





Distinct Pattern of *TP53* Mutations in Human Immunodeficiency Virus–Related Head and Neck Squamous Cell Carcinoma

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BACKGROUND: Human immunodeficiency virus–infected individuals (HIVIs) have a higher incidence of head and neck squamous cell carcinoma (HNSCC), and clinical and histopathological differences have been observed in their tumors in comparison with those of HNSCC patients without a human immunodeficiency virus (HIV) infection. The reasons for these differences are not clear, and molecular differences between HIV-related HNSCC and non-HIV-related HNSCC may exist. This study compared the mutational patterns of HIV-related HNSCC and non-HIV-related HNSCC. **METHODS:** The DNA of 20 samples of HIV-related HNSCCs and 32 samples of non-HIV-related HNSCCs was sequenced. DNA libraries covering exons of 18 genes frequently mutated in HNSCC (*AJUBA*, *CASP8*, *CCND1*, *CDKN2A*, *EGFR*, *FAT1*, *FBXW7*, *HLA-A*, *HRAS*, *KEAP1*, *NFE2L2*, *NOTCH1*, *NOTCH2*, *NSD1*, *PIK3CA*, *TGFBR2*, *TP53*, and *TP63*) were prepared and sequenced on an Ion Personal Genome Machine sequencer. DNA sequencing data were analyzed with Ion Reporter software. The human papillomavirus (HPV) status of the tumor samples was assessed with in situ hybridization, the MassARRAY HPV multiplex polymerase chain reaction assay, and p16 immunostaining. Mutation calls were compared among the studied groups. **RESULTS:** HIV-related HNSCC revealed a distinct pattern of mutations in comparison with non-HIV-related HNSCC. *TP53* mutation frequencies were significantly lower in HIV-related HNSCC. Mutations in HIV+ patients tended to be TpC>T nucleotide changes for all mutated genes but especially for *TP53*. **CONCLUSIONS:** HNSCC in HIVIs presents a distinct pattern of genetic mutations, particularly in the *TP53* gene. HIV-related HNSCC may have a distinct biology, and an effect of the HIV virus on the pathogenesis of these tumors should not be ruled out. *Cancer* 2018;124:84–94. © 2017 American Cancer Society.

KEYWORDS: head and neck cancer, human immunodeficiency virus (HIV), human papillomavirus (HPV), mutation, *TP53* gene.

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INTRODUCTION

Human immunodeficiency virus–infected individuals (HIVIs) have a higher cancer incidence than the general population. Kaposi sarcoma and non-Hodgkin lymphoma are the most common neoplasms among HIVIs and are AIDS-defining malignancies. However, since the advent of antiretroviral therapy, the incidence of other malignant tumors (non–AIDS-defining cancer) has increased, and they have become important causes of death for these patients.^{1–4}

Three factors are considered important for the elevated cancer incidence among HIVIs: high smoking rates, immunosuppression, and increased susceptibility to infection by oncoviruses. Smoking is the main preventable cause of cancer but is a common habit among HIVIs. The prevalence of tobacco smoke exposure is 2 times higher in this group versus the overall population.^{5,6} Immunosuppression is the main characteristic of human immunodeficiency virus (HIV) infection, and it is considered to be an important cause of cancer development. Organ transplant patients on immunosuppressive regimens demonstrate an increased incidence of malignant tumors in comparison with the general population. Their cancer incidence is similar to the frequency observed among HIVIs, and this suggests that immunodeficiency plays an important role in HIVI-related carcinogenesis.¹ An important common effect of tobacco abuse and immunosuppression is an increased susceptibility to infection by oncoviruses with the subsequent development of virus-related carcinogenesis.^{7–10} Virus-related tumors are almost 10 times more frequent in HIVIs versus the general US population.¹¹ Human herpesviruses and human papillomaviruses (HPVs) are responsible for the majority of these cases, and they lead to the development of not only sarcomas and lymphomas but also squamous cell carcinomas in the anogenital and head and neck regions.¹¹ Although human herpesviruses are related to AIDS-defining malignancies, HPVs are the major oncoviruses linked to non–AIDS-defining cancers.⁷

All these elements are also recognized as important risk factors for head and neck squamous cell carcinoma (HNSCC) development and may explain the higher incidence of these tumors among HIVIs.^{1,2,4,12–14} HNSCC is diagnosed at an earlier age and at a more advanced stage in HIVIs,¹⁵ and these tumors tend to be more aggressive¹⁶ and related to worse survival rates when they express high levels of TP53 in comparison with non-HIVIs.¹⁷ The efficacy of current treatment approaches to HIVI-related HNSCC is still a matter of debate.¹⁸ Radiotherapy treatment has been found to be less effective for the control of tumor relapse and related to worse overall survival and more treatment-related toxicity in HIVIs.¹⁸

Whether these differences in presentation and prognosis are related to the systemic effects of HIV-mediated immunosuppression or by particular biological characteristics of the primary tumor is still unclear.

Histopathological findings indicate that HIV-related HNSCC has unique features such as the enrichment of multinucleated giant tumor cells and the expression of HIV-related protein in some tumor cells.¹⁹ These observations indicate unique pathological processes that are associated with these tumors. To better understand the behavior of HIV-related HNSCC and possibly develop personalized treatment approaches, it is important to determine whether this represents a distinct molecular entity.

