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# Tumour Promoting Potentials of HAART backbone (NNRTIs and NRTIs) leading to increase cancer risk among AIDs patients

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## Abstract

**Introduction**:
Since the discovery of AIDS in 1980, the development of cancers has featured as a major problem in HIV patients. The extents of malignancies kept increasing among HIV infected population than the normal population. HAART widely became available in the 1990s, and has marked a notable decrease in the occurrence of diseases and death rate in HIV infected population but long-term use of it have been associated with increase cancer risk. Therefore, it will be of significance important to study the tumour promoting potentials of HAART Backbone (NNRTIs and NRTIs) leading to increase cancer risk in HIV infected population.

**Method**:
Cell differentiation potential was evaluated using U937 cells exposed to Nevirapine (NVP), Abacavir Sulphate (ABC) and Zidovudine (AZT) for 72hrs. Also, DNA breakage induced by NVP, ABC and AZT on HeLa cells at 40ug/ml and 50ug/ml using a modified FADU protocol was evaluated.

**Results:**
The highest transforming ability was recorded with AZT 50ug/ml as 98% of treated cells transformed into adherent cells after 72hrs exposure as compared to untreated cells. DNA breakage was not induced with NVP, ABC and AZT concentrations used on cells for 4hrs (40 and 50ug/ml).

**Conclusion**:
Overall, the research suggests that NVP, ABC and AZT have cell differentiation potential concluding that these drugs have tumour promoting potentials leading to increase cancer risk.

**Keywords:** Tumour promoting potential, DNA Damage, HAART classes, AIDs, Cancer

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# Introduction

HIV infected individuals have a 1000 times greater chanceof being diagnosed with Kaposi sarcoma, 70 times with non-Hodgkin lymphoma and 5 times with cervical cancer among women (Grulich et al., 2007). These 3 cancers are defined as AIDS defining cancers (ADCs) (Grulich et al., 2007). Clifford et al., (2005) and Engels et al., (2006) recorded major decrease in the risk of ADCs in AIDS population introduced to highly active antiretroviral therapy (HAART) which is a combination of different drug classes that manages the HIV virus reproduction effectively. This drug combination has resulted in overall progress of CD4 counts and it has also led to a reduction in the risk of other infections due to low immunity (Detels et al., 2001; Sterne et al., 2005), even if the normal immune system of the patient is not fully restored (Kaufmann et al., 2003, Tarwater et al., 2004). Over the past few years, a significant amount of research has tried to investigate the mechanism of action of these HAART classes, and discoveries found out that these drugs can both induce and block certain enzymes involved in drug metabolism (Antoniou and Tseng, 2005). However, the use of this therapy for a long time have proven to have adverse effect on HIV patient (D`Arminio et al., 2007) and is now becoming a major issue for researchers who are focusing on understanding the basic cellular mechanisms of these drug induced side effects which includes hypersensibility reactions, rash, hepatoxicity, dyslipidemia, insulin resistance and diabetes (Gills et al., 2007). Others include CNS toxic effects, peripheral neuropathies as well as nephrotoxicity and cardiovascular diseases risk (Montessori et al., 2004). Question such as toxicity face oncologists using HAART combination for the management of HIV infection and malignancies (Vaccher et al., 2001). (Palmer, 2003) suggested that toxicity of HAART may be influenced because the combination require many tablets to be taken multiple times which could possible lead to the combination of different toxicity of drugs on a patient.

Sugimura, (1992) defined carcinogenesis as a multi-stage process involving at least promotion, initiation and progression. Initiation induce changes in DNA and can be detected by various genotoxicity assays while promoters, can cause development of tumor through repeated application on cells. Kakunaga, (1973)., Reznikoff, (1973)., Mangiacasale et al., (2003) studied NNRTIs on tumor cell lines using *In vitro* assay concluding that these drugs have cytotoxic effect on cell lines. These HAART classes are dideoxy-nucleosides (d-drugs) that are being incorporated into cell DNA and function as chain terminators of DNA elongation thereby leading to genotoxicity manifestation such as formation of genetic mutation, chromosomal aberration and shortening of the telomere (IARC, 2000). The *in vitro* data propose that these HAART classes can induce, or strongly stop the growth of cancer (Gill et al., 2007; Olivero et al., 2005; D`Arminio et al., 2007) in HIV infected individual.

