mandel, T., Stenlund, A., & Ustav, M. (1996). Cis and trans requirements for stable episomal maintenance of the BPV-1 replicator. *Embo Journal, 15*(1), 1-11.

Piketty, C., Cochand-Priollet, B., Lanoy, E., Si-Mohamed, A., Trabelsi, S., Tubiana, R., . . . Group, t. V. S. (2013). Lack of regression of anal squamous intraepithelial lesions despite immune restoration under cART. *Aids, 27*(3), 401-406.

Piketty, C., Darragh, T. M., Costa, M. D., Bruneval, P., Heard, I., Kazatchkine, M. D., & Palefsky, J. M. (2003). High Prevalence of Anal Human Papillomavirus Infection and Anal Cancer Precursors among HIV-Infected Persons in the Absence of Anal Intercourse. *Annals of Internal Medicine, 138*(6), 453-459.

Piketty, C., Selinger-Leneman, H., Grabar, S., Duvivier, C., Bonmarchand, M., Abramowitz, L., . . . Mary-Krause, M. (2008). Marked increase in the incidence of invasive anal cancer among HIV-infected patients despite treatment with combination antiretroviral therapy. *Aids, 22*(10), 1203-1211.

Pita-Lopez, M. L., Ortiz-Lazareno, P. C., Navarro-Meza, M., Santoyo-Telles, F., & Peralta-Zaragoza, O. (2014). CD28-, CD45RA(null/dim) and natural killer-like CD8+ T cells are increased in peripheral blood of women with low-grade cervical lesions. *Cancer Cell International, 14*, 97.

Poljak, M., Marin, I. J., Seme, K., & Vince, A. (2002). Hybrid Capture II HPV Test detects at least 15 human papillomavirus genotypes not included in its current high-risk probe cocktail. *Journal of Clinical Virology, 25, Supplement 3*(0), 89-97.

Pourgheysari, B., Khan, N., Best, D., Bruton, R., Nayak, L., & Moss, P. A. H. (2007). The Cytomegalovirus-Specific CD4(+) T-Cell Response Expands with Age and Markedly Alters the CD4(+) T-Cell Repertoire. *Journal of Virology, 81*(14), 7759-7765.

Poynten, I. M., Machalek, D., Templeton, D., Jin, F., Hillman, R., Zablotzska, I., . . . Grulich, A. (2015). Comparison of age-specific patterns of sexual behaviour and anal HPV prevalence in homosexual men with patterns in women. *Sex Transm Infect.*

Riou, C., Tanko, R. F., Soares, A. P., Masson, L., Werner, L., Garrett, N. J., . . . Burgers, W. A. (2015). Restoration of CD4+ Responses to Copathogens in HIV-Infected Individuals on Antiretroviral Therapy Is Dependent on T Cell Memory Phenotype. *The Journal of Immunology, 195*(5), 2273-2281.

Riou, C., Treurnicht, F., Abrahams, M.-R., Mlisana, K., Liu, M. K. P., Goonetilleke, N., . . . Burgers, W. A. (2012). Increased memory differentiation is associated with decreased polyfunctionality for HIV but not for CMV-specific CD8+ T cells. *Journal of immunology (Baltimore, Md. : 1950), 189*(8), 3838-3847.

Rosen, B. J., Walter, L., Gilman, R. H., Cabrerra, L., Gravitt, P. E., & Marks, M. A. (2015). Prevalence and correlates of oral human papillomavirus infection among healthy males and females in Lima, Peru. *Sex Transm Infect.*

Rositch, A. F., Gravitt, P. E., Tobian, A. A. R., Newell, K., Quinn, T. C., Serwadda, D., . . . Reynolds, S. J. (2013). Frequent Detection of HPV before and after Initiation of Antiretroviral Therapy among HIV/HSV-2 Co-Infected Women in Uganda. *Plos One, 8*(1).

Ruel, T., Ssewanyana, I., Achan, J., Gasasira, A., Kamya, M. R., Kekitiinwa, A., . . . Charlebois, E. D. (2009). Dynamics of T cell activation accompanying CD4 recovery in antiretroviral treated HIV-infected Ugandan children. *Clinical Immunology, 131*(3), 410-414.

