Title: Distinct pattern of *TP53* mutations in HIV-related head and neck squamous cell carcinoma

Running title: *TP53* mutations in HIV-related HNSCC

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Precis for use in the Table of Contents: HNSCC in patients infected by HIV showed a specific pattern of DNA mutations when compared to non-HIV infected HNSCC individuals. This was particularly true for mutations in TP53, where tumors from HIV-infected patients showed a virus-related mutation fingerprint irrespectively of HPV co-infection.

Abstract: Background: HIV-infected individuals (HIVII) have a higher incidence of head and neck squamous cell carcinoma (HNSCC), and clinical and histopathological differences have been observed in their tumors when compared to HNSCC patients without HIV infection. The reasons for these differences are not clear and molecular differences between HIV-related and non-HIV-related HNSCC may exist. We compared the mutational patterns between HIV-related and non-HIV-related HNSCC. **Methods:** We sequenced the DNA of 20 samples of HIV-related and 32 samples of non-HIV-related HNSCCs. 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 PGM Sequencer. DNA sequencing data was analyzed by using the Ion Reporter Software. HPV status of the tumor samples was assessed by in situ hybridization, HPV MultiPlex PCR-MassArray assay and p16 immunostaining. Mutation calls were compared among the studied groups. **Results:** HIV-related HNSCC revealed a distinct pattern of mutations when compared to 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, considering all mutated genes but especially for *TP53*. **Conclusions:** HNSCC in HIVII presented 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 pathogenesis of these tumors should not be ruled out.

Keywords: TP53 Gene, Head and Neck Cancer, HIV, HPV, mutation

Introduction

HIV-infected individuals (HIVII) have a higher cancer incidence than the general population. Kaposi sarcoma and non-Hodgkin lymphoma are the most common neoplasms among HIVII and are AIDS-defining malignancies. However, after the advent of antiretroviral therapy (ART) the incidence of other malignant tumors (non-AIDS-defining cancer) increased and became an important cause of death in these patients.[1-4]

Three factors are considered important for the elevated cancer incidence among HIVII: 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 HIVII. Prevalence of tobacco smoke exposure among this group is two times higher than in the overall population.[5, 6] Immunosuppression is the main characteristic of HIV infection, and it is considered to be an important cause of cancer development. Organ-transplanted patients on immunosuppressive regimens demonstrate an increased incidence of malignant tumors as compared to the general population. Their cancer incidence is similar to the frequency observed among HIVII, suggesting that immunodeficiency plays an important role in HIVII-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 ten times higher in HIVII than in the general US population.[11] Human herpesviruses (HV) and human papillomaviruses (HPV) are responsible for the majority of these cases, leading to the development of sarcomas and lymphomas, but also squamous cell carcinomas in the anogenital and head and neck regions.[11] While human HV are related to AIDS-defining malignancies, HPV 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 HIVII.[1, 2, 4, 12-14] HNSCC is diagnosed at an earlier age and at a more advanced stage in HIVII [15], and they tend to be more aggressive[16] and related to worse survival rates when expressing high levels of TP53 than in non-HIVII.[17] The efficacy of current treatment approaches in HIVII-related HNSCC is still a matter of debate.[18] Radiotherapy treatment has been found to be less effective for control of tumor relapse and related to worse overall survival and more treatment-related toxicity in HIVII.[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 are still unclear.

Histopathological findings indicate that HIV-related HNSCC have 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. In order to better understand the behavior of HIV-related HNSCC and possibly develop personalized treatment approaches it is important to determine if they represent a distinct molecular entity.

Recently, integrated genomic studies in HNSCC have dramatically expanded our knowledge about the pathogenesis, progression and treatment of these tumors. 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 the 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. In order to accomplish this, we compared the pattern of mutations between HIV-related 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 Programs of Research Excellence (HNC-SPORE) HIV Supplement Consortium. A detailed description of patient recruitment, sample collection and clinical-pathologic data collection was described in a previous publication.[17] Briefly, formalin fixed paraffin embedded tissue (FFPE) from patients diagnosed with head and neck squamous cell carcinoma (HNSCC) and HIV infection were retrieved after 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 yield for sequencing were included in this study.

