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Research Article

Oral Cell Cancer: Could Intratumoral Microvessel Density (MVD) Hold the Key to Understanding the Antiangiogenic Effects of Efavirenz?

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Abstract

Introduction: in addition to some studies which suggested that Efavirenz [EFV] may have anti-proliferative effects on various cancer cell lines even at sub-therapeutic doses, EFV has been reported to strongly impact angiogenesis and disrupt neutral lipid homeostasis. The aim of this paper is to bring to light the potential role Intratumoral Microvessel Density (MVD) holds in understanding the Antiangiogenic Effects of Efavirenz on oral cell cancer.

Aims and objectives: to focus attention on the potential role Intratumoral Microvessel Density (MVD) holds in understanding the Antiangiogenic Effects of Efavirenz on oral cell cancer.

Methods: This paper aims to draw attention on the Intratumoral Microvessel Density (MVD) as a potential factor in understanding the Antiangiogenic Effects of Efavirenz on oral cell cancer.

Result : Our data analysis revealed that EFV treatments did not exhibit significant main effects on the viability of the cancer cell line ($F(6,20) = 0.7970$; $p = 0.5878$). One-way Analysis of Variance (ANOVA) was used to determine the efficacy of the drug, at 0.05 significance level ($p \leq 0.05$).

Discussion/Conclusion: The results indicate that Efavirenz holds promise as an anti-proliferative agent for treating oral cancer. Nevertheless, additional research is necessary to fully explain the impact of the Intratumoral Microvessel Density (MVD) in understanding the Antiangiogenic Effects of Efavirenz on oral cell cancer.

Keywords: Cal 27 cells ; Efavirenz; anti-mitotic; oral squamous cell; anti-angiogenic; AIDS -defining cancers; anti-proliferative; Apoptosis.

INTRODUCTION

Burden of cancers

Cancer remains the second leading cause of death worldwide, claiming approximately 10 million lives in 2020, with 70% of these fatalities occurring in low- and middle-income countries[1].

Globally, cancer incidence was estimated at 19.3 million cases, with nearly 10 million deaths reported (excluding nonmelanoma skin cancer) [2] . Behavioral and dietary factors play a significant role in cancerrelated mortality, with about one-third of deaths linked to five major risks: high body mass index, insufficient fruit and vegetable consumption, physical inactivity, tobacco use, and alcohol consumption. Among these, tobacco use stands out as the most critical, contributing to 22% of cancer deaths [3] .

Oral cancer

Oral cancer, a non-AIDS-defining cancer, occurs in the region between the vermilion border of the lips and the junction of the hard and soft palates, or the posterior third of the tongue[4]. Approximately 45% of all oral cavity cancers are tongue squamous cell carcinoma (TSCC). While TSCC predominantly affects older individuals with prolonged tobacco exposure, recent research suggests an increasing incidence among younger populations [5] . Early lesions, which are often curable, tend to be asymptomatic, underscoring the importance of early detection through screening to prevent advanced and

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fatal disease [6]. Treatment typically involves surgery, radiation, or a combination of both, with surgery being the primary approach for most cases of oral cavity cancer [4]. Despite advancements, the overall 5-year survival rate (across all sites and stages) remains just above 50% [4,7].

Oral cancer, the most common malignancy in the head and neck region, is an aggressive disease with a generally poor prognosis. Notably, oral squamous cell carcinoma (OSCC) ranks as the fifth most common cancer among males and the tenth most common among females [8]. Each year, more than 450,000 new cases of oral cancer are reported globally, yet only 40%–50% of patients survive beyond five years post-diagnosis [9].

In Africa, delayed diagnosis of OSCC significantly lowers survival rates, largely due to limited access to oral healthcare and insufficient awareness, particularly in low- and middle-income countries [8]. The primary causes include rising smoking habits, westernized lifestyles and diets, HIV/HPV infections, and inadequate health-related fiscal policies [10-11]. Among individuals aged 45 years, oral cancer incidence is reported at 7.3% in males and 7.8% in females [8].