Recently, integrated genomic studies in HNSCC have dramatically expanded our knowledge about the pathogenesis, progression, and treatment of this tumor type. For instance, these studies have revealed the genes and pathways most frequently affected in HNSCC and have identified HPV-related HNSCC as a distinct molecular entity.^{20–23} In this way, we believe that a genomic analysis of HIV-related HNSCC might reveal whether this group of tumors has a distinct pattern of DNA alterations that could indicate differences in their pathogenesis and progression. To accomplish this, we compared the pattern of mutations between HIV-related HNSCC and non–HIV-related HNSCC by sequencing a panel of genes frequently mutated in head and neck cancer.

MATERIALS AND METHODS

Patient Selection

The cohort of patients used in this study was obtained from the Head and Neck Cancer Specialized Program of Research Excellence Human Immunodeficiency Virus Supplement Consortium. IRB approval or exemption to share deidentified data with the study data center was obtained from sample collection sites. A detailed description of the patient recruitment, sample collection, and clinicopathologic data collection has been provided in a previous publication.¹⁷ Briefly, formalin-fixed, paraffin-embedded (FFPE) tissue from patients diagnosed with HNSCC and an HIV infection were retrieved after a retrospective review of medical records. HNSCC patients not infected by HIV were also retrieved as an age-, subsite-, and stage-matched control group. DNA was extracted from the FFPE tissues, and only samples with sufficient DNA yields for sequencing were included in this study.

HPV Testing

For the HPV status classification, we considered p16 expression with immunohistochemistry and HPV

detection with in situ hybridization (ISH) and the MassARRAY multiplex polymerase chain reaction (PCR) assay. A detailed description of these methods can be found in Walline et al's work.¹⁷ Briefly, p16 expression (CINtec; mtm Laboratories) was evaluated semiquantitatively on the basis of the expression intensity ([1] no staining, [2] low intensity, [3] moderate intensity, or [4] high intensity) and the proportion of positive cells ([1] <5%, [2] 5%-20%, [3] 21%-50%, or [4] 51%-100%). The p16 expression score was determined as the product of the intensity and the proportion of p16+ cells. Scores of 1 to 4 were considered negative/low, scores of 5 to 11 were considered moderate, and scores of 12 to 16 were considered high.¹⁷

HPV detection based on ISH was performed with the Inform HPV III assay (Ventana Medical Systems). This assay allows the detection of 12 oncogenic HPV types (HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, and HPV-66) but does not specify the HPV type.¹⁷

DNA extracted from tumors was used for PCR-based HPV detection with the MassARRAY HPV multiplex PCR assay, which was designed to detect 15 oncogenic HPV types (HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, HPV-59, HPV-66, HPV-68, and HPV-73). Samples that had p16 expression scores higher than 5 and were positive according to ISH- or PCR-based HPV detection methods were considered HPV+. Tumors for which HPV was detected in the absence of p16 overexpression were considered HPV-.

Library Preparation and DNA Sequencing

Ten nanograms of DNA was used as the input for the target DNA library preparation with the Ion AmpliSeq Library Kit. For the amplification of the targeted DNA, we used a customized pool of primers designed for the amplification of all exon regions of the following genes: *AJUBA*, *CASP8*, *CCND1*, *CDKN2A*, *EGFR*, *FAT1*, *FBXW7*, *HLA-A*, *HRAS*, *KEAP1*, *NFE2L2*, *NOTCH1*, *NOTCH2*, *NSD1*, *PIK3CA*, *TGFBR2*, *TP53*, and *TP63*. The target DNA libraries were sequenced with the Ion Personal Genome Machine sequencer platform. Variant calls were made on the Ion Reporter server with the AmpliSeq tumor-normal pair comprehensive cancer panel pipeline with customized filters. Although *HLA-A* is included in the sequencing assay, it is currently excluded from the analysis because of the difficulty in accurately calling mutations in this highly polymorphic gene.

TP53 Mutation Classification

TP53 gene mutations were classified according to 2 functional-impact and risk-classification systems proposed by Poeta et al²⁴ and Neskey et al.²⁵ With Poeta et al's system, the *TP53* mutations were classified as disruptive and nondisruptive mutations. Disruptive mutations were those inducing a disruption of p53 protein production (nonsense, frameshift, in-frame, and splice site mutations) or any missense mutation occurring within L2 or L3 DNA binding domains (codons 163-195 and 236-251) and changing the original amino acid polarity or charge category. For Neskey et al's classification, we used the online EAp53 server,²⁶ which classifies *TP53* mutations into high- and low-risk categories for HNSCC and determines a numeric risk score (Evolutionary Action score - EAscore). Only missense mutations are classifiable in this system.

Statistical Analysis

For the statistical analysis, we used IBM SPSS Statistics (version 22) and GraphPad Prism (version 6.07) for Windows. Associations between categorical variables were determined with Fisher's exact test. Associations between categorical and quantitative variables were determined with the Mann-Whitney test when categories had 2 values and with the Kruskal-Wallis test when categories had 3 or more values. Significant associations were considered when the *P* value was lower than .05. The log-rank test was used to determine differences among survival curves.

RESULTS

To understand the mutation frequencies and patterns in HIV+ HNSCC, we sequenced tumor samples from 20 HIV+ HNSCC patients. We also sequenced 32 HIV- patients as controls (Table 1). Among HIV+ cases, 11 (55%) were HPV-, and 9 (45%) were HPV+; among HIV- cases, 6 (18.75%) were HPV+, and 26 (81.25%) were HPV-. Sex and tissue sites did not differ among these groups, but HIV+HPV+ patients were significantly younger than HIV-HPV+ patients. All HIV+ patients were smokers or former smokers, and all except one were alcohol users. No information about tobacco and alcohol consumption was available for HIV- cases.

