Infectious Agents and Cancer

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A comparative analysis of somatic mutational profiles according to HIV status among women with cervical intraepithelial neoplasia 3 (CIN3): a focus on hotspots in *TP53*, *PIK3CA*, *PTEN*, and *EGFR*

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Abstract

Background Despite the success of antiretroviral therapy in HIV treatment, cervical cancer remains a leading malignancy in HIV-infected women. Additionally, co-infection by HIV and HPV further accelerates cervical cancer development. There are limited studies on the role of host somatic variations in HIV infected and HIV-negative women with cervical cancer. Therefore, this study aimed to investigate and compare host somatic genetic variation in cervical biopsies obtained from HIV infected and HIV-negative women with cervical intraepithelial neoplasia 3 to understand the genomic landscape. The distribution of HPV types was also investigated between HIV infected and HIV-negative women.

Methods The project used an age-matched case–control study utilizing archived cervical biopsies from 88 women (44 HIV infected, 44 HIV-negative) attending Groote Schuur Hospital Cancer Clinic between 2020 and 2022. HPV infection and type were confirmed using the Anyplex™ II HPV28 Detection kit. Six hotspot regions in the four commonly mutated genes (*TP53*, *PIK3CA*, *PTEN*, and *EGFR*) in cervical cancer were genotyped using PCR and Sanger Sequencing. Variant pathogenicity was assessed using SIFT, Polyphen-2, and ClinVar tools.

Results The median age was 37 years (IQR: 34–41) for HIV infected women and 35 years (IQR:32- 43) for HIV-negative women. Significantly more HIV-negative women (51% vs. 12%) reported tobacco smoking ($p < 0.0001$), menstruation irregularities (74% vs. 35%; $p = 0.005$), and contraception usage (77% vs. 59%; $p = 0.019$), when compared to their HIV-infected counterparts. Common HPV types identified were HPV16 (n = 43/88, 49%), HPV35 (n = 12/88, 14%), and HPV58 (n = 10/88, 11%). A total of 232 genetic variants were reported. HIV infected women had a significantly higher ($p = 0.0406$) burden of pathogenic variants (31%) compared to the HIV-negative (15%). The spectrum of observed mutations included stop-gain, missense, synonymous, and intronic changes. Most of the stop gain mutations in *TP53* and *PIK3CA* were reported among HIV infected women (n = 4/5), compared to HIV-negative women (n = 1/5). Damaging variants were more prevalent in women under 50 in both cohorts. We also report on rare HPV subtypes currently not included in the diagnostic HPV test kits in this cohort (HPV 82, 42, 43 and 53).

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Conclusion HIV-infection status and age appear to be risk factors for higher burden of pathogenic mutations in genes that predispose to cervical cancer. Mutation profiles in *PIK3CA and TP53* genes could be biomarkers of cervical cancer progression but more studies are needed.

Keywords CIN3, Cervical cancer, Human papillomavirus, HIV infected, HIV-negative

Introduction

Despite increasing screening and vaccination programs, cervical cancer remains a prevalent malignancy globally, especially in low and middle-income countries (LMICs) [1], with an estimated 604 127 new cases and 341 831 deaths worldwide in 2020 [1]. Cervical cancer is the fourth most diagnosed malignancy and cause of death in women globally [2]. In developing countries, cervical cancer is the second most diagnosed cancer and the cause of death of women after breast cancer [2]. In South Africa, cervical cancer is the second most diagnosed cancer in women, with an incidence rate of between 22.8 to 27 per 100 000 women, 5743 new cases, and 3027 deaths per annum [3].

Cervical cancer originates from precancerous lesions called cervical intraepithelial neoplasia (CIN), categorized into low-grade squamous intraepithelial lesion (LSIL) which is CIN1, and high-grade squamous intraepithelial lesions (HSIL) divided into CIN2 and CIN3 [4]. The main etiology of cervical cancer and its precursor CIN is infection with human papillomavirus (HPV). HPV is a common sexually transmitted infection affecting over half of sexually active individuals worldwide [5, 6]. There are over 200 HPV types, categorized as either high-risk HPV (HR-HPV) or low-risk HPV (LR-HPV) based on their cancer-inducing potential. HR-HPVs (e.g., HPV16 and HPV18), are associated with anogenital cancers, including cervical cancer, while LR-HPVs (e.g., HPV6 and HPV11), cause genital warts and low-grade cervical lesions [6]. Persistent infection with HR-HPV types causes the progression of CIN to invasive cervical cancer [7]. However, HR-HPV types alone are insufficient to drive cervical carcinogenesis [8]. Several risk factors influence the development of precancerous lesions to invasive cervical cancer. These include lifestyle, host genetics and being infected with multiple HR-HPV types [8]. These risk factors function as co-factors for cervical cancer by enhancing cancer development with HPV infection. The common risk factors for susceptibility to cervical cancer include early age of sexual debut, multiple sexual partners, tobacco smoking, long-term use of oral contraceptives, and immunosuppression [9, 10].

Human immunodeficiency virus (HIV) infection leads to immunosuppression [11]. The introduction of antiretroviral therapy (ART) has markedly changed the global epidemiology of HIV [11]. However, even in the presence of ART-controlled HIV, HIV-infected individuals still experience a high burden of premalignant and malignant lesions associated with HPV infection [12]. It has been shown that HIV infection increases the risk of incident and persistent HPV infection, rapid progression to HPV-associated lesions, and invasive cervical cancer [13]. Therefore, women infected with HPV and co-infected with HIV have a substantial risk of developing cervical cancer, estimated at six-fold compared to their HIV-negative counterparts [14]. Additionally, the morbidity of cervical cancer in HIV infected women is up to 8 times higher compared to among HIV-negative women [15, 16].