Lung cancer and other NADCs (Non-AIDS defining cancers) incidence in HIV/AIDs population was found out to be higher than (Rabkin and Yellin, 1994, Parker et al., 1998), equal to (Lyter et al., 1995 and Grulich et al., 2002), and lower than (Cooksley et al., 1999) the general population.

# Materials and Methods

## Test drugs

Nevirapine (NVP) SML0097, Zidovudine (AZT) PHR1292 and Abacavir sulphate (ABC) SML0089 were all purchased from Sigma. NVP were dissolved in dimethyl sulfoxide (DMSO) to get a final concentration of DMSO not more than 1% while ABC and AZT are soluble in water. All other reagents used were supplied courtesy of tissue culture laboratory, University of East London.

## Cells

HeLa cells and u937 cells were supplied courtesy of tissue culture laboratory, University of East London. HeLa cells were grown in Dulbelcco`s Modified Essential Medium (DMEM) supplied from Fisher Scientific. It was supplemented with 1% penicillin- streptomycin and 10% Fetal Bovine Serum (FBS) while U937 cells were grown in RPMI- 1640 supplemented with 10% Fetal Calf Serum (FCS). Medium was replaced every 3rd day. Cells were maintained in a humidified incubator of 37°C in a 5% $CO_2$. Cells were observed on an EVOS inverted microscope and emerge taken.

## Cell differentiation Assay

This was carried out to determine the ability of the tested drugs to differentiate semi adherent monocytes (U937) into adherent macrophages after 72hrs of exposure.

U937 cells were used and seeded in 24well plates at a density of 2 x$10^5$cells/ well. 500ul of cellsolution containing medium (RPMI- 1640) were added to the well plates and incubated for 24hrs in a humidified atmosphere of 37°C with 5% $CO_2$. After 24hrs, 500ul of test compound diluted with medium (50ug/ml, 100ug/ml for Abacavir and Zidovudine while 40ug/ml, 80ug/ml for Nevirapine) was added to each well plates and incubated for 72hrs in 4 replicates.

Cells treated with PMA were used as positive control while untreated cells without any treatment were used as negative control.

Number of adherent cells was also counted after 72hrs of drug exposure in each well plate. After observing and viewing the plates under the EVOS inverted microscope, medium was aspirated. 200ul of PBS was used in washing the cells followed by trpsinization using 250ul. Plates were incubated in a humidified atmosphere of 37°C with 5% $CO_2$ for 5min for cell detachment from flasks before transferring cells to Eppendorf Tubes. Samples were placed in ice to prevent cells from adhering to Eppendorf Tubes. Afterwards, cells were counted and result recorded.

## Quantification of DNA strand break

Automated FADU (Flourometric Analysis of DNA unwinding) assay was used to access DNA strand breaks. This was based on progressive DNA unwinding under highly controlled condition of alkaline pH, time and temperature.

Cells were treated using three groups: (T) Unwinding, (P) Control cells not subject to unwinding and (B) Cell exposed to total unwinding.

## All Sample group

HeLa cells was seeded in a 24 well plate with density of $2x10^5$ Cells per well. 500ul of cell solution containing medium (DMEM) were added to the well plates and incubated for 24hours in a humidified atmosphere of 37°C with 5% $CO_2$. After 24hours, 500ul of test compound diluted with medium (50ug/ml for Abacavir and Zidovudine while 40ug/ml for Nevirapine) was added to the well plates and incubated for 4hour. Medium was aspirated, cells were washed with 250ul of PBS and 100ul of trypsin/EDTA was added to each well and incubated for 3mins for detachment of cells from plates.100ul of lyses buffer was added to cells and cells were transferred to EppendorfTubes in ice to stop any further attachment of cells to Eppendorf Tubes.