Vascular Endothelial Growth Factor vs Use of Anti-angiogenic drugs in cancer management

Vascular Endothelial Growth Factor (VEGF) functions as an endothelial cell mitogen, playing a central role in both physiological and pathological angiogenesis. Of its various isoforms, VEGFA is the most extensively studied [12] . Beyond its role in promoting angiogenesis, VEGF also supports immune cell function within the tumor microenvironment and shapes the host response to tumors, highlighting its multifaceted influence on cancer progression [13] . VEGF signaling pathways are critical to tumorigenesis, particularly through their impact on cancer stem cell function [13-14]. Tumor growth and metastasis depends heavily on neovascularization, with VEGF serving as a potent pro-angiogenic factor driving this process. Neovascularization is a cornerstone in the development of cancerous tumors [15], and in cases where rapidly proliferating cancer cells outpace the supply of blood and oxygen, hypoxia ensues, disrupting angiogenic signals, this hypoxic state underscores the rationale for using anti-angiogenic agents as a therapeutic approach[16].

In the context of HIV-associated cancers, Kaposi's sarcoma (KS) stands out as an angiogenic tumor caused by Kaposi's sarcoma-associated herpesvirus (KSHV) [17]. KS is characterized by high vascularization, with its etiologic agent, KSHV, driving the angiogenic phenotypes of endothelial cells [18-19]. Upon infection of human endothelial cells, KSHV induces the production of VEGFA and VEGFC, alongside other key angiogenic factors such as platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) 2 [20]. This highlights the critical role of antiangiogenic agents in managing tumors by effectively targeting these angiogenic pathways.

Efavirenz

Efavirenz, marketed under the brand names Sustiva® and Stocrin®, is a synthetic purine derivative and a first-generation non-nucleoside reverse transcriptase inhibitor (NNRTI). It works by inhibiting the activity of viral RNA-directed DNA polymerase, also known as reverse transcriptase [21-22].

Beyond its antiretroviral properties, efavirenz (EFV) has shown notable toxicity against cancer cells [23]. It suppresses viral DNA replication [24] and exhibits antiangiogenic functions [25].

EFV has demonstrated the ability to inhibit the growth of various malignancies in cell culture, including colorectal, pancreatic, lung, glioblastoma, and leukemia [26]. Its use has also been associated with a reduction in the incidence of Kaposi's sarcoma and has been identified as having broader anti-cancer properties [27-28]. Notably, efavirenz, a key component of HAART, has been reported to significantly impact angiogenesis and disrupt neutral lipid homeostasis, even at sub-therapeutic doses [29]. Additionally, the prognosis of cancer in HIV patients improves with the use of EFV-containing regimens, highlighting its potential role in cancer management [30-31].

Efavirenz (EFV) has emerged as a pivotal element in cancer management, particularly for individuals living with HIV[31]. It has been repurposed to demonstrate effectiveness against various cancers, including prostate, pancreatic, and triple-negative breast cancer (TNBC). EFV is said to exerts its anticancer effects on TNBC by modulating the fatty acid metabolism pathway [32]. Furthermore, EFV induces apoptosis in Human Squamous Cell Carcinoma from Uterine Cervix (HCS-2) cells, highlighting its potential as a versatile anticancer agent [31].

EFV and HIV-cancers

Efavirenz (EFV) has shown inhibitory effects on the growth of various malignancies in cell culture, including colorectal, pancreatic, lung, glioblastoma, and leukemia [26], Its use has been associated with a reduced incidence of Kaposi's sarcoma and is increasingly recognized for its anti-cancer properties[27-28]. Remarkably, EFV has been reported to strongly impact angiogenesis and disrupt neutral lipid homeostasis, even at subtherapeutic doses [29]. Also, the prognosis of cancer in HIV patients improves with the use of EFV-containing regimens, further emphasizing its potential role in cancer management [30-31].

Tumor staging and angiogenesis

Angiogenesis plays a vital role in the progression of most solid tumors [33]. A significant correlation between increased vascularization and the development of oral carcinoma has been identified, underscoring the critical role of angiogenesis in its progression [34]. Some studies have shown that intratumoral microvessel density (MVD) is closely associated

with the prognosis of oral carcinoma [35]. High MVD has been linked to larger tumor size, higher relapse rates, and increased incidence of node metastases [35-36].

Furthermore, research suggests that the extent of neoangiogenesis is strongly associated with the histological grade of differentiation and the presence of locoregional metastases in carcinoma [37].