DNA was extracted from FFPE samples and sequenced on a custom Ion Torrent AmpliSeq panel containing 18 genes frequently altered in HNSCC. The identified mutations as well as the mutation frequencies described for HNSCC in The Cancer Genome Atlas (TCGA)²² are listed in Table 2, and summary oncprints are shown in Figure 1. Because HPV is known to alter the mutational landscape of HNSCC, the cohort was divided

TABLE 1. Clinical Characteristics of the Studied Head and Neck Cancer Patients

Characteristic	HIV+HPV+ (n = 9)	HIV+HPV- (n = 11)	HIV-HPV+ (n = 6)	HIV-HPV- (n = 26)	P ^a	P ^b	P ^c
Sex, No.							
Male	8	9	6	25	.309	1.000	.205
Female	1	2	0	1			
Age, mean ± SD, y	43.8 ± 10.5	52.3 ± 7.07	54.6 ± 7.03	54.3 ± 11.3	.061	.036	.384
Tumor site, No.							
Oral cavity	2	2	2	14	.188	.580	.228
Oropharynx	5	3	3	5			
Larynx	0	3	1	4			
Other head and neck sites	2	3	0	3			

Abbreviations: HIV, human immunodeficiency virus; HPV, human papillomavirus; SD, standard deviation.

^a Comparison among all 4 groups.

^b Comparison between HIV+ and HIV- patients with HPV+ tumors.

^c Comparison between HIV+ and HIV- patients with HPV- tumors.

into 4 groups for most analyses based on the HIV and HPV status. The associations were assessed through comparisons of all 4 groups together and also through comparisons of HIV+ and HIV- groups with the same HPV status. Overall, 84.6% of the patient tumors were found to have at least 1 mutation in the examined genes, and the number of patients with tumors harboring mutations was significantly different among the 4 groups ($P = .002$). Among the HPV+ cases, there was no difference in the number of tumors harboring mutations when HIV+ (55.6%) and HIV- patients (66.7%) were compared. However, among the HPV- cases, the number of mutations was higher in the HIV- group (100%) versus the HIV+ group (81.8%; Fig. 2A). The number of patients harboring mutations was significantly lower overall in the virus-related groups (HIV+ or/and HPV+) versus the HPV- cases from TCGA ($P < .001$) as well as the HIV-HPV- group ($P = .004$). The number of genes mutated per patient and the number of mutations per patient varied among the groups (Fig. 2B,C). Both variables showed higher values among HPV- patients, regardless of the HIV status.

No mutations were detected in 4 of the studied genes that are known to have low mutation frequencies in HNSCC (*AJUBA*, *CASP8*, *CCND1*, and *TGFBR2*). Mutations in the *TP53* and *NOTCH1* genes were detected in all groups. When we compared the frequencies of gene mutations among the groups, only *TP53* mutations were significantly different ($P < .001$; Table 2). HIV-HPV- patients had the highest frequency of mutations in the *TP53* gene (88.5%), whereas HIV+HPV+ patients had the least number of mutations in this gene (11.1%). A strikingly high frequency of *TP53* mutations was observed in HPV+ patients (33.3% of all 15 HPV+ cases) in comparison with other HNSCC HPV+ sequencing studies. We do not currently have an explanation for this high rate. Mutations in the *NFE2L2* gene

were found in 2 of 20 HIV+ patients (10%); this was a higher frequency of detection in comparison with the frequency for non-HIV patients (3.1%) and that reported for the TCGA cohort (6.5%). *NOTCH1* mutations were observed in 3 of 9 HIV+HPV+ patients (33.3%), and this was a higher mutation frequency in comparison with the frequency of our other groups and that reported in the TCGA data set. However, statistical significance was not reached for the *NFE2L2* and *NOTCH1* associations. *NSD1* mutations were differentially distributed among tumor sites ($P = .025$): they were observed in the larynx (2 mutated cases) and other head and neck sites (1 mutated case) but were absent in oral cavity and oropharynx cases. This is consistent with the distribution of *NSD1* mutations in TCGA. The frequency of mutations in any gene did not differ significantly between HIV+ and HIV- tumors with the same HPV status.

To assess qualitative differences in *TP53* mutations, we compared the types of *TP53* mutations among the groups (Fig. 3). Although HPV+ cases exhibited only missense *TP53* mutations, HPV- tumors also contained truncating and in-frame indel mutations. Among HIV+HPV- cases, truncating mutations were the most common (66.7%), whereas these mutations represented just 26.9% of the HIV-HPV- events. Next, we used 2 *TP53* functional-impact and risk-classification systems previously used for HNSCC (Table 3). With both systems, *TP53* mutations related to aggressive tumors (high-risk and disruptive) were more prevalent in HIV- and HPV- cases. The mutations in virus-related cases were predominantly classified as wild-type or low-risk/nondisruptive mutations. Truncating and frameshift mutations are not classifiable in Neskey et al's system²⁵ and are, therefore, called *other*. These were more prevalent in the HIV+ group (45% of mutated cases) versus the HIV- group (30.8% of wild-type cases).