Several studies have examined the association between cervical cancer among women with HIV and HPV. However, very few have compared the host somatic differences in HIV infected and HIV-negative women. Thus, we considered it important to evaluate genes that encode proteins essential for controlling normal cell growth, proliferation, and apoptosis (i.e. tumour suppressor genes, oncogenes, and DNA repair genes), which have been implicated in cervical carcinogenesis. Mutations in these genes lead to dysregulation including aberrant gene overexpression.

Multiple genes have been described as significantly mutated genes (SMG) in cancers by The Cancer Genome Atlas (TCGA) [17]. The SMGs in cervical cancer include tumour *TP53*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*), phosphatase and tensin homolog (*PTEN*), and epidermal growth factor receptor (*EGFR*) genes. These genes are also reported as significantly mutated in numerous studies [18, 19], including the TCGA. Genetic variation within the human host also plays a significant role in cancer progression and can be a potential insight into prognostic differences between the observed varying carcinogenesis rate in HIV infected women when compared to their HIV negative counterparts. This study therefore investigated host somatic genetic variations in *TP53*, *PIK3CA*, *PTEN* and *EGFR* in cervical biopsies obtained from HIV infected and HIV-negative women with histologically confirmed CIN3 with the aim to determine potential differences in genomic landscapes and HPV infection between HIV infected and HIV-negative women.

Methods

Study design and setting and ethics considerations

This was an age-matched case–control study where cases were women infected with HIV and controls were women who were not infected with HIV. Both cases and controls had histologically confirmed cervical intraepithelial neoplasia 3 (CIN3). The study employed archived cervical biopsies obtained from women who attended the Groote Schuur Hospital Cancer Clinic in Cape Town, South Africa between 2020 and 2022. Cervical biopsies were stored in the Division of Anatomical Pathology, Department of Pathology, Faculty of Health Sciences, University of Cape Town.

Study population and data collection

Eligible study participants were women 18 years or older, with histologically confirmed CIN3, and had biopsies collected prior to treatment. Women younger than 18 years, without histologically confirmed CIN3, nor archived cervical biopsies, and had been treated with chemotherapy or radiotherapy prior to tumour resection were excluded from the study. Demographic and clinical data were collected from patient's medical records, including viral load and CD4 count.

DNA extraction and HPV typing

Deoxyribonucleic Acid (DNA) was extracted from 4 µm thick formalin-fixed paraffin embedded (FFPE) curls using the Zymo genomic DNA extraction FFPE kit (Zymo Research, California, USA), following the manufacturer's protocol. DNA concentration and purity were assessed using Nanodrop® ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, United States). Gel electrophoresis using 1% agarose gel was used to assess the integrity of DNA. HPV genotyping was performed using the Anyplex™ II HPV28 Detection kit (Seegene, Seoul, South Korea), following the manufacturer's protocol. This kit tests for 19 high-risk HPV genotypes namely HPV type 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73, 82, and 9 low-risk HPV genotypes namely HPV type 6, 11, 40, 42, 43, 44, 54, 61, 70.

Gene selection and sequencing

This study reports alterations detected in the hotspot regions of selected genes (Table 1). To further support the selection of these genes, GeneMANIA (https://genemania.org/) a useful tool in determining the interactions between genes was used. Using GeneMANIA (Fig. 1), *PIK3CA, TP53, PTEN* and *EGFR* were selected as the most altered genes in cervical carcinogenesis pathways. Based on existing literature, somatic mutations in *TP53* (exon 4), *PIK3CA* (exon 9 and 20), *PTEN* (exon 5), and *EGFR* (exon 19 and 20) in the 88 tumour samples were analyzed using PCR and Sanger sequencing.

The primers used were ordered from Inqaba Biotechnical Industries (Pretoria, South Africa) (Supplementary Table 1). Each PCR mix contained 1X Reaction Buffer (Promega Cooperation, Madison, USA), 0.4 mM deoxyribonucleoside triphosphate (dNTPs)(Kappa Biosystems, Cape Town, South Africa), 0.03U Go Taq Polymerase (Promega, USA), 1.5 mM magnesium chloride (Thermo Scientific, Waltham, USA), 0.4 µM forward and reverse primers,and the total reaction volume was made up to 25 µl using nuclease-free water (Thermo Scientific, Waltham, USA). PCR was carried on an Applied Biosystems SimpliAmp™ Thermal Cycler (Thermo Scientific, USA) and cycling conditions included an initial denaturation at 95 °C for 3 min followed by 35 cycles of denaturation at 95 °C for 30 s, annealing for 30 s at appropriate temperature and extension at 72 °C for 1 min, then final extension at 72 °C for 5 min. Sanger sequencing was performed using 5 X Big-dye Terminator v3.1 (Life Technologies, CA, USA), 1 X Big-dye Terminator buffer (Life Technologies, CA, USA) and 1 µM of either forward or reverse primer. The sequencing reaction was performed in an Applied Biosystems SimpliAmp™ Thermal Cycler under the following conditions: initial denaturation at 98 °C for 5 min, followed by 25 cycles of denaturation at 96 °C for 30 s, annealing at 50 °C for 15 s, extension at

Table 1 Literature guided selection of tumour hotspots

Gene	Exon	Location	Function
TP53	4	DNA binding domain	Bind specific DNA sequences and integrate many cellular signals via protein–protein interactions to initiate the required cellular response
PIK3CA	9	Helical domain	It plays a role in unwinding the DNA double helix during DNA replication and repair
PIK3CA	20	Kinase domain	Responsible for the phosphorylation of phosphatidylinositol-4,5-bisphosphate (PIP2) into phosphatidylinositol-3,4,5-trisphosphate (PIP3)
PTEN	5	Phosphatase domain	It plays a role in *PTEN*'s enzymatic activity as a phosphatase which metabolizes PIP3 to PIP2 in PI3K/AKT/mTOR pathway
EGFR	19 and 20	Tyrosine domain	Plays a role in activating signaling pathways promoting proliferation