CAL 27

CAL 27 cells were derived from epithelial tissue collected in 1982 from a 56-year-old Caucasian male with a tongue lesion prior to treatment [38]. These cells exhibit epithelial characteristics, appearing as polygonal cells with a prominently granulated cytoplasm. Immunocytochemical analyses show strong positive staining with anti-keratin antibodies, although these cells demonstrate limited growth in semi-solid medium[39]. Identified as squamous cell carcinoma of the tongue, CAL 27 is among the most commonly used cell lines in oral squamous cell carcinoma (OSCC) research [40].

Justification

HIV remains a significant global health challenge, with South Africa being particularly affected [41]. HIV-related cancers further compound the disease burden, impacting both individual patients and the Department of Health. Scientific evidence highlights the essential role of antiretroviral drugs (ARVs) in reducing the incidence and severity of AIDS-defining cancers [29,42].

Efavirenz (EFV) is known to suppress viral DNA replication [24] and exhibit antiangiogenic properties [25].

However, the direct effect of EFV on cancer cell lines, as well as the role of intratumoral microvessel density (MVD) in demonstrating EFV's antiangiogenic functions, remains unclear. Therefore, the aim of this study is to evaluate these aspects.

METHODS

While efavirenz has shown beneficial effects in cancer treatment, its impact on oral squamous cell carcinoma—particularly the role of microvascular density in the efficacy of EFV's antiangiogenic functions— remains unexplored. This knowledge gap has prompted the need for the current study.

Experimental Groups

Test Groups cells were treated with EFV at various concentrations:

Test Group 1: 4 µg/mL each.
Test Group 2: 2 µg/mL each.
Test Group 3: 1µg/mL each.
Test Group 4: 0.5 µg/mL each.
Test Group 5: 0.25 µg/mL each.

Solvent Control groups were treated with equal volumes of physiological saline while positive Control Groups were not subjected to any experimental intervention; cells in these groups underwent normal growth processes.

Drug Treatment and Cell Incubation

Stock solutions of EFV at a concentration of 10µg/mL were prepared and subsequent serial dilutions were then carried out using physiological saline, and the resulting solutions were added to respective culture flasks containing CAL-27 cells to support cell health and growth, each culture media received 2-3 drops of penstrep solution containing penicillin (50 U/ml) and streptomycin (50 µg/ml). The cell cultures were placed in a controlled environment with 5% CO_2 at a temperature of 37°C. Over a period of 10 days, daily observations were conducted using an inverted microscope. On the 10th day, the cell cultures were harvested for the final assessment.

Cell Dissociation with Trypsin and Mycoplasma Contamination Test

The process of cell dissociation with trypsin was initiated when the cell growth reached a confluence of 90% of the total volume within the culture flask. This step ensured the controlled separation of cells from the culture substrate, allowing for further manipulation and analysis. To ensure the integrity of the cell lines, a test for mycoplasma contamination was carried out. This was performed on both day 5 and day 10 of the experiment using Hoechst dye from Sigma-Aldrich, St. Louis, USA. The presence of mycoplasma, a type of bacterial contamination, was assessed through this procedure, contributing to the reliability and accuracy of our experimental results. To assess the viability of CAL27 cells, a trypan blue assay was performed 48 hours after treatment. At harvest, any cell with confluence of less than 90% was eliminated from the study. To determine the concentration of cells per milliliter (ml) in the culture media, the approach outlined by Srivastava et al. [43] was adopted. utilization of hemocytometers and trypan blue staining-we took a 10µL sample from the cell culture and combined it with 90 µL of trypan blue solution in an autoclaved micro centrifuge tube. It was thorough mixed after which a 10µL aliquot of the blended mixture was placed on an automated hemocytometer. By tallying the cell count across all the squares on the hemocytometer, an average cell count was calculated. And to convert this average count into cells per milliliter, it was multiplied by the dilution factor (which, in this case, is 10). This calculation yielded the number of cells in millions per milliliter of the cell culture, providing a valuable metric for assessing cell concentration in the experimental setup.

Counting of Cells

As a part of the experimental process, a portion of the freshly prepared cell suspension was extracted from each

experimental group and the cell viability (the essential stain trypan blue was employed) thus selectively labeling and identifying dead cells. An automated cell counting method was employed (Luna-FL™ dual fluorescence cell counter developed by Logos Biosystems in the USA). This automated cell counter applies the same foundational principles as counting chambers or hemocytometers but integrates the use of fluorescent staining for enhanced accuracy.