## Group B Samples

300ul of 0.1M NaOH was added to lysed cells and placed on a vortex for 1 minute. Cells were maintained at room temperature for 30mins and neutralized by adding 300ul of 1M HCL and placed on a vortex for 1minutes.

## Group T Samples

300ul of 0.1 M NaOH were added to the lysed cells. 300ul of 1M HCL was added for neutralization and maintained at room temperature for the whole unwinding period and placed on a vortex for 1minute.

## Group P Samples

300ul of 0.1 M NaOH was added to the lysed cells without mixing. Cells were maintained under room temperature for 30mins.All samples were protected from other agents that can induce strands break under an alkaline environment. After DNA unwinding, the P-samples was neutralized by addition of 300ul of 1 *M* HCL and placed on a vortex for 1minute to render the samples homogeneous.

## *Fluorescent measurement of dsDNA*.

For all FADU 3 groups experiments carried out, Sybr safe fluorescent dye was used in staining the cells to determine the fraction of DNA breakage. 100ul of Sybr safe diluted with PBS was added to all samples

After mixing, the relative intensity of the fluorescence was read from a spectro-fluorimeter at 497nm excitation and 530nm emission.

## Statistical Analysis

The mean difference of all treated groups and untreated group was analyzed using student t-test. Significance difference of all groups compared to the control was analyzed using graph pad prism software.

For FADU experiment, the fraction of % ds-DNA (F) was calculated as F = P / T where T andP is the fluorescence intensities of T and P respectively.

# Results

## Evaluation of cell differentiation potential

Differentiation was carried out to observe the ability of (40ug/ml and 80ug/ml) Nevirapine, (50ug/ml and 100ug/ml) of Abacavir and Zidovudine to differentiate monocytes into macrophages after 72hrs drug exposure (Figure 1 & 2). Cells were observed using the EVOS inverted microscope and emerges taken.

Tumour promoting activity, 2 low concentration 40, 80ug/ml was used for NVP while 50, 100ug/ml was used for ABC and AZT as the aim of the assay was to observe its transformation ability as compared to the + control after 72hrs.and 80ug/ml, ABC and AZT 50 and 100ug/ml exposed to u937 cells showed to have tumour promoting ability as it could differentiate semi adherent monocytes into adherent macrophages after 72hrs as compared to the – control. From the table above, AZT 50ug/ml indicated to have more adherent cells as compared to NVP and ABC used.

The highest % adherent cells was indicated with AZT 50ug/ml which recorded 98% cell adherent and NVP 80ug/ml recording the lowest %cell adherent with 90.5% as compared to other drug concentrations.

## Evaluation of DNA strands break

Alkaline unwinding was carried out to quantify the amount of DNA strands break caused by the introduction of ABC, AZT 50ug/ml and NVP 40ug/ml as compared to the control (untreated cells) (Figure 3). FADU experiment was carried out in 4 replicates for each drug and sample.

The result indicated that U937 cells exposed to these drugs for 72hrs differentiated into round, large adherent cells from the pictures below as compared to the untreated cells (-control)