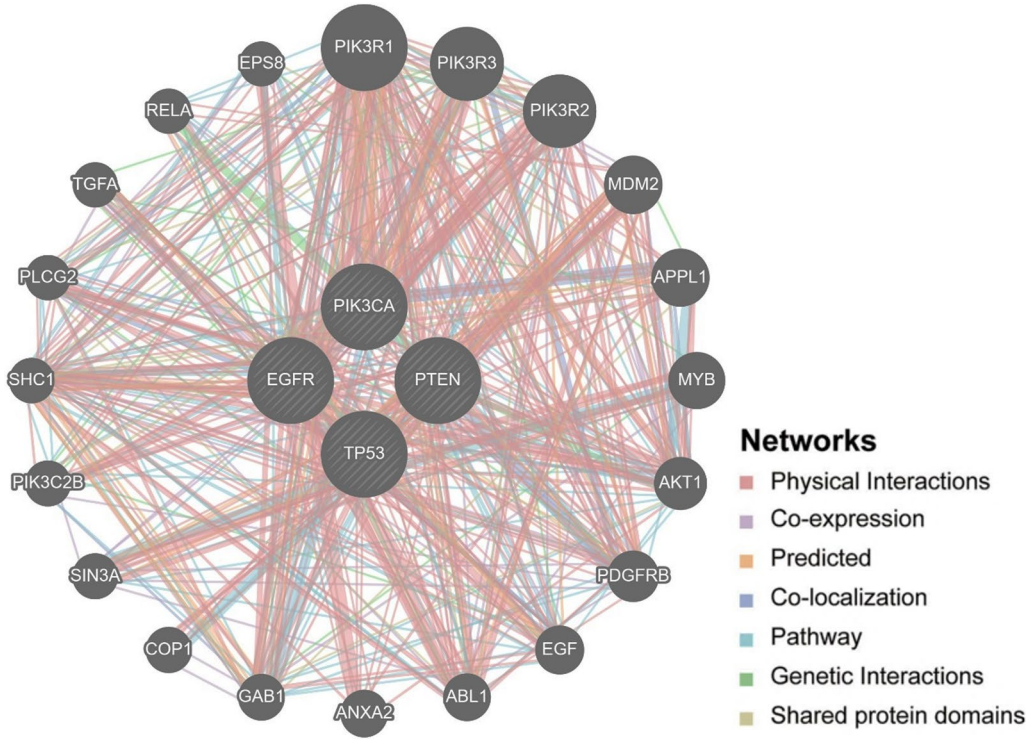


Fig. 1 A GeneMANIA gene–gene interaction network of the hotspot genes (*TP53, PIK3CA, PTEN* and *EGFR*). Different colors of the network nodes indicate different interactions which are physical interaction, co-expression, predicted, co-localization, common pathway, genetic interaction and shared protein domains

60 °C for 4 min, then final extension at 60 °C for 4 min. Capillary electrophoresis was done on the 3130xl DNA Analyzer (Applied Biosystems, CA, USA).

Data analysis and pathogenicity testing

Data from the somatic gene sequencing using Sanger sequencing was analyzed using the DNASTAR Lasergene SeqMan Pro alignment tool (v16.0.0), Blastn and dbSNP Pathogenicity testing for missense variants was done using Sorting Intolerant from Tolerant (SIFT) (https://sift.bii.astar.edu.sg/www/Extended_SIFT_chr_coords_submit.html), Polymorphism Phenotyping v2 (PolyPhen2) (http://genetics.bwh.harvard.edu/pph2/bgi.shtml), and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/).

Statistical analysis was performed using the STATA ® SE-64 software program, version 15.0. Continuous data was reported as mean ± standard deviation (SD) or median ± interquartile range (IQR) depending on whether the data is normally distributed or not normally distributed. The Shapiro-Wilks test was used to test for the normality of the data. Chi-square test was used to compare the differences in sociodemographic and clinical data between HIV infected and HIV-negative women. The p value < 0.05 was considered statistically significant.

Results

Clinical and demographic characteristics

Eighty-eight samples (n = 88) were retrieved for this study consisting of tumours derived from 44 HIV infected women (case group) and 44 HIV-negative women (control group). Clinical and demographic characteristics were available for 82 women (Table 2). Clinical files for the remaining 6 participants could not be located hence, were excluded from the clinical and demographic analyses. HIV-negative women showed higher rates for smoking history (20 HIV-negative versus 5 HIV infected; $p < 0.0001$), menstruation irregularity (26 HIV-negative versus 15 HIV infected; $p = 0.005$), and use of contraception (30 HIV-negative versus 24 HIV infected; $p = 0.019$) when compared to their HIV infected counterparts. The HIV infected women had controlled HIV viral load with 44% with non-detectable viral load, 37% having < 200 copies/ml, and only 12% (n = 5) had viral loads greater than 1000 copies/ml. Twenty-three percent (n = 10) had CD4 count greater than 500 cells/mm^3, and 8 individuals had CD4 < 200 cells/mm^3. These findings show that the study participants infected with HIV had satisfactory virological and immunological response to treatment.