TABLE 2. Mutation Findings According to the HIV and HPV Status

Mutation status	TCGA (279 Patients) ^a			HIV+HPV+ (9 Patients)			HIV+HPV- (11 Patients)			HIV-HPV+ (6 Patients)			HIV-HPV- (26 Patients)					
	HPV+ Frequency % (n = 36)	HPV- Frequency % (n = 243)	HPV- Frequency % (n = 243)	No. of Cases	Mutation %	No. of Mutations	No. of Cases	Mutation %	No. of Mutations	No. of Cases	Mutation %	No. of Mutations	No. of Cases	Mutation %	No. of Mutations	P ^b	P ^c	P ^d
WT	44	3	4	44.4	18.2	2	2	18.2	—	2	33.3	—	0	0	—	.002	1.000	.083
MUT	56	97	5	55.6	81.8	9	4	66.7	26	6	66.7	5	26	100	79	—	—	—
AJUBA	0	7	0	0	0	11	6	0	—	6	0	—	26	0	—	—	—	—
MUT	0	10	9	0	0	11	6	0	—	6	0	—	26	0	—	—	—	—
CASP8	6	10	0	0	0	11	0	0	—	0	0	—	26	0	—	—	—	—
MUT	0	0.4	9	0	0	11	6	0	—	6	0	—	26	0	—	—	—	—
CCND1	0	26	9	0	0	9	6	0	—	6	0	—	17	0	—	—	—	—
MUT	0	26	9	0	18.2	2	0	0	—	6	0	—	9	34.6	11	.090	—	.445
CDKN2A	0	5	0	0	0	11	6	0	—	6	0	—	24	7.7	2	1.000	—	1.000
MUT	0	26	9	0	0	9	6	0	—	6	0	—	24	7.7	2	.610	—	.567
EGFR	0	5	0	0	0	9	6	0	—	6	0	—	24	7.7	2	—	—	—
MUT	2.8	5	0	0	18.2	4	6	0	—	6	0	—	23	11.5	4	.881	—	1.000
FAT1	0	5	0	0	9.1	1	0	0	—	6	0	—	3	7.7	2	1.000	—	1.000
MUT	0	5	0	0	0	11	6	0	—	6	0	—	24	7.7	2	—	—	—
FBXW7	0	5	0	0	0	11	6	0	—	6	0	—	24	7.7	2	1.000	—	1.000
MUT	0	5	0	0	0	9	6	0	—	6	0	—	25	3.8	1	.306	—	.205
HRAS	0	20	6	0	18.2	2	0	0	—	6	0	—	19	3.8	1	.583	.604	.391
MUT	8	5	3	33.3	9.1	1	1	16.7	1	5	16.7	1	7	26.9	10	.924	1.000	1.000
KEAP1	0	5	1	11.1	9.1	1	6	0	—	6	0	—	22	15.4	10	—	—	—
MUT	0	5	0	0	0	10	6	0	—	6	0	—	4	3.8	1	.306	—	.205
NFE2L2	0	7	0	0	0	9	6	0	—	6	0	—	25	3.8	1	.876	1.000	1.000
MUT	0	20	6	0	0	10	5	0	—	6	0	—	22	15.4	4	—	—	—
NOTCH1	0	8	3	33.3	18.2	3	1	18.2	2	5	18.2	1	7	15.4	4	—	—	—
MUT	0	8	1	11.1	18.2	3	6	0	—	6	0	—	26	0	—	—	—	—
NOTCH2	0	11	0	0	0	9	6	0	—	6	0	—	26	0	—	—	—	—
MUT	8	19	1	11.1	18.2	11	6	0	—	6	0	—	26	0	—	—	—	—
NSD1	0	8	0	0	0	9	2	0	—	6	0	—	26	0	—	—	—	—
MUT	36	4	0	0	0	11	0	0	—	6	0	—	26	0	—	—	—	—
PIK3CA	2.8	84	1	11.1	81.8	9	2	66.7	9	2	66.7	4	23	88.5	26	<.001	.089	.623
MUT	0	2.5	0	0	9.1	1	0	0	—	6	0	—	3	11.5	3	.881	—	1.000
TGFBR2	0	0	0	0	9.1	1	0	0	—	6	0	—	3	11.5	3	—	—	—
MUT	2.8	84	1	11.1	81.8	9	2	66.7	9	2	66.7	4	23	88.5	26	<.001	.089	.623
TP53	2.8	84	1	11.1	81.8	9	2	66.7	9	2	66.7	4	23	88.5	26	<.001	.089	.623
MUT	0	2.5	0	0	9.1	1	0	0	—	6	0	—	3	11.5	3	.881	—	1.000
TP63	0	2.5	0	0	9.1	1	0	0	—	6	0	—	3	11.5	3	.881	—	1.000
MUT	0	2.5	0	0	9.1	1	0	0	—	6	0	—	3	11.5	3	.881	—	1.000

Abbreviations: HIV, human immunodeficiency virus; HPV, human papillomavirus; MUT, mutated; TCGA, The Cancer Genome Atlas; WT, wild type.

^aTCGA head and neck squamous cell carcinoma mutation frequencies per patient were taken from Lawrence et al.²²

^bThe P value was calculated on the basis of the HIV+HPV+, HIV+HPV-, HIV-HPV+, and HIV-HPV- groups.

^cThe P value was calculated on the basis of the HIV+HPV+ and HIV-HPV+ groups.

^dThe P value was calculated on the basis of the HIV+HPV- and HIV-HPV- groups.