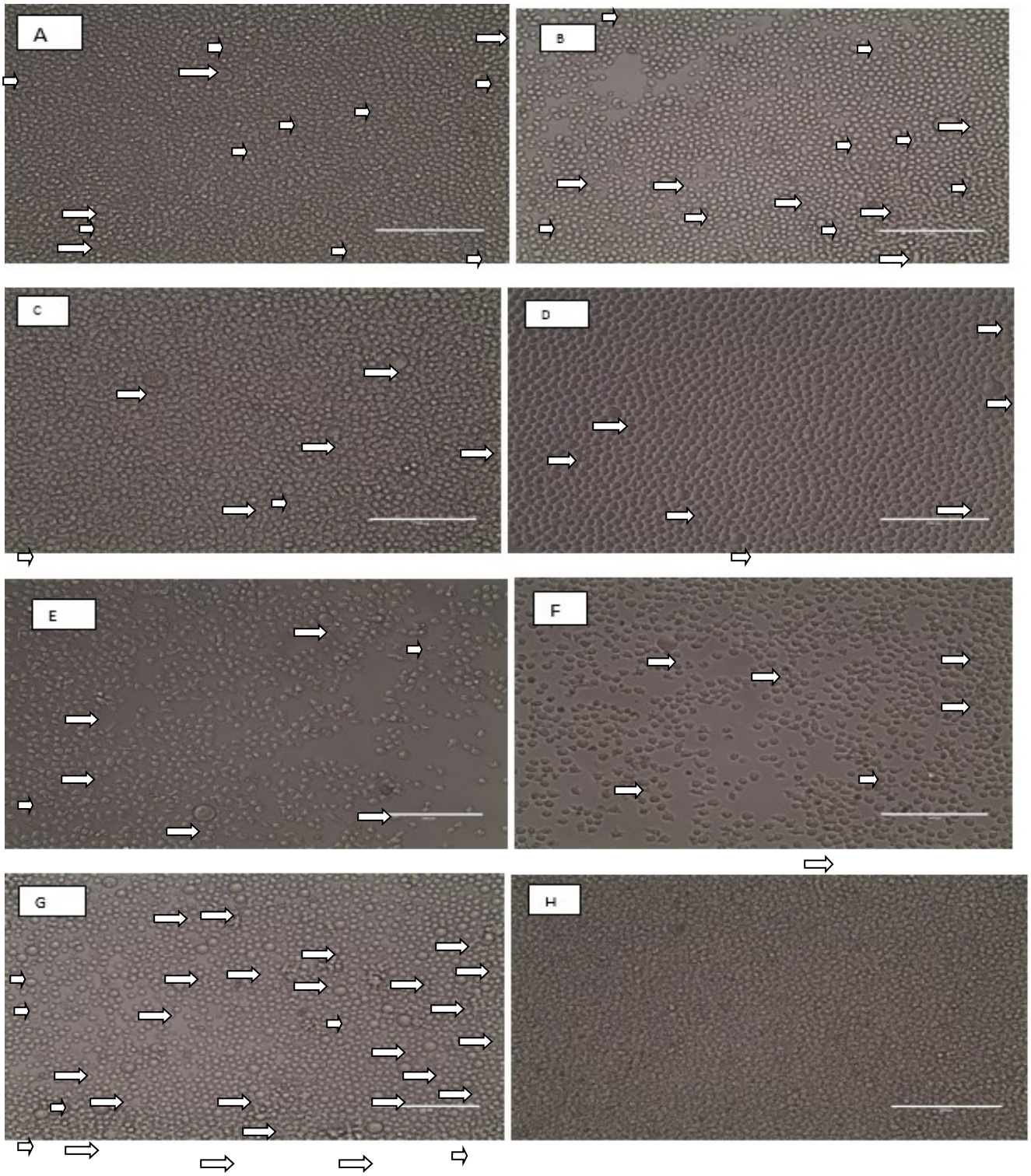

**Figure 1:** U937 cells exposed to A (Nevirapine 40ug/ml), B (Nevirapine 200ug/ml), C (Abacavir 50ug/ml), D (Abacavir 250ug/ml), E (Zidovudine 50ug/ml), F (Zidovudine 250ug/ml) G, (PMA treated), H (Untreated cells (Control)) for 72hrs.