HPV Testing

For HPV status classification, we considered p16 expression by immunohistochemistry and HPV detection by *in situ* hybridization (ISH) and multiplex PCR-MassArray. Detailed description of these methods can be found in Walline et al. (2016).[17] Briefly, p16 expression (CINtec, mtm Laboratories) was evaluated semi quantitatively considering expression intensity (1 – no staining; 2 – low; 3 – moderate; 4 – high) and proportion of positive cells (1: < 5%; 2: 5% to 20%; 3: 21% to 50%; 4: 51% to 100%). The p16 expression score was determined by the product of intensity by proportion of p16 positive cells. Scores of 1 to 4 were considered negative/low; 5 to 11 were considered moderate; and 12 to 16 were considered high.[17]

HPV detection based on *in situ* hybridization (ISH) was performed using the INFORM HPV III Assay (Ventana Medical Systems). This assay allows the detection of 12 oncogenic HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 66) but does not specify the HPV type.[17]

DNA extracted from tumors was used for PCR-based HPV detection using the HPV MultiPlex PCR MassArray, designed to detect 15 oncogenic HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and 73). Samples with p16 expression-score higher than 5 and positive in ISH or PCR based HPV detection methods, were considered HPV positive. Tumors where HPV was detected in the absence of p16 overexpression were considered HPV negative.

Library preparation and DNA sequencing

Ten nanograms of DNA were used as input for target DNA library preparation using the Ion AmpliSeq Library Kit. For amplification of the targeted DNA it was used a customized pool of primers designed for 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 in the Ion PGM Sequencer platform. Variant calls were made on the Ion Reporter server by using the Ampliseq tumor-normal pair CCP 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 two functional impact and risk classification systems proposed by Poeta et al. (2007)[24] and by Neskey et al. (2015)[25]. For Poeta et al. (2007)[24] system, the *TP53* mutations were classified as disruptive and non-disruptive mutations. Disruptive mutations were those that induce a disruption of p53 protein production (nonsense, frame shift, in frame and splice site mutations) or any missense mutation that occurs within L2 or L3 DNA binding domains (codons 163 to 195 and 236 to 251) and that changes original amino acid polarity or charge category. For the Neskey et al. (2015)[25] classification, we used the online software EAp53 server (http://mammoth.bcm.tmc.edu/cgi-bin/panos/EAp53.cgi) that classifies *TP53* mutations in high and low risk for HNSCC, and determines a numeric risk score (EAscore). Only missense mutations are classifiable in this system.

Statistical analysis

For statistical analysis we used the IBM SPSS Statistics version 22 and the GraphPad Prism version 6.07, for Windows. Associations between categorical variables were determined applying the Fisher's exact test. Associations between categorical and quantitative variables were determined using the Mann-Whitney Test, when categories have two values, and the Kruskal-Wallis Test, when categories have three or more values. Significant associations were considered when the p-value was lower than 0.05. The log-rank test was employed to access differences among survival curves.

Results

In order to understand the mutation frequencies and patterns in HIV+ HNSCC, we sequenced tumor samples from 20 HIV positive (HIV+) HNSCC patients. We also sequenced 32 HIV negative (HIV-) patients as controls (Table 1). Among HIV+ cases, 11 (55%) were HPV negative (HPV-) and 9 (45%) were HPV positive (HPV+), and among HIV- cases 6 (18.75%) were HPV+ and 26 (81.25%) were HPV-. Gender and tissue site were not different among these groups, but HIV+HPV+ patients were significantly younger than HIV-HPV+. All HIV+ patients were smokers or former smokers, and all except 1 were alcohol users. No information about tobacco and alcohol consumption were 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 mutation frequencies described in HNSCC in The Cancer Genome Atlas (TCGA)[22], are listed in Table 2 and summary oncoprints are found in Figure 1. Because HPV is known to alter the mutational landscape of HNSCC, the cohort was divided into 4 groups for most analyses based on the HIV and HPV statuses. The associations were assessed by comparing all four groups together, and also by comparing 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=0.002). Among the HPV+ cases, there was no difference in number of tumors harboring mutations when comparing HIV+ (55.6%) and HIV- (66.7%) patients. However, in the HPV- cases, the number of mutations was higher in the HIV- group (100%) when compared to HIV+ group (81.8%) (Figure 2a). The number of patients harboring mutations was significantly lower overall in the virus-related groups (HIV or/and HPV positive) as compared to the HPV negative cases from TCGA (p<0.001) as well as with the HIV-HPV- group (p=0.004). The number of genes mutated per patient and the number of mutations per patient varied among the groups (Figures 2b and 2c). Both variables showed higher values among HPV- patients, irrespective of HIV status.