Russell, S., Angell, T., Lechner, M., Liebertz, D., Correa, A., Sinha, U., . . . Epstein, A. (2013). Immune cell infiltration patterns and survival in head and neck squamous cell carcinoma. *Head Neck Oncol, 5*(3), 24.

Sackoff, J. E., Hanna, D. B., Pfeiffer, M. R., & Torian, L. V. (2006). Causes of Death among Persons with AIDS in the Era of Highly Active Antiretroviral Therapy: New York City. *Annals of Internal Medicine, 145*(6), 397-406.

Sainz, T., Serrano-Villar, S., Díaz, L., Tomé, M. I. G., Gurbindo, M. D., de José, M. I., . . . Muñoz-Fernández, M. Á. (2013). The CD4/CD8 ratio as a marker T-cell activation, senescence and activation/exhaustion in treated HIV-infected children and young adults. *Aids, 27*(9), 1513-1516.

Saison, J., Ferry, T., Demaret, J., Maucort Boulch, D., Venet, F., Perpoint, T., . . . Lyon, H. I. V. C. S. (2014). Association between discordant immunological response to highly active anti-retroviral therapy, regulatory T cell percentage, immune cell activation and very low-level viraemia in HIV-infected patients. *Clinical and Experimental Immunology, 176*(3), 401-409.

Saison, J., Maucort Boulch, D., Chidiac, C., Demaret, J., Malcus, C., Cotte, L., . . . Ferry, T. (2015). Increased Regulatory T-Cell Percentage Contributes to Poor CD4(+) Lymphocytes Recovery: A 2-Year Prospective Study After Introduction of Antiretroviral Therapy. *Open Forum Infectious Diseases, 2*(2), ofv063.

Sasagawa, T., Basha, W., Yamazaki, H., & Inoue, M. (2001). High-risk and multiple human papillomavirus infections associated with cervical abnormalities in Japanese women. *Cancer Epidemiology Biomarkers & Prevention, 10*(1), 45-52.

Sasagawa, T., Takagi, H., & Makinoda, S. (2012). Immune responses against human papillomavirus (HPV) infection and evasion of host defense in cervical cancer. *J Infect Chemother, 18*(6), 807-815.

Schacker, T. W., Nguyen, P. L., Beilman, G. J., Wolinsky, S., Larson, M., Reilly, C., & Haase, A. T. (2002). Collagen deposition in HIV-1 infected lymphatic tissues and T cell homeostasis. *The Journal of Clinical Investigation, 110*(8), 1133-1139.

Schiffman, M. H. (1992). Recent Progress in Defining the Epidemiology of Human Papillomavirus Infection and Cervical Neoplasia. *J Natl Cancer Inst, 84*(6), 394-398.

Schneider, M. F., Gange, S. J., Williams, C. M., Anastos, K., Greenblatt, R. M., Kingsley, L., . . . Muñoz, A. (2005). Patterns of the hazard of death after AIDS through the evolution of antiretroviral therapy: 1984–2004. *Aids, 19*(17), 2009-2018.

Schwarcz, L., Chen, M. J., Vittinghoff, E., Hsu, L., & Schwarcz, S. (2013). Declining incidence of AIDS-defining opportunistic illnesses: results from 16 years of population-based AIDS surveillance. *Aids, 27*(4), 597-605.

Schwarz, E., Freese, U. K., Gissmann, L., Mayer, W., Roggenbuck, B., Stremlau, A., & zur Hausen, H. (1985). Structure and transcription of human papillomavirus sequences in cervical carcinoma cells. *Nature, 314*(6006), 111-114.

Serrano-Villar, S., Sainz, T., Lee, S. A., Hunt, P. W., Sinclair, E., Shacklett, B. L., . . . Deeks, S. G. (2014). HIV-Infected Individuals with Low CD4/CD8 Ratio despite Effective Antiretroviral Therapy Exhibit Altered T Cell Subsets, Heightened CD8+ T Cell Activation, and Increased Risk of Non-AIDS Morbidity and Mortality. *Plos Pathogens, 10*(5), e1004078.

Shankar, P., Russo, M., Harnisch, B., Patterson, M., Skolnik, P., & Lieberman, J. (2000). Impaired function of circulating HIV-specific CD8+ T cells in chronic human immunodeficiency virus infection. *Blood, 96*(9), 3094-3101.