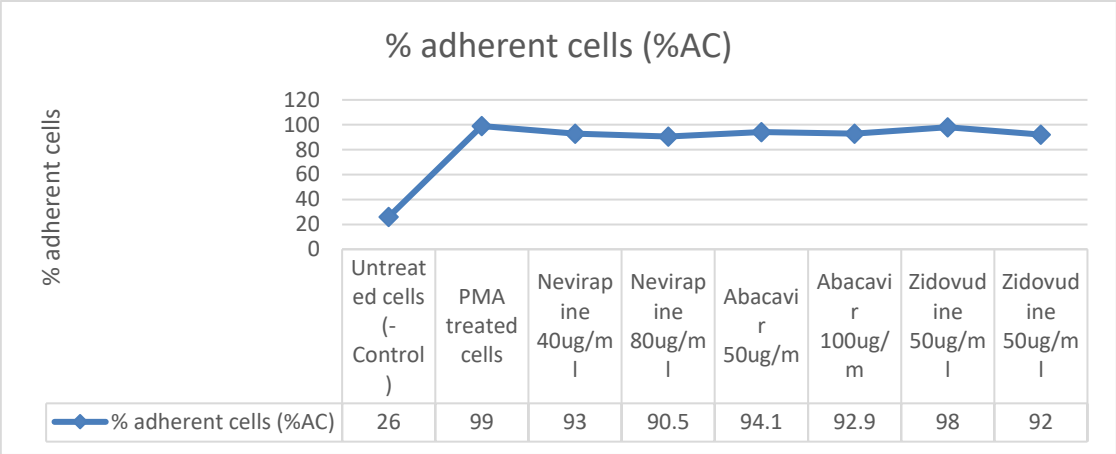


**Figure 2**: Graph showing number of % adherent cells exposed to NVP, ABC and AZT using 2 concentrations and maintained for 72hrs.

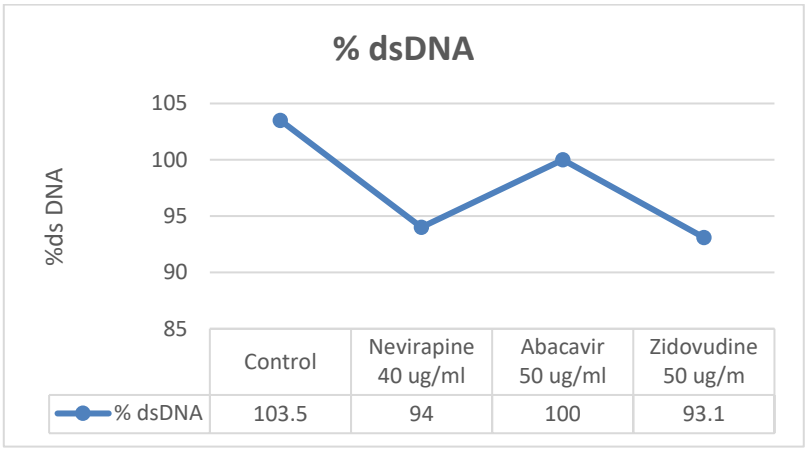


**Figure 3:** Graph showing fraction of %DNA strand break of HeLa cells after 4hrs of exposure to NVP, ABC and AZT at lower concentration

% Fluorescent measurement of ds-DNA was calculated as: F (fraction of dsDNA) = P/ Tx 100 where P and T are fluorescence intensities of Sample P and T respectively.

The result above indicates no significant difference in HeLa cells exposed to NVP, ABC and AZT as there is no decrease in the % dsDNA of cells as compared to the control. A reduction in the intensity of fluorescence indicates an increase in DNA unwinding and consequently, a higher amount of DNA strands break. % dsDNA is expressed as percentage of dsDNA fraction in control cells without induced DNA damage.

## Discussion

U937 cells exposed to NVP 40 and 80ug/ml, ABC 50 and 100ug/ml and AZT 50 and 100ug/ml with a positive PMA control for 72hrs indicated changes on the cells morphologically as compared to untreated cells (-control). After 72hrs, small, round, semi adherent U937 cells (monocytes) transformed into large, oval, adherent cells (macrophages) after treatment with NVP, ABC and AZT as compared to PMA treated cells and untreated cells. This indicates NVP, ABC and AZT transforming ability in all concentration used as compared to PMA treated and Untreated cells.