Data Analysis

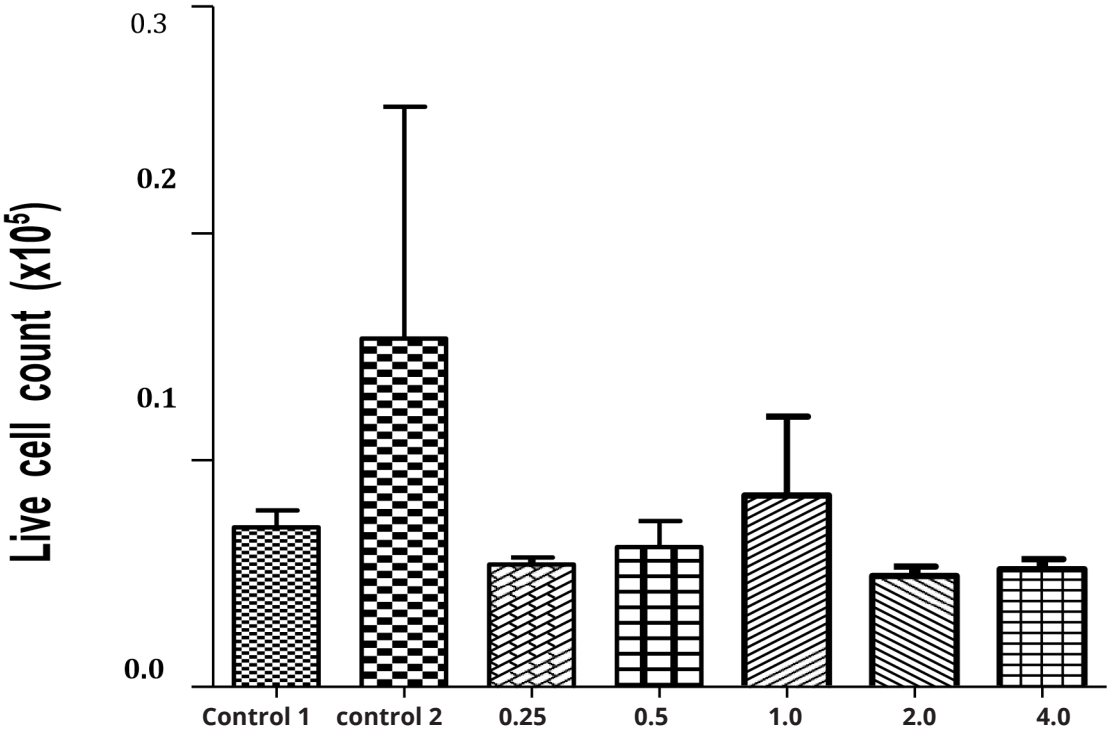
Results obtained were analyzed using the one-way Analysis of Variance (ANOVA) and obtained values used to compare the efficacies of the drugs, at 0.05 significance level ($p \leq 0.05$).

RESULTS

The results were collected at a wavelength of 595.0. Ultraviolet (UV) radiation induces squamous cell carcinoma and exert their toxic effects by causing DNA damage, which depends mainly on the wavelength [44] and a study has shown that Cell viability increased with increasing wavelength [45].

Our data analysis revealed that EFV treatments did not exhibit significant main effects on the viability of the cancer cell line ($F(6,20) = 0.7970$; $p = 0.5878$, **Fig. 1**).

Figure 1. Different conc. Of EFV in in µg/ml.s.



The effects of Efavirenz on cell Division. The graph shows the average number of live Cal-27 cancer cells following incubation with DMEM, DMSO and different concentrations of EFV in µg/ml. Data shown represents mean ± SEM; n=3 per group. **P<0.05* compared to control, *#P<0.05* compared to other treatment groups; One-way ANOVA, followed by Bonferroni post hoc comparison test.

DISCUSSION

Sample size determination

Sample size in this study was determined by the flask size, as the volume of the flask dictates the relative number of cells it can accommodate. If the same flask size is used for all experimental groups, and the flask reaches at least 90% confluence, the sample size can be considered equal and adequate [46]. In this study, the same flask size was used across all experimental groups. Furthermore, Pollard et al. [47] suggested that in cell culture experiments, as the sample size increases with cell growth and repeated experimentation, the mean of the results will approach the true mean (i.e., the mean of the whole

population). They concluded that, for statistical significance in a descriptive analytical study such as this, the experimental cells should be cloned from the same cell group, which was done in this study. Additionally, each group was performed in triplicates, and all experimental cell groups achieved at least 90% confluency. Some studies argued that determining sample size in cell culture does not need to be more specific, as treatments are applied simultaneously to all cells in a well, rather than independently to each individual cell[46,48], as well as the Cellto-cell connections trigger the release of signaling molecules, and cells compete for the same nutrients in the culture media [48] .