Shiels, M. S., Cole, S. R., Kirk, G. D., & Poole, C. (2009). A meta-analysis of the incidence of non-AIDS cancers in HIV-infected individuals. *Journal of acquired immune deficiency syndromes (1999), 52*(5), 611-622.

Sirera, G., Videla, S., López-Blázquez, R., Llatjos, M., Tarrats, A., Castellà, e., . . . Clotet, C. (2008). Highly active antiretroviral therapy and incidence of cervical squamous intraepithelial lesions among HIV-infected women with normal cytology and CD4 counts above 350 cells/mm3. *Journal of Antimicrobial Chemotherapy, 61*(1), 191-194.

Sirera, G., Videla, S., Pinol, M., Canadas, M. P., Llatjos, M., Ballesteros, A. L., . . . Clotet, B. (2006). High prevalence of human papillomavirus infection in the anus, penis and mouth in HIV-positive men. *Aids, 20*(8), 1201-1204.

Solomon, D., Davey, D., Kurman, R., & et al. (2002). The 2001 bethesda system: Terminology for reporting results of cervical cytology. *JAMA, 287*(16), 2114-2119.

Stanley, M. A. (2012). Epithelial cell responses to infection with human papillomavirus. *Clin Microbiol Rev, 25*(2), 215-222.

Steele, J. C., Mann, C. H., Rookes, S., Rollason, T., Murphy, D., Freeth, M. G., . . . Roberts, S. (2005). T-cell responses to human papillomavirus type 16 among women with different grades of cervical neoplasia. *British Journal of Cancer, 93*(2), 248-259.

Stern, P. L., & Kitchener, H. C. (2008). *Vaccines for the Prevention of Cervical Cancer*: Oxford University Press.

Straight, S. W., Hinkle, P. M., Jewers, R. J., & Mccance, D. J. (1993). The E5 Oncoprotein of Human Papillomavirus Type-16 Transforms Fibroblasts and Effects the down-Regulation of the Epidermal Growth-Factor Receptor in Keratinocytes. *Journal of Virology, 67*(8), 4521-4532.

Streeck, H., Brumme, Z. L., Anastario, M., Cohen, K. W., Jolin, J. S., Meier, A., . . . Altfeld, M. (2008). Antigen Load and Viral Sequence Diversification Determine the Functional Profile of HIV-1–Specific CD8(+) T Cells. *Plos Medicine, 5*(5), e100.

Stubenrauch, F., & Laimins, L. A. (1999). Human papillomavirus life cycle: active and latent phases. *Semin Cancer Biol, 9*(6), 379-386.

Svare, E. I., Kjaer, S. K., Worm, A. M., Osterlind, A., Meijer, C. J., & van den Brule, A. J. (2002). Risk factors for genital HPV DNA in men resemble those found in women: a study of male attendees at a Danish STD clinic. *Sex Transm Infect, 78*(3), 215-218.

Syrjänen, S., Lodi, G., von Bültzingslöwen, I., Aliko, A., Arduino, P., Campisi, G., . . . Jontell, M. (2011). Human papillomaviruses in oral carcinoma and oral potentially malignant disorders: a systematic review. *Oral Diseases, 17*, 58-72.

Takehara, K., Toda, T., Nishimura, T., Sakane, J., Kawakami, Y., Mizunoe, T., . . . Taniyama, K. (2011). Human Papillomavirus Types 52 and 58 Are Prevalent in Uterine Cervical Squamous Lesions from Japanese Women. *Pathology Research International, 2011*, 7.

Tassiopoulos, K., Landay, A., Collier, A. C., Connick, E., Deeks, S. G., Hunt, P., . . . Bosch, R. (2012). CD28-Negative CD4(+) and CD8(+) T Cells in Antiretroviral Therapy–Naive HIV-Infected Adults Enrolled in Adult Clinical Trials Group Studies. *The Journal of Infectious Diseases, 205*(11), 1730-1738.

Teichmann, J., Stephan, E., Discher, T., Lange, U., Federlin, K., Stracke, H., . . . Bretzel, R. G. (2009). Changes in calciotropic hormones and biochemical markers of bone metabolism in patients with human immunodeficiency virus infection. *Metabolism - Clinical and Experimental, 49*(9), 1134-1139.