The % of adherent cells transformed with Nevirapine 40ug/ml after 72hrs was 93% as compared to cells treated with Nevirapine 40ug/ml indicating a significant difference in cells as compared to cells with no treatment. 90.5%of U937 cells treated with Nevirapine 80ug/ml for 72hrs was observed to be adherent with significance from untreated cells.

Abacavir 50ug/ml transformed 94.1% and Abacavir 100ug/ml transformed 92.1% of treated cells into adherent cells indicating a significance difference in the cells as compared to untreated cells.

The highest transformation ability was observed in Zidovudine 50ug/ml as 98% of treated cells transformed into adherent cells after 72hrs and 92% transformed with Zidovudine 100ug/ml indicating significant difference on cells as compared to untreated cells.

HeLa cells treated with Nevirapine 40ug/ml, Abacavir 50ug/ml and Zidovudine 50ug/ml indicated no significant difference on cell as compared to untreated cells suggesting no evidence of drug effect on cell DNA as compared to untreated cells.

% double stranded (dsDNA) breakage induced by Nevirapine 40ug/ml on HeLa cells recorded 94%, Abacavir 50ug/ml recorded 100% and Zidovudine recorded 93.1%as compared to untreated cells. This study indicates no evidence of dsDNA breakage as compared to untreated cells. The lower the intensity, the more DNA unwinding and consequently, more DNA breakage but all drugs used did not show any difference from the untreated cells suggesting drug concentration used did not induce dsDNA breakage on cells after 4hrs.

These finding could be linked to the fact that these drugs are incorporated in to cellular DNA (Olivero et al., 2005) which may result in the occurrence of unsuitable DNA damage, which can lead to impaired function of the cells thereby contributing to the genotoxic effects of the drug. Effect of the drugs may produce genotoxic manifestation that include mutagenesis (IARC, 2000) thereby leading to the cause of cancer (Gills et al., 2007) and increasing the risk of cancers in patients taking these drugs (D`Arminio et al., 2007).

## Conclusion

Nevirapine, Abacavir Sulphate and Zidovudinemight induce tumour formation as they have indicated to have tumour promoting potential by inducing cell differentiation. No evidence of DNA damage was indicated by these drugs with the concentrations used.

More indebt research should be carried out on these HAART classes and other HAART classes that were not covered for better understanding of HAART mechanism which may be linked to increase cancer risk in HIV infected patients.

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## References

1. Antoniou T and Tseng A.L (2005): Interactions between anti-retroviral and anti- neoplastic drug therapy. *ClinPharmacokinet*; 44(2):111–45.

2. Clifford G.M., Polesel J., Rickenbach M., and Kofler A., et al (2005): Cancer risk in the Swiss HIV cohort study; Associations with immunodeficiency, smoking and highly active antiretroviral therapy. *J Natl Cancer Inst*, 97: 425 – 32.

3. Cooksley C.D., Hwang L.Y., Waller D.K., and Ford C.E, (1999): HIV related malignancies; community based study

using linkage of cancer registry data. *Int J STD AIDS*, 10:795–802.

4. D'Arminio M. A., Abrams D., and Pradier C, et al (2007): HIV induced immunodeficiency and risk of fatal AIDs defining and Non-defining malignancies; Results from the DAD study. Presented at the 14th Conference on Retroviruses and Opportunistic Infections, Los Angeles, CA, February 25-28, (abstr 84)

5. Detels R., Tarwater P., Phair J.P et al (2001): Effectiveness of potent antiretroviral therapies on the incidence of opportunistic infections before and after AIDs diagnosis. *AIDs*, 15: 347-55.

6. Engels E.A., Pfeiffer R.M., Virgo P, et al (2006): Trends in cancer risk among persons with AIDs in the United states 1980-2002. *AIDS*, 20: 1645-54.