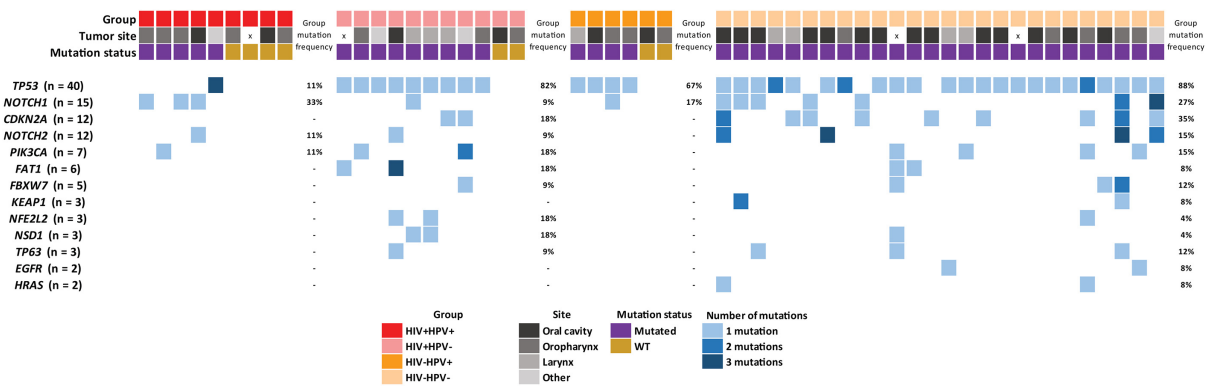


Figure 1. Oncoprint showing the frequencies of the mutations detected in each studied group. HIV indicates human immunodeficiency virus; HPV, human papillomavirus; WT, wild type.

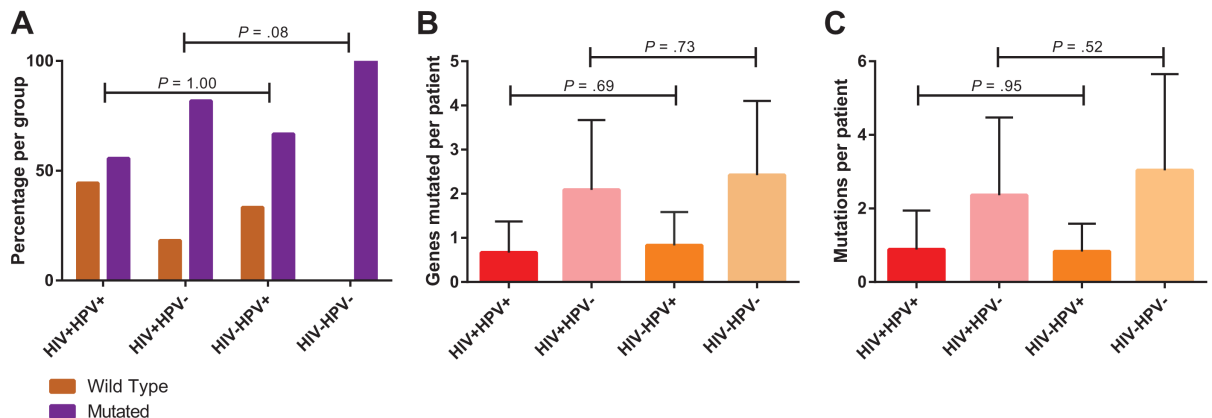


Figure 2. Overall mutation pattern according to each studied group. (A) Frequencies of mutated and wild-type cases for the studied genes (y-axis) according to each studied group. Groups with the same HPV status were compared, and a few HIV+ cases with mutations were found with HPV- tumors. (B) Number of genes mutated per patient in each studied group. No differences in the number of genes mutated per patient were observed between groups with the same HPV status. (C) Number of overall mutations per patient. No differences in the number of mutations per patient were observed between groups with the same HPV status. HIV indicates human immunodeficiency virus; HPV, human papillomavirus.

We also assessed the types of nucleotide changes in each studied group for all genes as well as the *TP53* gene individually (Fig. 4). C>T mutations were the most prevalent nucleotide changes observed in all groups and for the majority of the genes. Only the genes *NFE2L2*, *NSD1*, and *PIK3CA* showed more C>G, C>A, and T>C nucleotide changes, respectively. No significant difference in the distribution of nucleotide changes was observed among the 4 groups ($P = .93$; Fig. 4A) or between HIV+ and HIV- groups with the same HPV status ($P = .24$ for HPV+ tumors and $P = .98$ for HPV- tumors). No difference in the pattern of nucleotide change distribution was observed when patients were grouped according to their HIV status ($P = .95$; Fig. 4B).

As for nucleotide changes in the *TP53* gene, C>T changes were the most commonly observed, but no statistical difference was observed among the 4 groups ($P = .80$; Fig. 4C) or between HIV+ and HIV- samples with the same HPV status ($P = .34$ for HPV+ tumors and $P = .92$ for HPV- tumors). Although the frequency of C>T changes was higher among tumors with an HIV infection (66.7%) versus HIV- tumors (43.3%), the difference between them was not significant ($P = .95$; Fig. 4D).

Considering the high frequency of C>T mutations in these samples, we investigated the 5' flanking nucleotide for each mutation (Fig. 4E). HIV+HPV- samples exhibited a higher frequency of TpC>T mutations