No mutations were detected in four 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. Comparing the frequency of gene mutations among the groups, only *TP53* mutations were significantly different (p<0.001) (Table 2). HIV-HPV- (88.5%) patients had the highest frequency of mutations in the *TP53* gene, while HIV+HPV+ (14.3%) patients had the least number of mutations in this gene. A strikingly high frequency of *TP53* mutations was observed in HPV+ patients (33.3% of all 15 HPV+ cases) when compared to other HNSCC HPV+ sequencing studies. We do not currently have an explanation for this high rate. Mutations in *NFE2L2* gene were found in 2 of 20 HIV+ patients (10%), which were a higher detected frequency as compared to non-HIV patients (3.1%) and that reported in the TCGA cohort (6.5%). *NOTCH1* mutations were observed in 3 of 9 HIV+HPV+ patients (33.3%), which were a higher mutation frequency compared to our other groups and that reported in the TCGA dataset. However, statistical significance was not reached in the *NFE2L2* and *NOTCH1* associations. *NSD1* mutations were differentially distributed among tumor sites

(p=0.025), being observed in larynx (two mutated cases) and other H&N sites (one mutated case) but 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.

In order to assess qualitative differences in *TP53* mutations, we compared the types of *TP53* mutations among the groups (Figure 3). While 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 (55.5%), while these mutations represented just 26.9% of the HIV-HPV- events. Next, we employed two TP53 functional impact and risk classification systems previously utilized for HNSCC (Table 3). In both systems, *TP53* mutations related to aggressive tumors ("high risk" and "disruptive") were more prevalent in HIV and HPV negative cases. The mutations in virus-related cases were predominantly classified as wild-type or low risk/non-disruptive mutations. Truncating and frame shift mutations are not classifiable in the Neskey et al. 2015(15) system and are therefore referred to as "Others". These were more prevalent among HIV+ (45% of mutated cases) than in HIV- group (30.8% of wild type cases).

We also assessed the type of nucleotide changes in each studied group for all genes, as well as for the *TP53* gene individually (Figure 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 four groups (p=0.93) (Fig.4a), neither between HIV+ and HIV- groups with the same HPV status (p=0.24 among HPV+ tumors and p=0.98 among HPV-). No difference in the pattern of nucleotide change distribution was observed when patients were grouped according to HIV status (p=0.95) (Fig.4b).

Regarding nucleotide changes in the TP53 gene, C>T changes were the most commonly observed, but no statistical difference was observed among the four groups (p=0.80) (Fig.4c), neither between HIV+ and HIV- samples with the same HPV status (p=0.34 among HPV+ tumors and p=0.92 among HPV-). Although the frequency of C>T changes was higher among tumors with HIV infection (66.7%) than in the HIV- tumors (43.3%), the difference between them was not significant (p=0.95) (Fig.4d).

Considering high frequency of C>T mutations in these samples, we investigated the 5' flanking

nucleotide for each mutation (Fig.4e). HIV+HPV- samples exhibited higher TpC>T mutations (26.3%) than tumors of HIV-HPV- negative patients (10.2%), when all mutated genes were taken into account. Considering HPV+ tumors, TpC>T changes were observed only among HIV+ patients (42.9% of all mutations), being absent in HIV- ones (p=0.02). When tumors were grouped according their HIV status, irrespective of HPV infection, the frequency of TpC>T among HIV+ patients (34.8%) was significantly higher than in HIV- patients (10.9%) (p=0.02) (Fig.4f). The same trend was observed when only the *TP53* gene was considered, where only HIV+ tumors exhibited TpC>T nucleotide changes (p = 0.02) (Fig.4h).