Tommasino, M. (2013). The human papillomavirus family and its role in carcinogenesis. *Semin Cancer Biol.*

Torti, C., Prosperi, M., Motta, D., Digiambenedetto, S., Maggiolo, F., Paraninfo, G., . . . Frank, I. (2012). 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, 18*(5), 449-458.

Trautmann, L., Janbazian, L., Chomont, N., Said, E. A., Gimmig, S., Bessette, B., . . . Sekaly, R. P. (2006). Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction. *Nat Med, 12*(10), 1198-1202.

Tuboi, S. H., Brinkhof, M. W., Egger, M., Stone, R. A., Braitstein, P., Nash, D., . . . Schechter, M. (2007). Discordant responses to potent antiretroviral treatment in previously naive HIV-1-infected adults initiating treatment in resource-constrained countries: the antiretroviral therapy in low-income countries (ART-LINC) collaboration. *J Acquir Immune Defic Syndr, 45*(1), 52-59.

Vacharotayangul, P., Rungsiyanont, S., Lam-Ubol, A., Pankam, T., Rodbamrung, P., Naorungroj, S., & Phanuphak, N. (2015). Higher prevalence of oral human papillomavirus infection in HIV-positive than HIV-negative Thai men and women. *Cancer Epidemiol, 39*(6), 917-922.

van Aar, F., Mooij, S. H., van der Sande, M. A. B., Meijer, C. J. L. M., King, A. J., Verhagen, D. W. M., . . . Schim van der Loeff, M. F. (2014). Twelve-month incidence and clearance of oral HPV infection in HIV-negative and HIV-infected men who have sex with men: the H2M cohort study. *Bmc Infectious Diseases, 14*, 668.

van Esch, E. M. G., van Poelgeest, M. I. E., Trimbos, J. B. M. Z., Fleuren, G. J., Jordanova, E. S., & van der Burg, S. H. (2015). Intraepithelial macrophage infiltration is related to a high number of regulatory T cells and promotes a progressive course of HPV-induced vulvar neoplasia. *International Journal of Cancer, 136*(4), E85-E94.

van Rijn, V. M., Mooij, S. H., Mollers, M., Snijders, P. J. F., Speksnijder, A. G. C. L., King, A. J., . . . van der Loeff, M. F. S. (2014). Anal, Penile, and Oral High-Risk HPV Infections and HPV Seropositivity in HIV-Positive and HIV-Negative Men Who Have Sex with Men. *Plos One, 9*(3), e92208.

Veldman, T., Horikawa, I., Barrett, J. C., & Schlegel, R. (2001). Transcriptional activation of the telomerase hTERT gene by human papillomavirus type 16 E6 oncoprotein. *Journal of Virology, 75*(9), 4467-4472.

Videla, S., Darwich, L., Cañadas, M.-P., Coll, J., Piñol, M., García-Cuyás, F., . . . Group, o. b. o. H.-H. S. (2013). Natural History of Human Papillomavirus Infections Involving Anal, Penile, and Oral Sites Among HIV-Positive Men. *Sex Transm Dis, 40*(1), 3-10.

Visser, J., Nijman, H. W., Hoogenboom, B. N., Jager, P., van Baarle, D., Schuuring, E., . . . Daemen, T. (2007). Frequencies and role of regulatory T cells in patients with (pre)malignant cervical neoplasia. *Clinical and Experimental Immunology, 150*(2), 199-209.

Vivar, N., Ruffin, N., Sammicheli, S., Hejdeman, B., Rethi, B., & Chiodi, F. (2011). Survival and Proliferation of CD28- T Cells During HIV-1 Infection Relate to the Amplitude of Viral Replication. *Journal of Infectious Diseases, 203*(11), 1658-1667.

Walker, A. S., Doerholt, K., Sharland, M., Gibb, D. M., & Committee, f. t. C. H. P. S. S. (2004). Response to highly active antiretroviral therapy varies with age: the UK and Ireland Collaborative HIV Paediatric Study. *Aids, 18*(14), 1915-1924.

Walker, B. R., Colledge, N. R., Ralston, S. H., & Penman, I. (2013). *Davidson's Principles and Practice of Medicine*: Elsevier Health Sciences UK.