Table 2 Demographic and clinical characteristics of HIV infected and HIV-negative women with CIN3

Characteristic	HIV infected N = 43	HIV-negative N = 39	*p* value
Median age (IQR)	37 (34–41)	35 (32–43)	0.382
Ethnicity			
African	6 (14%)	5 (13%)	0.131
Mixed Ancestry	3 (7%)	2 (5%)	
Unknown	34 (79%)	32 (82%)	–
Comorbidities			
Yes	16 (37%)	18 (46%)	0.45
No	13 (30%)	14 (36%)	
Unknown	14 (33%)	7 (18%)	–
Family history of cancer			
Yes	1 (2%)	7 (18%)	0.715
No	1 (2%)	4 (10%)	
Unknown	41 (98%)	28 (72%)	–
Smoking history			
Yes	5 (12%)	20 (51%)	< 0.0001*
No	33 (52%)	19 (49%)	
Unknown	5 (12%)	0	–
Alcohol history			
Yes	6 (14%)	6 (15%)	0.784
No	11 (26%)	9 (23%)	
Unknown	26 (60%)	24 (62%)	–
BMI			
Average 20–24	11 (26%)	5 (13%)	0.243
Above average 25–29	1 (2%)	2 (5%)	
Unknown	31 (72%)	32 (82%)	–
Menarche			
10–15 years	6 (14%)	12 (31%)	0.119
16–20 years	4 (9%)	1 (2%)	
> 20 years	0	1 (2%)	
Unknown	33 (77%)	25 (65%)	–
Menstruation irregularity			
Monthly	15 (35%)	3 (8%)	0.005*
Skipped for 1–3 months	1 (2%)	5 (13%)	
Amenorrhea	8 (19%)	21 (53%)	
Menopause	6 (14%)	3 (8%)	
Unknown	13 (30%)	7 (18%)	–
Type of contraceptive			
Oral	0	1 (2%)	0.019*
Implant	2 (5%)	5 (13%)	
Injectable	18 (42%)	19 (49%)	
Tubal ligation	4 (9%)	5 (13%)	
None	15 (35%)	2 (5%)	
Unknown	4 (9%)	7 (18%)	–
Duration of contraceptive use			
1–5 years	2 (5%)	1 (2%)	0.902
6–10 years	2 (5%)	1 (2%)	
> 10 years	3 (7%)	3 (8%)	
Unknown	36 (83%)	35 (88%)	–
Parity			
Primiparity	5 (12%)	2 (5%)	0.179
Multiparity	38 (88%)	37 (95%)	
Age at first conception			
15–20 years	6 (14%)	5 (13%)	0.186
21–25 years	4 (9%)	4 (10%)	
26–30 years	0	2 (5%)	
Unknown	33 (77%)	28 (72%)	–
Viral load (copies/ml), n (%)			
Less than detectable	19 (44%)		–
< 200	16 (37%)		
200–999	3 (7%)		
> 1000	5 (12%)		
CD4 count (cells/mm^3), n (%)			
< 200	8 (19%)		–
200–500	12 (28%)		
> 500	10 (23%)		
Unknown	13 (30%)		

SD Standard deviation, *IQR* interquartile range, *BMI* body mass index

* Represents statistical significance

Overall prevalence of HPV infection

The 88 participants with CIN3 were screened for HPV and 87 were successfully genotyped with one sample failing. We report on 11 HR-HPV types (16, 31, 33, 35, 39, 45, 51, 52, 53, 58 and 82) and 4 LR-HPV types (6, 11, 42 and 43) (Fig. 2A). HPV16 was the most prevalent type (n = 43/87, 49%), followed by HPV35 (n = 12/87, 14%) and HPV58 (n = 10/87, 11%). HIV infected women exhibited significantly higher rates of HPV co-infections compared to HIV-negative women ($p = 0.002$) (Fig. 2B). HR-HPV18 was not detected in this study cohort.

Overall variants identified in a cohort

Genotyping identified four types of mutations: stop-gain, missense, synonymous and intronic. A total of 232 variants were identified (Fig. 3). Overall, HIV infected women had less mutation burden (n = 105) compared to HIV-negative women (n = 127), however, they had more damaging mutations, which includes both missense and stop gain variants. Stop gain variants were limited to *TP53* and *PIK3CA with* three identified in *TP53* and two in *PIK3CA*. Four (80%) of the five stop-gain variants were identified in HIV infected women. *TP53* had a higher frequency of missense and stop-gain