Differences in 5-years survival rates were accessed among patients considering their HIV/HPV groups, *TP53* status (WT vs mutated) and *TP53* mutation type (ins/del vs transition vs transversion). No significant differences among survival curves were observed in any comparison (p = 0.45; p = 0.30; p = 0.46, respectively). Presence of mutations in other genes (alone or in combination) were also not associated with survival in these patients. Due to the absence of TNM information for HIV negative patients, the influence of tumor clinical stage on these results were not possible to access.

Discussion

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

HIVII are a unique group of patients in the context of HNSCC. They have a higher exposure to tobacco and alcohol, have impaired immune functions and consequently are more prone to infection by high-risk HPV.[4-7, 14] In this way, these patients have multiple key risk factors for cancer development, particularly HNSCC. Not surprisingly, HIVII have higher rates of HNSCC than the general population. It has been described that HIVII with HNSCC present at more advanced tumor stage and have reduced survival rates.[15, 16, 18, 26] However, it is not clear if these findings are caused by intrinsic features of the tumor cells or if they are related to host factors related to HIV infection.[22, 23, 27] We aimed to investigate this by comparing the pattern of mutations in HIV-related and non-HIV-related tumors in genes frequently mutated in HNSCC. Because HPV infection causes a distinct subtype of HNSCC [22, 23, 27] we considered HPV status as a key variable as well.

Our observations regarding genetic alterations in HNSCC from HIVII are unique in the literature. The only similar analysis we could find was performed by Souza et al. (2013)[28] that compared the pattern of *TP53* mutations in DNA obtained from cervical swabs (normal and altered cytology) from HIV-infected and non-infected patients (all HPV+), and they found that the frequency of *TP53* mutations was similar between both groups (~19%).

Souza et.al. (2013)[28] also found that the genomic position of *TP53* mutations changed with HIV status. HIV+ patients had more mutations located in exon 7, while HIV- patients had more mutations in exon 6. Our small sample size made it difficult to compare the genomic location of *TP53* mutations but we did examine the types of mutations. HIV+HPV- patients presented a higher frequency of truncating mutations, and the missense mutations were more frequently classified as low risk and non-disruptive. Many TP53 mutations have been studied for their gain-of-function properties which can provide novel characteristics to the tumors. While missense mutations in the p53 DNA binding domain can promote loss of DNA binding activity, this event may also change the protein structure leading to new potential protein interactions, which in turn may result in p53 gain-of-function.[29] The pattern of *TP53* mutations in HIV+ cases suggest 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 pattern 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 was considered a hallmark of tobacco-related DNA mutations in lung cancer and also in some HNSCCs.[21, 22, 30, 31] In fact, only one HPV+ case in our cohort exhibited C>A transversions. Among the HIV+HPV- cases, the percentage of C>A changes was similar to HIV-HPV- cases (12.5% and 9.6%, respectively), which might be expected considering that all HIV+ patients were smokers. C>T transition have been demonstrated as being more predominant in virally transformed tumors, including HNSCC.[22, 32] However, we demonstrated that a specific subtype of C>T changes, the TpC>T mutations, were enriched in the HIV infected patients, conferring 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 HPV co-infection.