Wang-Johanning, F., Lu, D. W., Wang, Y., Johnson, M. R., & Johanning, G. L. (2002). Quantitation of human papillomavirus 16 E6 and E7 DNA and RNA in residual material from ThinPrep Papanicolaou tests using real-time polymerase chain reaction analysis. *Cancer, 94*(8), 2199-2210.

Wang, C.-c., Silverberg, M., & Abrams, D. (2014). Non-AIDS-Defining Malignancies in the HIV-Infected Population. *Current Infectious Disease Reports, 16*(6), 1-7.

Wilkin, T. J., Palmer, S., Brudney, K. F., Chiasson, M. A., & Wright, T. C. (2004). Anal intraepithelial neoplasia in heterosexual and homosexual HIV-positive men with access to antiretroviral therapy. *Journal of Infectious Diseases, 190*(9), 1685-1691.

Woo, Y. L., Sterling, J., Damay, I., Coleman, N., Crawford, R., Van Der Burg, S. H., & Stanley, M. (2008). Characterising the local immune responses in cervical intraepithelial neoplasia: a cross-sectional and longitudinal analysis. *BJOG: An International Journal of Obstetrics & Gynaecology, 115*(13), 1616-1622.

Woo, Y. L., van den Hende, M., Sterling, J. C., Coleman, N., Crawford, R. A. F., Kwappenberg, K. M. C., . . . van der Burg, S. H. (2010). 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, 126*(1), 133-141.

Woodman, C. B. J., Collins, S. I., & Young, L. S. (2007). The natural history of cervical HPV infection: unresolved issues. *Nat Rev Cancer, 7*(1), 11-22.

Yin, D. E., Warshaw, M. G., Miller, W. C., Castro, H., Fiscus, S. A., Harper, L. M., . . . McKinney, R. E. (2014). Using CD4 Percentage and Age to Optimize Pediatric Antiretroviral Therapy Initiation. *Pediatrics*.

Yukl, S., Gianella, S., Sinclair, E., Epling, L., Li, Q., Duan, L., . . . Wong, J. K. (2010). Differences in HIV Burden and Immune Activation within the Gut of HIV+ Patients on Suppressive Antiretroviral Therapy. *The Journal of Infectious Diseases, 202*(10), 1553-1561.

Zaravinos, A., Mammas, I. N., Sourvinos, G., & Spandidos, D. A. (2009). Molecular detection methods of human papillomavirus (HPV). *Int J Biol Markers, 24*(4), 215-222.

Zhang, D. Y., Yin, Y. P., Feng, T. J., Hong, F. C., Jiang, N., Wang, B. X., & Chen, X. S. (2014). HPV Infections among MSM in Shenzhen, China. *Plos One, 9*(5), e96364.

Zhang, S. M., Buring, J. E., Lee, I. M., Cook, N. R., & Ridker, P. M. (2005). C-Reactive Protein Levels Are Not Associated with Increased Risk for Colorectal Cancer in Women. *Annals of Internal Medicine, 142*(6), 425-432.

LIST OF PUBLICATIONS AND PAPERS PRESENTED

Conference Attended

Leng, C.Y., Low, H.C., Chong, M.L., Sulaiman, H., Azwa, I., Woo, Y.L., Kamarulzaman, A. (2014). High frequency of Human Papillomavirus infection in anal canal despite immune reconstitution by combination antiretroviral therapy. Presented at 3rd National AIDS conference (NAC), Kuantan, Malaysia.

Published Manuscript

Low, H. C., Silver, M. I., Brown, B. J., **Leng, C. Y.**, Blas, M. M., Gravitt, P. E., & Woo, Y. L. (2015). Comparison of Hybribio GenoArray and Roche Human Papillomavirus (HPV) Linear Array for HPV Genotyping in Anal Swab Samples. *Journal of Clinical Microbiology*, *53*(2), 550–556.

Leng, C.Y., Low, H.C., Chua, L.L., Chong, M.L., Sulaiman, H., Azwa, I., Rajasuriar, R., Roberts, J.M., Kamarulzaman, A., Woo, Y.L. Human papillomavirus 16 (HPV16) and HPV52 E6-specific immunity in HIV-infected adults on combination antiretroviral therapy (cART). *Hiv Medicine*.