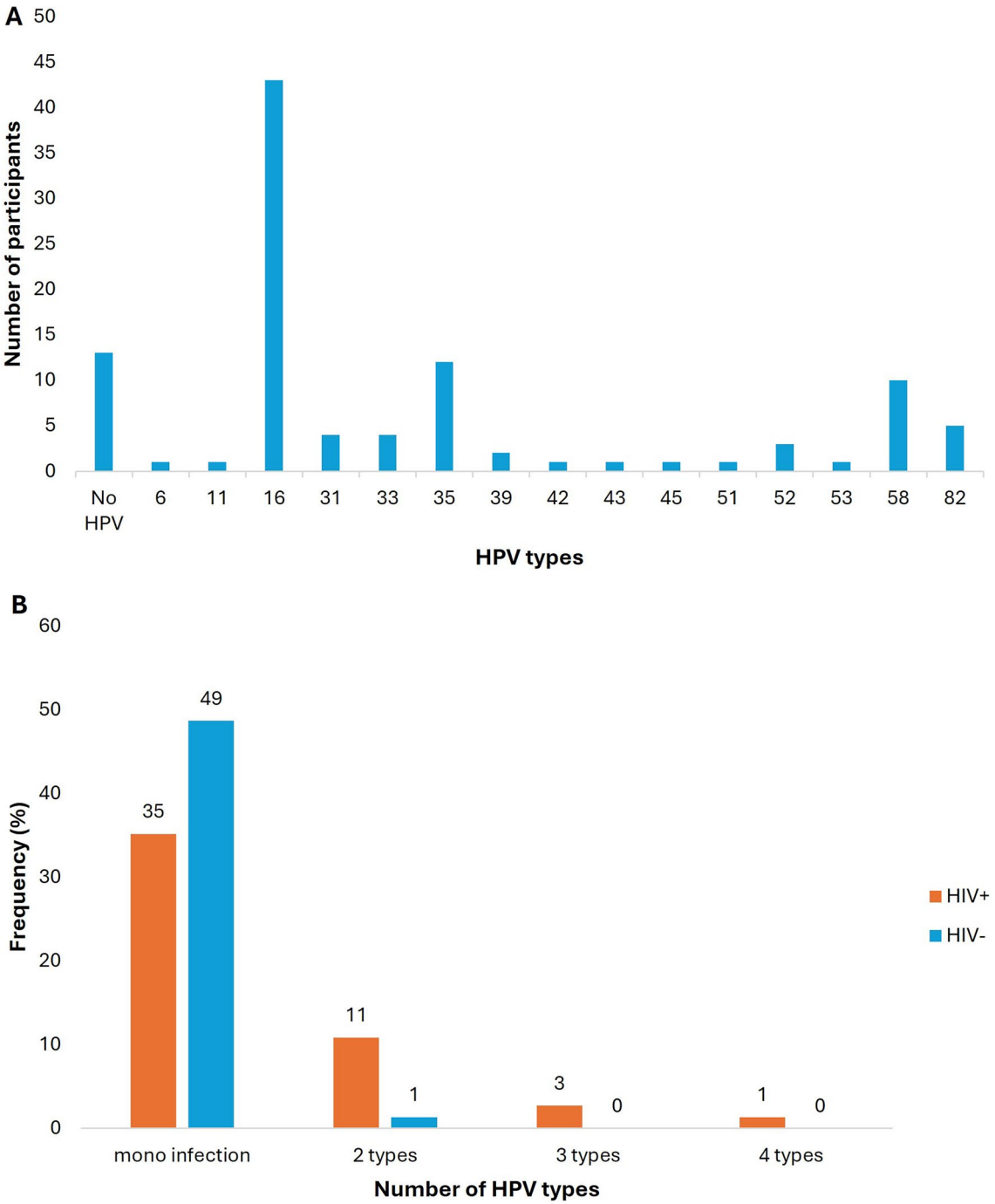
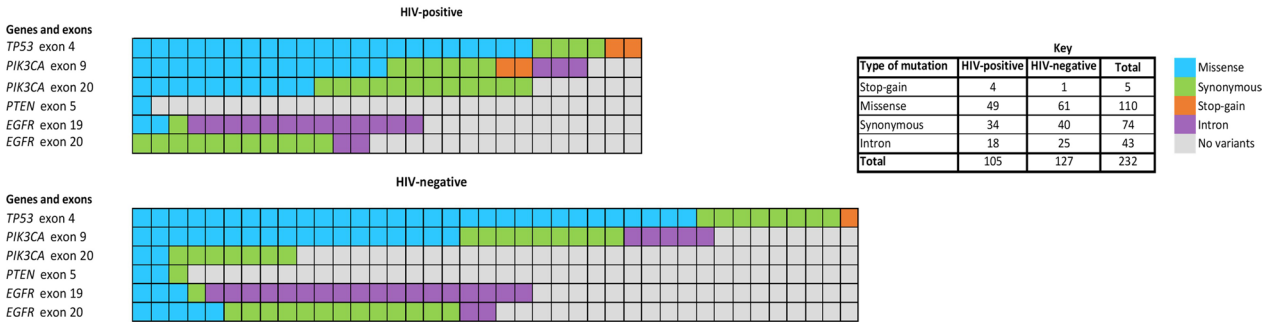


Fig. 2 HPV genotypes. **A** Distribution of HPV genotypes identified in the study cohort. **B** Distribution of HPV genotypes in women with confirmed CIN3 stratified by HIV status, showing mono-infections and co-infections. The co-infections were predominantly limited to HIV infected women

mutations than all other genes (Fig. 3). *PIK3CA* presented with all four types of mutations, most of which were missense mutations. *PTEN* had no stop-gain nor intronic mutations, while *EGFR* had no stop-gain



Type of mutation	HIV-positive	HIV-negative	Total
Stop-gain	4	1	5
Missense	49	61	110
Synonymous	34	40	74
Intron	18	25	43
Total	105	127	232

Fig. 3 Distribution of variants according to HIV status among the women. Each oncoprint box represents a mutation, and different colors represent different mutation types, while each row represents a gene and exon. The key shows the total number of variants identified for HIV infected and HIV-negative participants for each mutation type and the description of different colors in the oncoprint

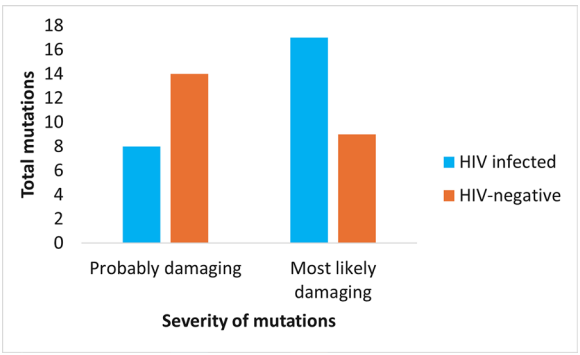


Fig. 4 Overall number of pathogenic variants. A total of 48 pathogenic variants were identified in the cohort. The probably damaging mutations were reported as pathogenic on one pathogenicity testing tool, while most likely damaging variants were reported pathogenic in two or more testing tools. Mostly likely damaging variants were more prone on the HIV infected women

mutations but exhibited a higher frequency of intronic mutations.

Genomic landscapes of pathogenic variants

Missense variants were further analyzed to determine whether the amino acid substitution was damaging to the protein function. Sorting Intolerant from Tolerant (SIFT), Polymorphism Phenotyping v2 (Polyphen-2), and ClinVar tools were used to test for the pathogenicity of the missense variants. Variants were categorized based on their reported damaging status in SIFT or PolyPhen2 or ClinVar. Those reported as damaging in one tool were classified as probably damaging, while those reported by at least two tools were classified with high confidence as most likely damaging. Variants not reported as damaging in any tool were classified as unlikely damaging.