Cytosine deamination is a defense mechanism against retrovirus infection exerted by the APOBEC

family of enzymes. Human APOBEC3G is induced by HIV infection in order to impair virus infectivity by promoting mutations in its DNA.[33-35] 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, are observed in numerous human cancers, including HNSCC, and are commonly described in the *TP53* gene.[36-40] Interestingly, oncogenic pathogens such as HPV and *Helicobacter pylori* have proved to cause APOBEC3G-mediated mutations in oral and gastric epithelium.[36, 40] 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.[41-43] One of these HIV-encoded products, the Nef protein, is believed to interact with p53 and inhibit its function, thus promoting HIV infection and replication. Greenway et al. (2002)[44] observed that Nef inhibits p53-dependent apoptosis, and McLemore et al. (2010)[19] found the expression of this protein in the cytoplasm of 7 HNSCC samples. These results might indicate that HIV infection would have a direct role in HNSCC pathogenesis independent of HPV infection.[45] HIV DNA, RNA and viral particles have been detected in the oral mucosa of HIVII[46] and it has been demonstrated that epithelial cells from the oral mucosa lining are susceptible to HIV infection.[47] 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 of the genetic differences observed among these patients, no significant difference in survival was observed in our analysis, considering 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 in 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 represent a distinct genomic entity. The alterations observed in this study must be validated in larger cohorts, but they suggest that HIVII are biologically distinct tumors. More studies are needed to understand the unique etiology, pathogenesis, and biology of these tumors and to determine if there are unique therapeutic modalities that will benefit these HNSCC patients.

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Figure Legends

Fig.1 – Oncoprint showing the frequency of mutations detected in each studied group.

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Fig. 3 – Mutation maps of the *TP53* gene in each studied group. Green dots represent missense mutations; red dots represents truncating mutations; black dot represents in-frame indel; Truncating and in-frame indels are observed only among HPV negative cases. The frequency of truncating mutations among HIV positive cases is significantly higher than in HIV negative patients.

Fig. 4 – Distribution of nucleotide changes among the studied groups. **Fig.4a –** Frequency of nucleotide changes in all mutated genes among the four studied groups. **Fig.4b** – Frequency of nucleotide changes in all mutated genes among tumors from HIV+ and HIV- patients, irrespective of HPV status. **Fig.4c** – Frequency of nucleotide changes in *TP53* gene among the four studied groups. **Fig.3d** – Frequency of nucleotide changes in *TP53* gene among tumors from HIV+ and HIV- patients, irrespective of HPV status. **Fig.4e –** Frequency of each 5' nucleotide flanking

C>T mutations in all mutated genes, considering the four studied groups. **Fig.4f** - Frequency of each 5' nucleotide flanking C>T mutations in all mutated genes among tumors from HIV+ and HIV- patients, irrespective of HPV status. **Fig.4g** - Frequency of each 5' nucleotide flanking C>T mutations in *TP53* gene, considering the four studied groups. **Fig.4h** - Frequency of each 5' nucleotide flanking C>T mutations in TP53 gene among tumors from HIV+ and HIV- patients, irrespective of HPV status.

Table 1 – Clinical characteristics of the studied head and neck cancer patients

		HIV+ HPV+ N=9	HIV+ HPV- N=11	HIV- HPV+ N=6	HIV- HPV- N=26	p-value *	p-value **	p-value ***
Gender	*Male*	8	9	6	25	0.309	1.000	0.205
	Female	1	2	0	1			
Age	*Mean±SD*	43.8±10.5	52.3±7.07	54.6±7.03	54.3±11.3	0.061	**0.036**	0.384
Tumor site	*Oral cavity*	2	2	2	14	0.188	0.580	0.228
	Oropharynx	5	3	3	5			
	Larynx	0	3	1	4			
	Other H&N sites	2	3	0	3			

SD – Standard Deviation; *p-value*- comparison among all four groups; p-value ** - comparison between HIV+ and HIV- patients among* ***HPV+*** *tumors; p-value*** - comparison between HIV+ and HIV- patients among* ***HPV-*** *tumors*