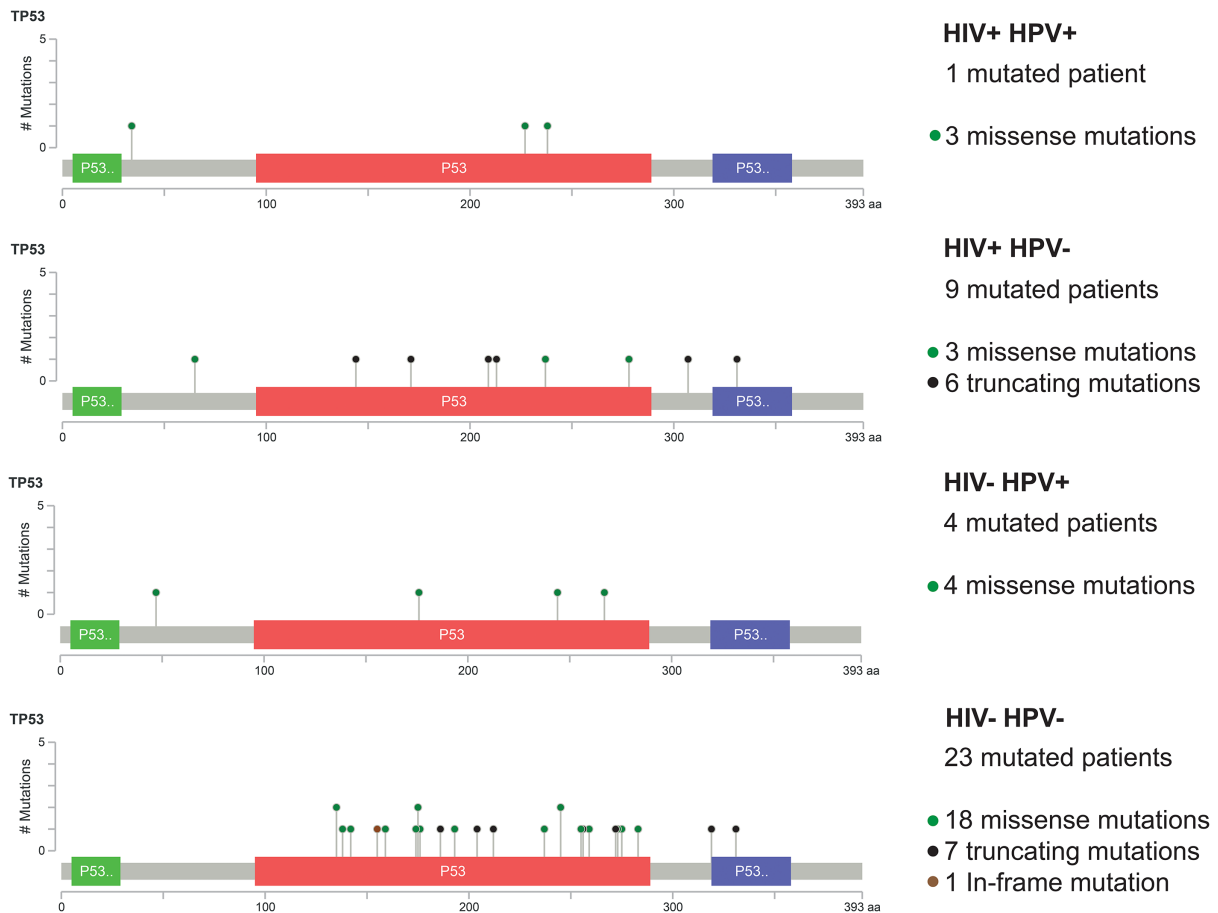


Figure 3. Mutation maps of the *TP53* gene in each studied group. Green dots represent missense mutations, red dots represent truncating mutations, and black dots represent in-frame indels; truncating and in-frame indels were observed only among HPV-cases. The frequency of truncating mutations was significantly higher among HIV+ cases versus HIV- patients. HIV indicates human immunodeficiency virus; HPV, human papillomavirus.

(26.3%) than tumors of HIV-HPV- patients (10.2%) when all mutated genes were taken into account. As for HPV+ tumors, TpC>T changes were observed only among HIV+ patients (42.9% of all mutations) and were absent among HIV- ones ($P = .02$). When tumors were grouped according their HIV status, regardless of the HPV infection status, the frequency of TpC>T was significantly higher among HIV+ patients (34.8%) versus HIV- patients (10.9%; $P = .02$; Fig. 4F). The same trend was observed when only the *TP53* gene was considered: only HIV+ tumors exhibited TpC>T nucleotide changes ($P = .02$; Fig. 4H).

Differences in 5-year survival rates were determined for patients with respect to their HIV/HPV group, *TP53* status (wild-type vs mutated), and *TP53* mutation type (insertion/deletion, transition, or transversion). No significant differences between survival curves were observed in any comparison ($P = .45$, $P = .30$, and $P = .46$,

respectively). The presence of mutations in other genes (alone or in combination) was also not associated with survival in these patients. Because of the absence of TNM information for HIV- patients, the influence of the tumor clinical stage on these results was not able to be determined.

DISCUSSION

In this study, we observed an overall decreased frequency of mutations in HIV+ HNSCCs, and HIV-related HNSCCs had a distinct pattern of genetic mutations in the *TP53* gene characterized by truncating mutations and TpC>T nucleotide changes.

HIVIs are a unique group of patients in the context of HNSCC. They have greater exposure to tobacco and alcohol, have impaired immune function, and consequently are more prone to infection by high-risk HPV.^{4-7,14} In this way, these patients have multiple key risk

TABLE 3. Association Between the TP53 Mutation Score and the Clinical Characteristics of Head and Neck Cancer Patients