The pathogenicity analysis results, including stop-gain variants, are presented in (Supplementary Tables 2 and 3). After pathogenicity testing, 48 variants were identified as pathogenic. HIV-negative women had 25 variants, while HIV-negative women had 23 variants, ($p = 0.4321$). However, a higher mutation burden of stop-gain variants was identified in HIV infected women. Additionally, HIV

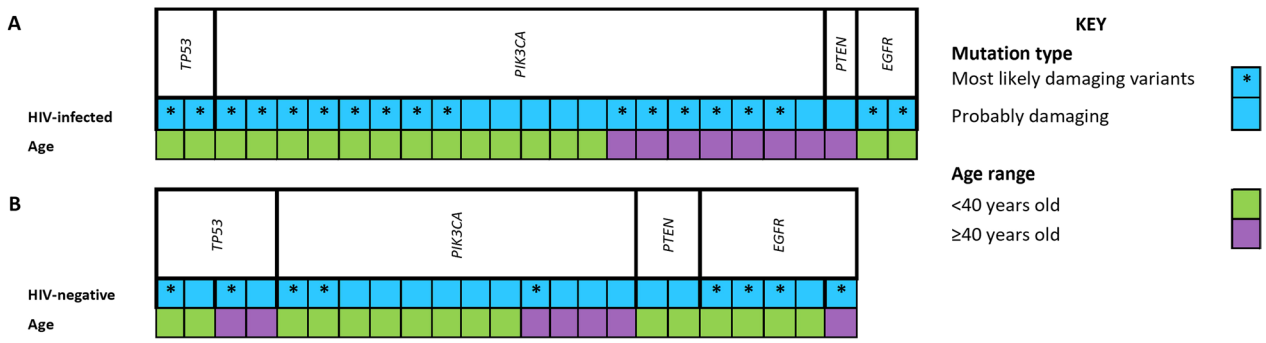


Fig. 5 Overall number of pathogenic variants per age group (< 40 years old and ≥ 40 years old) per HIV status. Showing **A** HIV infected, **B** HIV-negative. The boxes with asterisks represent the most likely damaging variants, while those without asterisks are probably damaging

infected women (n = 17) had more most likely damaging variants compared to HIV-negative women (n = 9) ($p = 0.0406$) (Fig. 4). Three genes, T*P53*, *PIK3CA* and *EGFR* presented with damaging variants. Most likely damaging variants were most common in *PIK3CA* among HIV infected women when compared to HIV-negative women ($p = 0.0071$).

Genomic landscapes of pathogenic variants versus age

Most-likely damaging variants were further stratified based on HIV status and age (Fig. 5). Age was divided into < 40 years old and ≥ 40 years old. Four stop-gain variants were identified in HIV infected women, and three of those were among women younger than 40 years, while for HIV-negative women one stop-gain was identified also in a woman younger than 40 years.

Discussion

The interaction of HIV infection and HPV on host somatic genetic variation which leads to CIN3 remains to be fully elucidated. The median age observed in this study cohort was 36 (IQR: 33–43), which is lower than the median age reported in other studies conducted in women with cervical cancer. A study by Taku and colleagues [20], reported a median age of 40 (IQR: 33–48). While another study by Kuguyo and colleagues [21], reported a median age of 51 (IQR: 42–62). Other studies have shown that participants with cervical cancer tend to be relatively young, with the median age at diagnosis of 47 years; however, almost 50% of cases diagnosed in this study were under the age of 35 years [22]. This means that in our cohort, there seems to be an earlier onset of cervical cancer whose molecular drivers need to be decoded and understood.

There are several factors that influence susceptibility to cervical cancer. Here we report significant differences between HIV infected and HIV-negative women in smoking history ($p < 0.0001$), menstruation irregularity ($p = 0.005$), and use of contraception ($p = 0.019$). Smoking history is a risk factor for various cancers, including cervical cancer [23]. Smoking has been independently shown to accelerate the risk of developing cervical cancer, and individuals who smoke are said to have four times higher risk of developing cervical cancer compared to nonsmokers, even after adjusting for other risk factors such as sexual behavior and history of infections [24]. These findings suggest that smoking drives cervical cancer by impairing the immune system and damaging the cervical epithelium through increased modification of DNA in the cervical epithelium [25].

In this study, we report of more smokers among HIV-negative women compared to their HIV infected counterparts, an observation probably driven by adjusted behavior among the HIV infected group. Among HIV-negative women irregular menstruation patterns were pronounced as was use of contraception. Irregular menstruation and the use of contraceptives have been associated with the development of cervical cancer [26]. This may suggest that contraceptives affect hormonal imbalances, which may lead to irregular menstruation, thereby promoting the development of aberrant cervical cells further increasing [27]. In other studies, women using hormonal contraceptives have been reported to have an increased chance of engaging in unprotected sex, placing them at a higher risk of HPV infection, a major etiology of cervical cancer [28]. In the current study, a higher incidence of multiple HPV infections was observed among HIV infected women using contraception. However, no instances of multiple HPV infections were found in HIV-negative women using contraceptives.

Cervical cancer is considered an AIDS-defining illness [29], indicating an increased risk among HIV infected women compared to their HIV-negative counterparts. A study by Bosch and colleagues [30], revealed a two to 22-fold increased risk of cervical cancer in HIV infected women compared to HIV-negative women. Similarly, Stelzle and co-workers [14] reported a six-fold increase in cervical cancer in HIV infected women compared to HIV-negative women. This increased risk is due to factors such as recurring oncogenic HPV infection, higher viral loads and decreased CD4 cell counts [31]. A higher viral load in HIV infected women is associated with increased risk of cervical dysplasia and progression to invasive cervical cancer [32], which is a result of immune suppression that leads to failure to fight off AIDS-defining illness or opportunistic diseases. Thus, it is important to monitor and manage viral load in HIV infected women to facilitate early detection and prevention of cervical cancer. In this study there was insufficient data to categorize HIV-infected individuals based on viral load (< 200 HIV copies/ml) and CD4 count (> 200 cells/mm^3) due to a small sample size and inadequate medical history of the CD4 count. Out of 44 HIV infected individuals, 14 of them did not have the CD4 count data. Therefore, categorization by both viral load and CD4 count was not possible. However, each component was looked at independently. Out of 44 HIV-infected participants 36 had viral load < 200 HIV copies/ml, and eight had viral load > 200 HIV copies/ml. Of the eight participants that had viral load > 200 HIV copies/ml, one patient had one pathogenic variant in *EGFR* gene. A lower CD4 count has been linked with cervical cancer in women living with HIV [33]. Konopnicki and colleagues [34], showed that a CD4 count higher than 500 cells/μl for 18 months among HIV infected women were significantly associated with reduced risk of HPV infection, which is a driver of