Table 2 – Mutation findings according HIV and HPV statuses

		*TCGA** *279 patients*		**HIV+ HPV+** 9 patients			**HIV+ HPV-** 11 patients			**HIV- HPV+** 6 patients			**HIV- HPV-** 26 patients					
		HPV+ N=36 Freq.	HPV- N=243 Freq.	# Cases	Mut. Freq.	# Mut.	# Cases	Mut. Freq.	# Mut.	# Cases	Mut. Freq.	# Mut.	# Cases	Mut. Freq.	# Mut.	*p-value***	*p-value ****	*p-value *****
Mutation status	*WT*	*44*	*3*	*4*	*44.4*	-	*2*	*18.2*	-	*2*	*33.3*	-	*0*	*0*	-	**0.002**	1.000	0.083
	Mut	*56*	*97*	*5*	*55.6*	*8*	*9*	*81.8*	*26*	*4*	*66.7*	*5*	*26*	*100*	*79*			
AJUBA	*WT*			9		-	11		-	6		-	*26*		-	-	-	-
	Mut	0	7	0	0	-	0	0	-	0	0	-	0	0	-			
CASP8	*WT*			9		-	11		-	6		-	*26*		-	-	-	-
	Mut	6	10	0	0	-	0	0	-	0	0	-	0	0	-			
CCND1	*WT*			9		-	11		-	6		-	*26*		-	-	-	-
	Mut	0	0.4	0	0	-	0	0	-	0	0	-	0	0	-			
CDKN2A	*WT*			9		-	9		-	6		-	17		-	0.090	-	0.445
	Mut	0	26	0	0	-	2	18.2	2	0	0	-	9	34.6	11			
EGFR	*WT*			9		-	11		-	6		-	24		-	1.000	-	1.000
	Mut	0	5	0	0	-	0	0	-	0	0	-	2	7.7	2			
FAT1	*WT*			9		-	9		-	6		-	24		-	0.610	-	0.567
	Mut	0	26	0	0	-	2	18.2	4	0	0	-	2	7.7	2			
FBXW7	*WT*			9		-	10		-	6		-	23		-	0.881	-	1.000
	Mut	2.8	5	0	0	-	1	9.1	1	0	0	-	3	11.5	4			
HRAS	*WT*			9		-	11		-	6		-	24		-	1.000	-	1.000
	Mut	0	5	0	0	-	0	0	-	0	0	-	2	7.7	2			
KEAP1	*WT*			9		-	11		-	6		-	24		-	1.000	-	1.000
	Mut	0	5	0	0	-	0	0	-	0	0	-	2	7.7	3			
NFE2L2	*WT*			9		-	9		-	6		-	25		-	0.306	-	0.205
	Mut	0	7	0	0	-	2	18.2	2	0	0	-	1	3.8	1			
NOTCH1	*WT*			6		-	10		-	5		-	19		-	0.583	0.604	0.391
	Mut	8	20	3	33.3	3	1	9.1	1	1	16.7	1	7	26.9	10			
NOTCH2	*WT*			8		-	10		-	6		-	22		-	0.924	1.000	1.000
	Mut	0	5	1	11.1	1	1	9.1	1	0	0	-	4	15.4	10			
NSD1	*WT*			9		-	9		-	6		-	25		-	0.306	-	0.205
	Mut	8	11	0	0	-	2	18.2	2	0	0	-	1	3.8	1			
PIK3CA	*WT*			8		-	9		-	6		-	22		-	0.876	1.000	1.000
	Mut	36	19	1	11.1	1	2	18.2	3	0	0	-	4	15.4	4			
TGFBR2	*WT*			9		-	11		-	6		-	*26*		-	-	-	-
	Mut	2.8	4	0	0	-	0	0	-	0	0	-	0	0	-			
TP53	*WT*			8		-	*2*		-	*2*		-	3		-	**<0.001**	0.089	0.623
	Mut	2.8	84	1	11.1	3	*9*	*81.8*	9	*4*	*66.7*	4	23	88.5	26			
TP63	*WT*			9		-	10		-	6		-	23		-	0.881	-	1.000
	Mut	0	2.5	0	0	-	1	9.1	1	0	0	-	3	11.5	3			

TCGA HNSCC mutation frequency per patient according to Lawrence et al. (2015); p-value was calculated based on the groups HIV+HPV+, HIV+HPV-, HIV-HPV+ and HIV-HPV- ; p-value*** was calculated based on the groups **HIV+HPV+** and **HIV-HPV+**; p-value**** was calculated based on the groups **HIV+HPV-** and **HIV-HPV-**; WT – Wild type; Mut – Mutated; Freq. – Frequency; # cases = number of cases; Mut. Freq. – mutation frequency; # Mut. – number of mutations*