Characteristic	TP53 Mutation Score: Neskey et al ²⁵				P	P ^b	TP53 Mutation Score: Poeta et al ²⁴				P	P ^b
	WT	Low Risk	High Risk	Other ^a			WT	Nondisruptive	Disruptive			
Sex, No.												
Male	14	9	14	11	1.000	—	14	24	10	1.000	—	
Female	1	0	1	2	—	—	1	2	1	—	—	
Age, mean ± SD, y	47.2 ± 12.2	54.5 ± 8.36	53.8 ± 9.09	53.6 ± 11.1	.419	—	47.2 ± 12.2	53.6 ± 10.2	54.7 ± 7.53	.354	—	
Tumor site, No.												
Oral cavity	7	1	6	6	.119	—	7	9	4	.303	—	
Oropharynx	7	3	5	1	—	—	7	7	2	—	—	
Larynx	0	4	2	2	—	—	0	5	3	—	—	
Other head and neck sites	1	1	2	4	—	—	1	5	2	—	—	
HIV status, No.												
+	10	3	2	5	.011	—	10	6	4	.020	—	
-	5	6	13	8	—	—	5	20	7	—	—	
HPV status, No.												
+	10	2	3	0	.001	—	10	3	2	<.001	—	
-	5	7	12	13	—	—	5	23	9	—	—	
Virus infection status, No.												
Present	12	5	4	5	.022	—	12	8	6	.010	—	
Absent	3	4	11	8	—	—	3	18	5	—	—	
HIV/HPV status, No.												
+/+	8	0	1	0	.001	.071	8	1	0	.001	.071	
-/+	2	2	2	0	—	—	2	2	2	—	—	
+/-	2	3	1	5	—	.210	2	5	4	—	.448	
-/-	3	4	11	8	—	—	3	18	5	—	—	

Abbreviations: HIV, human immunodeficiency virus; HPV, human papillomavirus; SD, standard deviation; WT, wild type.

^aOther refers to mutations not classifiable by Neskey et al's system.²⁵

^bThe P value was calculated through the comparison of groups with the same HPV status.

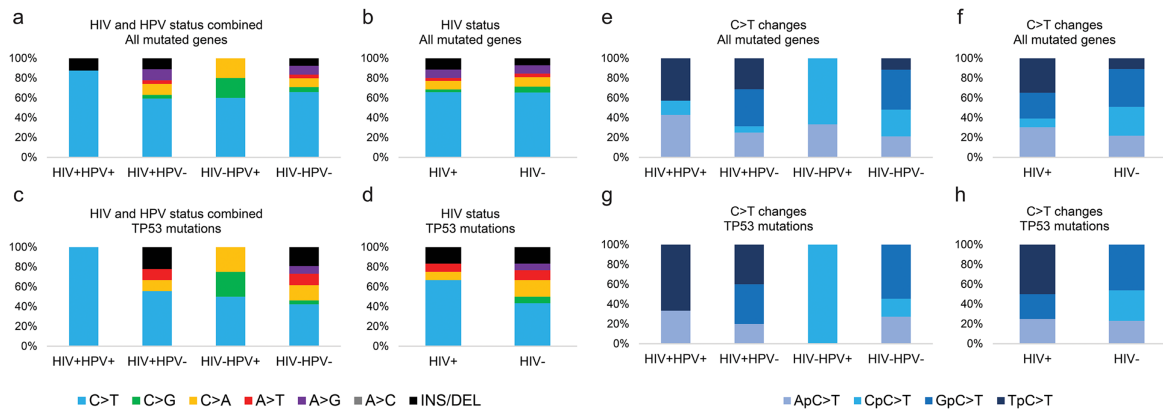


Figure 4. Distribution of nucleotide changes among the studied groups. (A) Frequency of nucleotide changes in all mutated genes among the 4 studied groups. (B) Frequency of nucleotide changes in all mutated genes among tumors from HIV+ and HIV- patients, regardless of the HPV status. (C) Frequency of nucleotide changes in the TP53 gene among the 4 studied groups. (D) Frequency of nucleotide changes in the TP53 gene among tumors from HIV+ and HIV- patients, regardless of the HPV status. (E) Frequency of each 5' nucleotide flanking C>T mutations in all mutated genes for the 4 studied groups. (F) Frequency of each 5' nucleotide flanking C>T mutations in all mutated genes among tumors from HIV+ and HIV- patients, regardless of the HPV status. (G) Frequency of each 5' nucleotide flanking C>T mutations in the TP53 gene for the 4 studied groups. (H) Frequency of each 5' nucleotide flanking C>T mutations in the TP53 gene among tumors from HIV+ and HIV- patients, regardless of the HPV status. HIV indicates human immunodeficiency virus; HPV, human papillomavirus.

factors for the development of cancer and particularly HNSCC. Not surprisingly, HIVIIs have higher rates of HNSCC than the general population. It has been

reported that HIVIIs with HNSCC present at a more advanced tumor stage and have reduced survival rates.^{15,16,18,27} However, it is not clear whether these

findings are caused by intrinsic features of the tumor cells or are related to host factors related to the HIV infection.^{22,23,28} We aimed to investigate this by comparing the patterns of mutations in HIV-related and non-HIV-related tumors in genes frequently mutated in HNSCC. Because an HPV infection causes a distinct subtype of HNSCC,^{22,23,28} we considered the HPV status as a key variable as well.

Our observations regarding genetic alterations in HNSCC from HIVIs are unique in the literature. The only similar analysis that we could find was performed by Souza et al,²⁹ who compared the patterns of *TP53* mutations in DNA obtained from cervical swabs (normal and altered cytologies) from HIV-infected and uninfected patients (all HPV+). They found that the frequencies of *TP53* mutations were similar in the 2 groups (approximately 19%).

Souza et al²⁹ also found that the genomic position of *TP53* mutations changed with the HIV status. HIV+ patients had more mutations located in exon 7, whereas HIV- patients had more mutations in exon 6. Our small sample size made it difficult to compare the genomic locations of *TP53* mutations, but we did examine the types of mutations. HIV+HPV- patients presented with a higher frequency of truncating mutations, and the missense mutations were more frequently classified as low-risk and nondisruptive. Many *TP53* mutations have been studied for their gain-of-function properties, which can provide novel characteristics to the tumors. Although missense mutations in the p53 DNA binding domain can promote a loss of DNA binding activity, this event may also change the protein structure and lead to new potential protein interactions, which in turn may result in p53 gain of function.³⁰ The pattern of *TP53* mutations in HIV+ cases suggests that there are fewer gain-of-function *TP53* mutations, and it is intriguing to speculate that this could be related to the immune status of the individuals or HIV itself.