cervical cancer development. In this study 30 participants had CD4 count information, and eight had CD4 < 200 cells/mm^3, while 22 had CD4 > 200 cells/mm^3. Of the eight participants that had that CD4 < 200 cells/mm^3, one patient had one pathogenic variant in *EGFR* gene. These findings suggest that identified pathogenic variants might not necessarily be linked to viral load or CD4 count, as only one pathogenic variant was found in one individual in the immunocompromised cohort.

Distribution of HPV types

This study identified prevalence and distribution of HR-HPV and LR-HPV types among HIV infected and HIV-negative women with CIN3. Seventy-four (84.09%) of women enrolled in this study were positive for HPV DNA. Thirteen (14.77%) of the women were negative for HPV DNA and one sample (1.14%) failed HPV genotyping. The women that did not have HPV DNA could be due to the choice of kit used which only targeted 28 HPV types. HPV16 was the most prevalent in the study cohort, which aligns with the previous study by Stoler and colleagues [35]. The reported high prevalence of both HPV 35 and 58 is similar to observations earlier among South African participants with CIN3 [36]. We also report a significantly higher rate of HPV-coinfection in HIV infected women compared to HIV-negative women (p value = 0.002), further confirming the observations by Mcharo and colleagues [37], who reported multiple HPV infections in HIV infected women compared to HIV-negative women (p = 0.0006). These findings suggest that HIV infected women may be more susceptible to multiple strains of HPV due to the compromised immunity [38, 39]. Thus, the distribution of HPV subtypes appears to be affected by local demographics, which is important in healthcare decision-making, particularly, in the selection of HPV vaccines. In addition, we report on four HPV subtypes HPV 82, 42, 43 and 53 that are ordinarily not detected using commercial HPV DNA diagnostic test kits such as BD Onclarity™, Abbot Alinity™, and Roche cobas ®. All four HPV types (82, 42, 43 and 53) were found in multiple infections, while HPV82 was also reported as a mono-infection. Our study therefore implores wider HPV screening that covers more HPV subtypes, for proper stratification of participants and management.

Genomic landscapes

The main etiological factor for cervical cancer is HPV [40]. Additionally, oncogenic mutations can also trigger neoplastic transformation, leading to abnormal protein or changing the protein's expression level coded by the mutated gene [19]. In this study genomic profiles of four frequently mutated genes, *TP53*, *PIK3CA*, *PTEN,* and *EGFR* in cervical cancer were evaluated, comparing results between HIV infected and HIV-negative women with CIN3. Of the 232 mutations found in the cohort, 38% were found in *PIK3CA*, 31% in *EGFR*, 29% in *TP53* and 2% in *PTEN*. Similarly, a study by Sharmin and coworkers, found a high frequency of mutation in *EGFR* and *PIK3CA*, respectively [41]. However, a study by Wright and colleagues, found a lower *EGFR* mutation frequency compared to the current study (3.8% versus 31%), and a lower mutation frequency for *PIK3CA* compared to our study (31.3% versus 38%) [42]. *PIK3CA* frequency of mutations in the current study was also higher than that identified in a study by Spaans and colleagues [43], (38% versus 20%). *TP53* also had a higher frequency of mutations when compared to a study by Wang and colleagues, (29% versus 12%), while *PTEN* had a lower frequency of mutations in this study (2% versus 16%) [44].

When looking at the frequencies of mutations stratified by HIV status, *PIK3CA* mutations were more frequent in HIV infected women (53% compared to 47% HIV-negative). This finding contradicts a previous study that identified a higher proportion of *PIK3CA* mutations in HIV-negative women compared to HIV infected women (45% compared to 29% HIV infected), resulting in a 1.3 times higher expression of *PIK3CA* in HIV-negative women [45]. Conversely, higher mutation frequencies for *TP53*, *EGFR*, and *PTEN* were observed in HIV-negative women (*TP53*: 41% versus. 59%, *EGFR*: 41% versus. 59%, *PTEN*: 20% versus. 80%), respectively. It is important to compare the somatic profiles of HIV infected and HIV-negative women with CIN3 to better understand how cancer progresses between these two groups. Currently, there is limited research in this area.

Genomic landscapes of Pathogenic variants

HIV infected women had a lower mutation burden (n = 105), compared to their HIV-negative counterparts (n = 127), mutation burden refers to the total number of genetic mutations present in a tumour, regardless of whether such mutations have detrimental effects [46]. A more nuanced approach considers the burden of damaging mutations. Thus, the identified missense variants were tested for pathogenicity using SIFT, Polyphen-2 and ClinVar.

A total of 48 pathogenic variants in the entire cohort (Sup. Table 2 and 3), were reported to be pathogenic There were significantly more (31% versus 15%) likely damaging mutations among the HIV infected women than among the HIV-negative women, respectively (p = 0.0406). The higher burden of most-likely damaging variants in HIV infected women potentially indicate increased susceptibility to CIN3 and cervical cancer development compared to HIV-negative women. This is supported by a study by Mpata and Nkosi that reported

HIV infected women being 2–10 times more likely to develop precancerous lesions [47]. These pathogenic mutations may be drivers of carcinogenesis through alteration functions of the proteins concerned.