Table 3 – Association between TP53 mutations score and clinical characteristics of the head and neck cancer patients

		Neskey et al. (2015) TP53 mutation score						Poeta et al. (2007) TP53 mutation score				
		WT	Low risk	High risk	Other‡	p-value	p-value*	WT	Non-disruptive	Disruptive	p-value	p-value*
Gender	*Male*	14	9	14	11	1.000	-	14	24	10	1.000	-
	Female	1	0	1	2		-	1	2	1		-
Age	*Mean±SD*	47.2±12.2	54.5±8.36	53.8±9.09	53.6±11.1	0.419	-	47.2±12.2	53.6±10.2	54.7±7.53	0.354	-
Tumor site	*Oral cavity*	7	1	6	6	0.119	-	7	9	4	0.303	-
	Oropharynx	7	3	5	1		-	7	7	2		-
	Larynx	0	4	2	2		-	0	5	3		-
	Other H&NS	1	1	2	4		-	1	5	2		-
HIV status	Positive	10	3	2	5	**0.011**	-	10	6	4	**0.020**	-
	Negative	5	6	13	8		-	5	20	7		-
HPV status	Positive	10	2	3	0	**0.001**	-	10	3	2	**<0.001**	-
	Negative	5	7	12	13		-	5	23	9		-
Virus infection status	Present	12	5	4	5	**0.022**	-	12	8	6	**0.010**	-
	Absent	3	4	11	8		-	3	18	5		-
HIV/HPV status	+/+	8	0	1	0	**0.001**	0.071	8	1	0	**0.001**	0.071
	-/+	2	2	2	0			2	2	2		
	+/-	2	3	1	5		0.210	2	5	4		0.448
	-/-	3	4	11	8			3	18	5		

‡ Refers to mutations not classifiable by Neskey et al. (2015) system; p-value was calculated by comparing groups with the same HPV status ; H&NS – Head and neck sites*

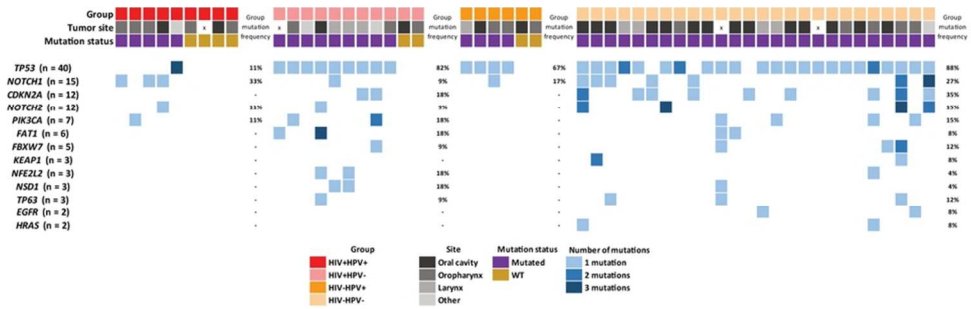
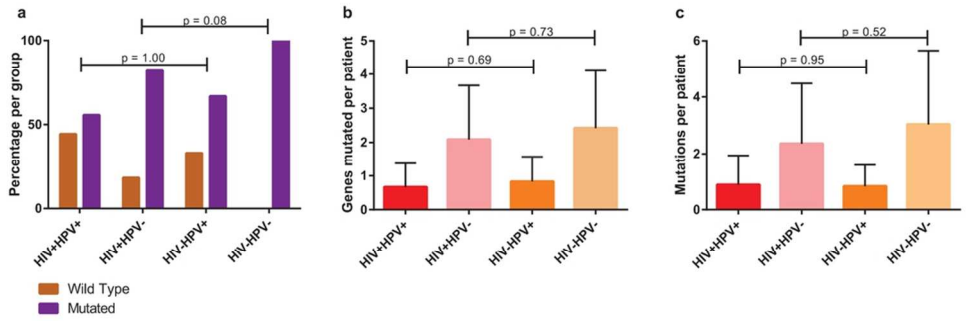


Fig.1 – Oncoprint showing the frequency of mutations detected in each studied group.