Cancer staging and antiangiogenic impact of EFV

Squamous cell carcinoma can present with various growth patterns, including exophytic, ulcerative, and infiltrative. The infiltrative and ulcerative types are most commonly observed due to the loose tissue planes surrounding the intrinsic tongue musculature, which allow cancer cells to spread more easily and cause symptoms once the tumor size interferes with tongue mobility [49]. Although EFV has demonstrated antiangiogenic effects [24], its impact on CAL 27 cells appears to be minimal, despite established evidence of its antiangiogenic properties in other contexts. This suggests that tumor staging could influence the antiangiogenic effect of EFV, necessitating further research to confirm this hypothesis.

Notably, EFV has been shown to affect breast cancer stem cells, which are thought to be key drivers of cancer metastasis and have been linked to resistance and recurrence in patients undergoing traditional drug and radiation therapies [50]. Given the established antiangiogenic effects of EFV on various cancers and the role of microvessel density (MVD) in cancer growth, the key question that requires clarification is: "What is the role of MVD in demonstrating the antiangiogenic properties of EFV in oral cell cancer?"

CONCLUSION

The prevalence of squamous cell carcinoma underscores the importance of angiogenesis in its development and progression. Specifically, intratumoral microvessel density (MVD) has been shown to correlate with prognosis, with higher MVD linked to larger tumor size, increased relapse rates, and node metastases. While previous studies have suggested that EFV possesses antiangiogenic properties, these effects were not observed in this study. Further research is needed to explore whether tumor staging influences the antiangiogenic effects of EFV in this context. This would help fully understand the potential antitumor effects of EFV and assess its efficacy as a cancer treatment.

REFERENCES

1. American cancer society. [2024]. The Global Cancer Burden. Available at: https://www.cancer.org/about-us/our- global- health-work/global-cancer-burden.html. Assessed on 16/01/24.

2. Sung H, Ferlay J, Siegel R, Laversanne M,Soerjomataram I,Jemal A,Bray F.(2021).'Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries'. CANCER. https://doi.org/10.3322/caac.21660. available at: https://acsjournals.onlinelibrary.wiley.com/doi/full/10.3322/caac.21660 [accessed on 26/2/21].

3. Safiri S, Nejadghaderi SA, Abdollahi M, Carson-Chahhoud K, Kaufman JS, Bragazzi NL, Moradi-Lakeh M, Mansournia MA, Sullman MJM, Almasi-Hashiani A, Taghizadieh A, Collins GS, Kolahi AA. [2022]. Global, regional, and national burden of cancers attributable to tobacco smoking in 204 countries and territories, 1990-2019. Cancer Med.11(13):2662-2678. doi: 10.1002/cam4.4647. Epub 2022 May 27. PMID: 35621231; PMCID: PMC9249976.

4. Bradley A.S 2019. Oral Squamous Cell Carcinoma. Available at: https://www.msdmanuals.com/professional/ear,- nose,-and-throat-disorders/tumors-of-the-head-and-neck/oral-squamous-cell-carcinoma [accessed on 7/3/21].

5. Khadijah M,Krithika C,Nadeem J,Bharathi R, Thayumanavan B, Leena S. (2019). 'Meta-analysis on risk factors of squamous cell carcinoma of the tongue in young adults'. 23(3): 450–457.doi: 10.4103/jomfp.JOMFP_118_19. PMID: 31942130. PMCID: PMC6948024.

6. Bradley AS.[2022]. Oral Squamous Cell Carcinoma. Available at: https://www.msdmanuals.com/professional/ear,- nose,-and-throat-disorders/tumors-of-the-head-and-neck/oral-squamous-cell-carcinoma. assessed on 18/4/23.

7. Thavarool S, Muttath G, Nayanar S, Duraisamy K, Bhat P, Shringarpure K, Nayak P, Tripathy J, Thaddeus A, Philip S, Satheesan B.(2019).'Improved survival among oral cancer patients: findings from a retrospective study at a tertiary care cancer centre in rural Kerala, India'. World J Surg Onc.17. https://doi.org/10.1186/s12957-018- 1550-z.