Another interesting qualitative observation is the different patterns of nucleotide changes in all mutated genes but especially in the *TP53* gene among HIV+ and HIV- tumors. The presence of C>A changes in the *TP53* gene is considered a hallmark of tobacco-related DNA mutations in lung cancer and also in some HNSCCs.^{21,22,31,32} In fact, only 1 HPV+ case in our cohort exhibited C>A transversions. Among the HIV+HPV- cases, the percentage of C>A changes was similar to that among the HIV-HPV- cases (12.5% vs 9.6%), and this might be expected because all the HIV+ patients were smokers. C>T transitions have been

demonstrated as being more predominant in virally transformed tumors, including HNSCC tumors.^{22,33} However, we demonstrated that a specific subtype of C>T changes, the TpC>T mutations, were enriched in the HIV-infected patients, and they conferred a virus-related mutation fingerprint for these cases. Interestingly, the increased number of TpC>T changes among HIV+ tumors was independent of the presence of an HPV co-infection.

Cytosine deamination is a defense mechanism against retrovirus infections exerted by the APOBEC family of enzymes. Human APOBEC3G is induced by an HIV infection to impair virus infectivity by promoting mutations in its DNA.³⁴⁻³⁶ However, the mutagenic potential of APOBEC3G is not restricted to the viral genome, and cytosine deamination may also occur in human DNA. The APOBEC-related pattern of mutations, characterized by TpC>T transitions, can be observed in numerous human cancers, including HNSCC, and they are commonly described in the *TP53* gene.³⁷⁻⁴¹ Interestingly, oncogenic pathogens such as HPV and *Helicobacter pylori* have been proved to cause APOBEC3G-mediated mutations in oral and gastric epithelium.^{37,41} The enrichment of TpC>T changes in HIV-related tumors might suggest that HIV infection contributes to the *TP53* mutation pattern observed in this cohort.

Some studies have demonstrated that HIV-encoded proteins may interact with the p53 protein and modulate its function in different ways.⁴²⁻⁴⁴ One of these HIV-encoded products, the Nef protein, is believed to interact with p53 and inhibit its function and thus promote HIV infection and replication. Greenway et al⁴⁵ observed that Nef inhibits p53-dependent apoptosis, and McLemore et al¹⁹ found the expression of this protein in the cytoplasm of 7 HNSCC samples. These results might indicate that an HIV infection would have a direct role in HNSCC pathogenesis independent of an HPV infection.⁴⁶ HIV DNA, RNA, and viral particles have been detected in the oral mucosa of HIVIs,⁴⁷ and it has been demonstrated that epithelial cells from the oral mucosa lining are susceptible to HIV infection.⁴⁸ These findings might suggest a direct oncogenic effect of the virus on oral epithelial cells. More extensive sequencing would be necessary to confirm this.

Despite the genetic differences observed among these patients, no significant difference in survival was observed in our analysis with respect to the HIV and HPV status, *TP53* mutation status, and *TP53* mutation type. We believe that our small sample size might limit

definitive conclusions on the impact of such variables on the survival of HNSCC patients. The impact of such variables should be further investigated in larger cohorts.

Considering these results, we conclude that HIV-related HNSCC likely represents a distinct genomic entity. The alterations observed in this study must be validated in larger cohorts, but they suggest that these tumors in HIVIs are biologically distinct. More studies are needed to understand the unique etiology, pathogenesis, and biology of these tumors and to determine whether there are unique therapeutic modalities that would benefit these HNSCC patients.

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CONFLICT OF INTEREST DISCLOSURES

Robert L. Ferris reports working on an advisory board and a clinical trial for Astra-Zeneca/MedImmune and receiving research funding from the company, working on an advisory board for Lilly, working on an advisory board and a clinical trial for Bristol-Myers Squibb and receiving research funding from the company, working on an advisory board and a clinical trial for Merck, working on an advisory board for Pfizer, and receiving research funding from VentiRx Pharmaceuticals outside the submitted work.

AUTHOR CONTRIBUTIONS

Frederico O. Gleber-Netto: Acquisition, analysis, or interpretation of the data; drafting of the article; and statistical analysis. **Mei Zhao:** Acquisition, analysis, or interpretation of the data. **Sanchit Trivedi:** Acquisition, analysis, or interpretation of the data. **Jiping Wang:** Acquisition, analysis, or interpretation of the data. **Samar Jasser:** Acquisition, analysis, or interpretation of the data. **Christina McDowell:** Acquisition, analysis, or interpretation of the data. **Humam Kadara:** Acquisition, analysis, or interpretation of the data. **Jiexin Zhang:** Acquisition, analysis, or interpretation of the data. **Jing Wang:** Acquisition, analysis, or interpretation of the data. **William N. William, Jr:** Acquisition, analysis, or interpretation of the data. **J. Jack Lee:** Acquisition, analysis, or interpretation of the data. **Minh Ly Nguyen:** Critical revision of the article for important intellectual content. **Sara I. Pai:** Acquisition, analysis, or interpretation of the data and critical revision of the article for important intellectual content. **Heather M. Walline:** Acquisition, analysis, or interpretation of the data. **Dong M. Shin:** Acquisition, analysis, or interpretation of the data and critical revision of the

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