7. Gills J.J., Lopiccolo J., and Tsurutani J, et al (2007): Nelfinavir, A lead HIV protease inhibitor is a broad spectrum anticancer agent that induces endoplasmic reticulum stress, autophagy and apoptosis in vitro and in vivo. *Clin Cancer Res* 2007; 13:5183- 5194

8. Grulich A.E, Li Y., McDonald A., Law M., Correll P., and Kaldor J, (2002): Rates of Non AIDs defining cancer in people with HIV infection before and after AIDs diagnosis. *AIDS*, 16:1155–1161.

9. Grulich A.E., Van Leeuwen M.T, Falster M.O., and Vajdic C.M, (2007): Incidence of cancers in people with HIV/AIDS compared with immune suppressed transplant recipients: a meta-analysis. *Lancet*, 370(9581):59–67.

10. IARC (2000): IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: World Health Organization. *IARC Scientific Publications*, 76: 1-521.

11. Kakunaga T., (1973): Quantitative system for assay of malignant transformation by chemical carcinogens using a clone derived from BALB/c 3T3. *International Journal of Cancer*, 12:463–473.

12. Kaufmann G.R., Perrin L., Furrer H., and Telenti A, et al (2003): CD4 T lymphocyte recovery in individuals with advanced HIV-1 infection receiving potent antiretroviral therapy for 4years; the Swiss HIV Cohort Study. *Arch Intern Med*, 163: 2187 – 95

13. Reznikoff C.A, Brankow D.W, Heidelberger C., (1973): Establishment and characterization of cloned line of C3H mouse embryo cells sensitive to postconfluence inhibition of division, Cancer Res. 33: 3231–3238.

14. Lyter D.W., Bryant J., Thackeray R., Rinaldo C.R., and Kingsley L.A, (1995): Incidence of human immunodeficiency virus-related and nonrelated malignancies in a large cohort of homosexual men. *J ClinOncol*, 13:2540–2546.

15. Mangiacasale R., Pittoggi C., Mattei E., and Lorenzini R, et al (2003): Exposure of normal and transformed cells to nevirapine, a reverse transcriptase inhibitor, reduces cell growth and promotes differentiation. *Oncogene*, 22: 2750–2761.

16. Montessori V., Natasha P., Marianne H., Linda A., and Julio S.G.M, (2004): Adverse effects of antiretroviral therapy for HIV infection, *CMAJ*: 170 (2)

17. Olivero O.A., Tejera A.M., and Fernandez J.J, et al (2005): Zidovudine induces S-phase arrest and cell cycle gene expression changes in human cells. *Mutagenesis*, 20:139-146

18. Palmer C., (2003): HIV treatments and highly active antiretroviral therapy: Sexual Health Physician, Infectious Diseases Unit, Nambour; *Australian Prescriber* Vol. 26 No. 3

19. Parker M.S., Leveno D.M., Campbell T.J., Worrell J.A., and Carozza S.E, (1998): AIDS-related bronchogenic carcinoma: fact or fiction? *Chest*, 113:154–161.

20. Rabkin C.S, and Yellin F, (1994): Cancer incidence in a population with a high prevalence of infection with human immunodeficiency virus type 1. *J Natl Cancer Inst*, 86:1711–1716.

21. Sugimura T, (1992): Multistep carcinogenesis: a 1992 perspective, *Science* 259: 603–607.

22. Sterne J.A.C., Hernan M.A., Ledergerber B., Tilling K., Weber R., and Sendi P, (2005): Long-term effectiveness of potent antiretroviral therapy in preventing AIDS and death: a prospective cohort study, for the Swiss HIV Cohort Study. *Lancet*, 366:378-84.

23. Tarwater PM., Gallant J.E., Gore M.E., Phair J.P., and Detels R, et al (2004): Prognosis value of plasma HIV RNA among HAART users. *AIDS*, 18: 2419 – 23.

24. Vaccher E., Spina M., and Di Gennaro G., et al (2001): Concomitant CHOP chemotherapy and HAART in patients with HIV related Non Hodgkin lymphoma. *Cancer*: (in press).

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