8. Botha P, Schoonees A, Pontes C.(2018).'Mapping oral cancer research in South Africa'. SADJ.73: 384 – 394. http://dx.doi.org/10.17159/2519-0105/2018/v73no6a1.

9. Ren ZH, Hu CY, He HR, Li YJ, Lyu J. [2020].Global and regional burdens of oral cancer from 1990 to 2017: Results from the global burden of disease study. Cancer Commun (Lond). 40(2-3):81-92. doi: 10.1002/cac2.12009. Epub 2020 Feb18. PMID: 32067418; PMCID: PMC7163731.

10. Adeola H, Afrogheh A, Hille J.(2018) '.The burden of head and neck cancer in Africa: the status quo and research prospects'. S. Afr. dent. j. vol.73 n.8. http://dx.doi.org/10.17159/2519-0105/2018/v73no8a1.

11. Ndayisabye H, Ndagijimana A, Biracyaza E, Umubyeyi E.[2022].Factors Associated With Oral Cancer Adverse Outcome at the Rwanda Military Hospital, a Retrospective Cross-Sectional Study. Front. Oral. Health, https://doi.org/10.3389/froh.2022.844254.

12. Jiang, X., Wang, J., Deng, X. et al. The role of microenvironment in tumor angiogenesis. J Exp Clin Cancer Res 39, 204 (2020). https://doi.org/10.1186/s13046-020-01709-5.

13. Mercurio AM. (2019). 'VEGF/Neuropilin Signaling in Cancer Stem Cells'. Int J Mol Sci. 20(3): 490. doi: 10.3390/ijms20030490. PMID: 30678134. PMCID: PMC6387347.

14. Lizárraga-Verdugo E, Avendaño-Félix M, Bermúdez M, Ramos-Payán R, Pérez-Plasencia C, Aguilar-Medina M.(2020).'Cancer Stem Cells and Its Role in Angiogenesis and Vasculogenic Mimicry in Gastrointestinal Cancers'. Front. Oncol. https://doi.org/10.3389/fonc.2020.00413

15. Llorián-Salvador M, González-Rodríguez S.(2018).' Painful Understanding of VEGF'. Front. Pharmacol. https://doi.org/10.3389/fphar.2018.01267.

16. Abdalla A, Xiao L, Ullah M, Yu M, Ouyang C, Yang G.(2018).' Current Challenges of Cancer Anti-angiogenic Therapy and the Promise of Nanotherapeutics'. Theranostics. 8(2):533-548. Published 2018 Jan 1. doi:10.7150/thno.21674.

17. Rivera-Soto R, Damania B. [2019]. Modulation of Angiogenic Processes by the Human Gammaherpesviruses, Epstein– Barr Virus and Kaposi's Sarcoma-Associated Herpesvirus. Front. Microbiol., |https://doi.org/10.3389/fmicb.2019.01544.

18. Cesarman E, Damania B, Krown SE, Martin J, Bower M, Whitby D.[2019]. Kaposi sarcoma. Nat Rev Dis Primers. 31;5(1):9. doi: 10.1038/s41572-019-0060-9. PMID: 30705286; PMCID: PMC6685213.

19. Bishop BN, Lynch DT.[2022].Kaposi Sarcoma. StatPearls [Internet]. National Library of Medicine. Available at: https://www.ncbi.nlm.nih.gov/books/NBK534839/ assessed on 18/4/23.

20. Yang M, Xiaoqiong L,Yu S, Chungang Z, Lei Z.[2018]. VEGF-A/VEGFR-2 and FGF-2/FGFR-1 but not PDGF- BB/PDGFR- β play important roles in promoting immature and inflammatory intraplaque angiogenesis. https://doi.org/10.1371/journal.pone.0201395.

21. Costa B, Vale N. Efavirenz: History, Development and Future. Biomolecules. 2022 Dec 31;13(1):88. doi: 10.3390/biom13010088. PMID: 36671473; PMCID: PMC9855767.

22. Braz VA, Holladay LA, Barkley MD.[2010] Efavirenz binding to HIV-1 reverse transcriptase monomers and dimers. Biochemistry;49(3):601-10. doi: 10.1021/bi901579y. PMID: 20039714; PMCID: PMC2896556.