84x25mm (300 x 300 DPI)



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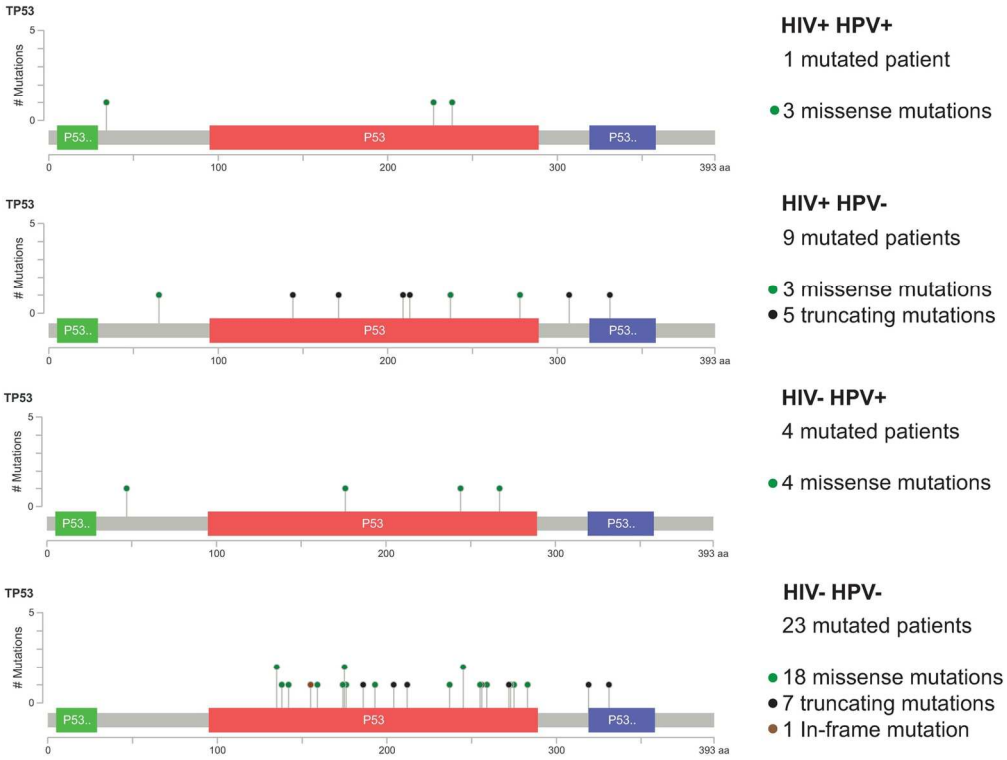
93x34mm (300 x 300 DPI)



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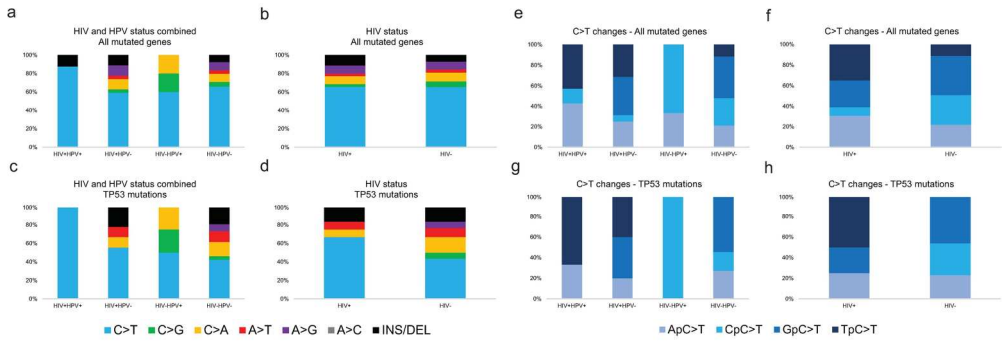
149x114mm (300 x 300 DPI)



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186x137mm (300 x 300 DPI)