Overall, the most likely damaging variants were significantly more prevalent in *PIK3CA* among HIV infected women (n = 13) compared to HIV-negative (n = 3) ($p = 0.0071$). *PIK3CA* is a significantly mutated gene in cervical cancer reported in multiple studies, with a frequency of 5.7–35.7% [48]. The majority of the *PIK3CA* most likely damaging variants were found on exon 20 which is a kinase domain responsible for the phosphorylation of phosphatidylinositol-4,5-bisphosphate (PIP2) into phosphatidylinositol-3,4,5-trisphosphate (PIP3) [49]. The mutations found in this region can result in neoplastic transformation, which results in cancer progression by altering the normal activation of PI3K/AKT/mTOR pathway [50]. The increase in *PIK3CA* kinase activity results in the overactivation of PI3K/AKT/mTOR signaling pathway. The overexpression of mutant *PIK3CA* proteins is believed to result in tumour progression and resistance to standard therapy [51, 52]. Thus, understanding the function and mutations of *PIK3CA* is important to determine its effectiveness as a target of molecular targeted therapy or as a predictive biomarker in both gene therapy and radiation therapy [53]. *PTEN* and *TP53* are among the genes that are significantly mutated in cervical cancer, and they are either mutated or functionally inactive in cervical cancer [54, 55]. *PTEN* did not have any most likely damaging variants for HIV infected and HIV-negative women. There was no significant difference in the number of *TP53* most likely damaging variants between HIV infected and HIV-negative women. Abnormal activation of *EGFR* (mutations/amplification/overexpression) has been reported in different human cancers, including cervical cancer [56]. In the present study, *EGFR* had most likely damaging variants present in both HIV infected and HIV-negative women; however, there was no statistical significance. Overall, in the current study, we report a higher number of *PIK3CA* pathogenic mutations in CIN3 compared to other sequenced genes (*TP53, PTEN* and *EGFR*).

Pathogenic variants were mostly identified in women younger than 40 years old (Fig. 5). The reason for more pathogenic variants in younger women may include changes in sexual behavior and the burden of associated sexually transmitted infections, though the exact causes remain unclear [57, 58]. All the stop-gain variants were identified in a younger cohort of women for both HIV infected and HIV-negative women. This study cohort was virally suppressed and have good CD4 count showing that the progression to CIN3 at such a young age of virtually immunocompetent HIV infected women in this population needs to be further investigated. Something is driving the rapid progression that has not yet been identified. More multidisciplinary and concerted efforts are required.

Some of the limitations of this study were that it focused on a small set of genes and that our genetic characterization did not cover each gene from start to end but focused on hotspot regions. Future studies should therefore explore a broader genomic landscape between HIV infected and HIV-negative women and seek to cover entire gene regions. Differential expression studies are necessary for the understanding of the impact of the pathogenic variants on the protein level.

Conclusion

In this study, we were particularly interested in the hotspot regions of four significantly mutated genes in cervical cancer because these regions exhibit a higher frequency of mutations than other regions within the target genes. The results indicate that HIV infected women have a higher number of pathogenic variants compared to HIV-negative women. These variants include a higher mutation burden of stop-gain variants in HIV infected women. These preliminary results indicate that HIV infection potentially has an impact on the genetic profiles, resulting in increased genetic alterations that may be the cause of rapid progression to invasive cervical cancer in these women. Concerning HIV status, HPV and host genetics, our study highlights possible interaction between HIV and HPV on the occurrence of CIN3 in younger women and postulates host genetics (mutation burden) as a possible enabler. Understanding this interaction helps come up with molecular-based interventions that may enhance improved treatment and management of cervical cancer in HIV infected women.

Abbreviations

ART	Antiretroviral therapy
HIV	Human immunodeficiency virus
AIDS	Acquired immunodeficiency syndrome
HPV	Human papillomavirus
LMICs	Low-income and middle-income countries
CIN	Cervical intraepithelial neoplasia
LSIL	Low-grade squamous intraepithelial lesion
HSIL	High-grade squamous intraepithelial lesion
HR-HPV	High-risk HPV
LR-HPV	Low-risk HPV
SMG	Significantly mutated genes
TCGA	The cancer genome atlas
TP53	Tumour protein 53
PIK3CA	Phosphatidylinositol-4,5- bisphosphate 3-kinase, catalytic subunit alpha
RBD	Ras Binding domain
PI3K/Akt/mTOR	Phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin
PIP2	Phosphatidylinositol-4,5-bis-phosphate
PIP3	Phosphatidylinositol-3,4,5-triphosphate
PTEN	Phosphatase and tensin homolog
EGFR	Epidermal growth factor receptor

DNA	Deoxyribonucleic acid
HREC	Human research ethics committee
FFPE	Formalin-fixed paraffin-embedded
PCR	Polymerase chain reaction
IARC	International agency research on cancer
FastAP	FastAp thermosensitive alkaline phosphatase
ExoI	Exonuclease I
dNTP	Deoxynucleotide triphosphate
EDTA	Ethylenediaminetetraacetic acid
SIFT	Sorting intolerant from tolerant
PolyPhen2	Polymorphism phenotyping v2
SD	Standard deviation
IQR	Interquartile range

Supplementary Information

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Additional file 1.

Additional file 2.

Additional file 3.

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Author contributions
C.D., and N.S., conceptulaised the project, N.M., led the project experimentation, wrote the first draft. N.S., H.-T.W., C.D., and R.N., supervised the lead author (N.M.), commented on the draft manuscript. All authors reviewed the manuscript.

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Data availability
The supplementary files contain most of the data generated and analyzed during the current study. Other details on data for this study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Ethical approval was sought from the Human Research Ethics Committee (HREC) of the Faculty of Health Sciences, University of Cape Town (HREC Ref:560/2022).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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