23. Hecht M, Erber S, Harrer T, Klinker H, Roth T, Parsch H, Fiebig N, Fietkau R, Distel L. (2018). 'Efavirenz Has the Highest Anti-Proliferative Effect of Non-Nucleoside Reverse Transcriptase Inhibitors against Pancreatic Cancer Cells'. PLoS One. 2015; 10(6): e0130277.

24. Abrahams MR, Joseph SB, Garrett N, Tyers L, Moeser M, Archin N, Council OD, Matten D, Zhou S, Doolabh D, Anthony C, Goonetilleke N, Karim SA, Margolis DM, Pond SK, Williamson C,Swanstrom R. (2019). 'The replication-competent HIV-1 latent reservoir is primarily established near the time of therapy initiation'. Science translational medicine. 11(513), eaaw5589. https://doi.org/10.1126/scitranslmed.aaw5589.

25. McNeil R., Penny B., Hosie M. 2017: Antiretroviral therapy and HIV-associated cancers: Antiangiogenic effect of efavirenz on chick chorioallantoic membrane. Tropical Journal of Pharmaceutical Research / Vol. 16 No. 11 (2017) /. DOI:10.4314/tjpr.v16i11.23.

26. Bárbara C, Nuno V.[2023].Efavirenz: History, Development and Future. Biomolecules, available at:file:///C:/Users/gifta/Downloads biomolecules-13-00088.pdf assessed on 18/4/23.

27. Colafigli M, Ciccullo A, Borghetti A, Fanti I, Melis F, Modica S, Uccella I, Bonadies A, Ferraresi V, Anzalone E, Pennica A, Migliano E, Rossetti B, Madeddu G, Cauda R, Cristaudo A, Di Giambenedetto S, Latini A.[2019]. Impact of Antiretroviral Therapy on the Risk of Recurrence in HIV-

1 Infected Patients with Kaposi Sarcoma: A Multicenter Cohort Experience. J Clin Med;8(12):2062. doi: 10.3390/jcm8122062. PMID: 31771172; PMCID: PMC6947508.

28. Freeman EE, Busakhala N, Regan S, Asirwa FC, Wenger M, Seth D, Moon KC, Semeere A, Maurer T, Wools-Kaloustian K, Bassett I, Martin J. [2020]. Real-world use of chemotherapy for Kaposi's sarcoma in a large community-based HIV primary care system in Kenya. BMC Cancer ;20(1):71. doi: 10.1186/s12885-019-6506- 3. PMID: 31996161; PMCID: PMC6990575.

29. Zizioli D, Ferretti S, Tiecco G, Mignani L, Monti E, Castelli F, Quiros-Roldan E, Zanella I.[2023] Comparison of Efavirenz and Doravirine Developmental Toxicity in an Embryo Animal Model. Int J Mol Sci. ;24(14):11664. doi:10.3390/ijms241411664. PMID: 37511423; PMCID: PMC10380689.

30. Spencer D, Krause R, Rossouw T, Moosa M, Browde S, Maramba E, Jankelowitz L, Mulaudzi M, Ratishikana-Moloko M, Modupe O, and Mahomed A. (2019). 'Palliative care guidelines for the management of HIV-infected people in South Africa'. Southern African journal of HIV medicine. 20(1), 1013. https://doi.org/10.4102/sajhivmed.v20i1.1013.

31. Marima R, Hull R, Dlamini Z, Penny C. [2020]. Efavirenz and Lopinavir/Ritonavir Alter Cell Cycle Regulation in Lung Cancer. Frontiers in Oncology. DOI:10.3389/fonc.2020.01693.

32. Fong C.[2022]. Efavirenz free radicals, oxidative stress and CNS toxicity in HIV infections and anti-cancer therapies: quantum mechanical study. Available at: https://hal.science/hal-03744488/document. Assessed on 16/01/24.

33. National Cancer Institute [2018]. Angiogenesis Inhibitors. Available at: https://www.cancer.gov/about-cancer/treatment/types/immunotherapy/angiogenesis-inhibitors-fact-sheet assessed on 17/4/23.

34. Zuazo-Gaztelu I, Casanovas O. [2018]. Unraveling the Role of Angiogenesis in Cancer Ecosystems. Frontiers in Oncology. DOI: 10.3389/fonc.2018.00248.

35. Szafarowski T, Sierdzinski J, Szczepanski MJ, Whiteside TL, Ludwig N, Krzeski A. [2018]. Microvessel density in head and neck squamous cell carcinoma. Eur Arch Otorhinolaryngol. 275(7):1845-1851. doi: 10.1007/s00405-018- 4996-2. Epub 2018 May 10. PMID: 29748768; PMCID: PMC5992238.

36. Dong Y, Ma G, Liu Y, Lu S, Liu L. [2020]. Prognostic Value of Microvessel Density in Head and Neck Squamous Cell Carcinoma: A Meta-Analysis. Dis Markers.2020:8842795. doi: 10.1155/2020/8842795. PMID: 33062071; PMCID: PMC7539077.

37. Lugano R, Ramachandran M, Dimberg A. [2020]. Tumor angiogenesis: causes, consequences, challenges and opportunities. Cell Mol Life Sci. 77(9):1745-1770. doi: 10.1007/s00018-019-03351-7. Epub 2019 Nov 6. PMID: 31690961; PMCID: PMC7190605.

38. ATCC [no date]. CAL 27. available at: https://www.atcc.org/products/crl-2095 . Assessed on 5/5/23.

39. BCRJ [no date]. Available at: https://bcrj.org.br/celula/cal-27/ . assessed on 4/5/23.

40. Freitas RD, Dias RB, Vidal MT, Valverde LD, Costa RG, Damasceno AK, Sales CB, da Rocha L D, dos Reis MG, Soares MB, Coletta RD, Pereira TA, Bezerra DP, Rocha CA. (2020).'Inhibition of CAL27 Oral Squamous Carcinoma Cell by Targeting Hedgehog Pathway With Vismodegib or Itraconazole'. Front. Oncol. https://doi.org/10.3389/fonc.2020.563838.

41. Allinder S., Fleischman J.[2019]. The World's Largest HIV Epidemic in Crisis: HIV in South Africa. Center for strategic and international studies. Available at: https://www.csis.org/analysis/worlds-largest-hiv-epidemic-crisis-hiv- south- africa. Assessed on 16/01/24.

42. Yarchoan R, Uldrick TS.[2018]. HIV-Associated Cancers and Related Diseases. N Engl J Med.15;378(11):1029-1041. doi: 10.1056/NEJMra1615896. PMID: 29539283; PMCID: PMC6890231.

43. Srivastava N, Shukla N, Trivedi A, Nadar S, Srivastava P.(2020). In-silico Identification of Novel Drug Target for Osteoarthritis in human using System Network Biology Approaches. DOI:10.26434/chemrxiv.12548030. available at : https://www.researchgate.net/publication/342451652 accessed on 2/6/21.

44. Pygmalion M, Ruiz L, Popovic e, Gizard j, Pascal P, Marat x, Lucet-Levannier k, Muller b, Galey j.[2010]. Skin cell protection against UVA by Sideroxyl, a new antioxidant complementary to sunscreens,Free Radical Biology and Medicine. https://doi.org/10.1016/j.freeradbiomed.2010.08.009.

45. Masuma R, Kashima S, Kurasaki M, Okuno T.[2013].

Effects of UV wavelength on cell damages caused by UV irradiation in PC12 cells,Journal of Photochemistry and Photobiology B: Biology. https://doi.org/10.1016/j.jphotobiol.2013.06.003.

46. Lazic SE, Clarke-Williams CJ, Munafò MR.(2018). What exactly is 'N' in cell culture and animal experiments? PLoS Biol 16(4): e2005282. https://doi.org/10.1371/journal.pbio.2005282.

47. Pollard DA, Pollard TD, Pollard KS.[2019]. Empowering statistical methods for cellular and molecular biologists. Mol Biol Cell. 1;30(12):1359-1368. doi: 10.1091/mbc.E15-02-0076. PMID: 31145670; PMCID: PMC6724699.

48. Lazic SE.(2016). Experimental Design for Laboratory Biologists: Maximising Information and Improving Reproducibility.Cambridge, UK: Cambridge University Press.

49. Gosselin BJ. (2021). Malignant Tumors of the Mobile Tongue. Available at: https://emedicine.medscape.com/article/847428-overview , assessed on 10/4/23.

50. Chiou P, Ohms S, Board P, Dahlstrom J, Rangasamy D, Casarotto M. [2021]. The Antiviral Drug Efavirenz in Breast Cancer Stem Cell Therapy. Cancers (Basel).13(24): 6232. doi: 10.3390/cancers13246232. PMCID: PMC8699628.PMID: 34944852.