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Original Article

The Genomic Landscape of Vulvar Squamous Cell Carcinoma

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Summary: Vulvar squamous cell cancer (VSC) accounts for 90% of vulvar cancers. Next-generation sequencing studies of VSC imply human papillomavirus (HPV) and p53 status play separate roles in carcinogenesis and prognosis. We sought to describe the genomic landscape and analyze the immunologic profiles of VSC with respect to HPV and p53 status. A total of 443 VSC tumors underwent tumor profiling. Next-generation sequencing was performed on genomic DNA isolated from formalin-fixed paraffin-embedded tumor samples. PD-L1, microsatellite instability were tested by fragment analysis, IHC, and next-generation sequencing. Tumor mutational burden—high was defined as >10 mutations per MB. HPV 16/18 positive (HPV+) status was determined using whole exome sequencing on 105 samples. Three cohorts were identified from 105 samples with known HPV: HPV+, HPV −/p53wt, and HPV−/p53mt. Where HPV and *p53* status were examined, *TP53* mutations were exclusive of HPV+ tumors. In all, 37% of samples were HPV+. Among the 66 HPV− tumors, 52 (78.8%) were HPV−/p53mt and 14 (21.2%) were HPV−/p53wt. The HPV−/p53wt cohort had a higher rate of mutations in the *PI3KCA* gene (42.9% HPV−/p53wt vs 26.3% HPV+ vs. 5.8% HPV−/p53mt, $q=0.028$) and alterations in the PI3K/AkT/mTOR pathway (57.1% HPV−/p53wt vs. 34.2% HPV+ vs. 7.7% HPV−/p53mt, $q=0.0386$) than the other 2 cohorts. Ninety-eight VSC tumors with HPV16/18 information underwent transcriptomic analysis and immune deconvolution method. No differences were observed in immune profiles. The HPV−/p53wt VSC tumors had significantly higher rates of mutations in the *PI3KCA* gene and alterations in the PI3K/AkT/mTOR pathway, a potential target that merits further investigation in this subgroup. **Key Words:** Vulvar squamous carcinoma—Genomic landscape—Human papillomavirus.

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The authors declare no conflict of interest.

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Vulvar squamous cell carcinoma (VSC) compromises over 90% of vulvar cancers. As many as 40% of patients with VSC who are initially managed surgically will have a recurrence, which is often fatal (1). Nonsurgical treatment of VSC includes radiation, cytotoxic systemic therapy, or both. Patients who are not candidates for surgical management, as well as those who present with recurrence, have poor overall survival (2).

VSC is currently grouped into 2 major categories based on the pathways of carcinogenesis. "Usual type" is thought to be driven by high-risk strains of the human papillomavirus (HPV), and "differentiated type" is attributed mostly to *TP53* driver mutations (p53mt) (3,4). As these 2 types have distinct etiologies, they are rarely found concurrently (5,6). Recent studies have suggested these molecular identifiers may also play a role in prognosis (7–9).

Next-generation sequencing (NGS) has expanded our understanding of the molecular heterogeneity of cancers historically associated with HPV infection (10,11). However, there is a relative dearth of NGS studies of VSC. Most of the genomic analyses of VSC tumors are limited by sample size and/or the number of genes evaluated (6,12,13). Contemporary analyses suggest at least 3 distinct genomic types of VSC: HPV +/p53wt, HPV−/p53wt, and HPV−/p53mt (13). Within these types, we postulated there may be other unexplored genetic differences identifiable through NGS that could be of benefit for the understanding of etiology, improvements in prognostication, and identification of potential targets for treatment in VSC. Therefore, we sought to further explore the differences in the genomic landscape between the HPV+/p53wt vulvar cancers and their HPV− counterparts, HPV −/p53mt, and the less explored HPV−/p53wt.

MATERIALS AND METHODS

Sample Collection From Participants

A total of 443 VSC tumors underwent comprehensive tumor profiling at Caris Life Sciences. This study was conducted in accordance with the guidelines of the Declaration of Helsinki, Belmont Report, and US Common Rule. In keeping with 45 CFR 46.101 (b), this study was performed utilizing retrospective, deidentified clinical data from patients with VSC. Therefore, this study was deemed Institutional Review Board exempt, and no patient consent was necessary from the subjects.

Genomic and Transcriptomic Analysis

NGS and whole exome sequencing (WES) was performed on genomic DNA isolated from formalin-fixed paraffin-embedded (FFPE) tumor samples using either the TruSeq Amplicon Cancer panel (47 genes; Illumina Inc.) (N = 59, 13.3%), NextSeq platform (592 whole-gene targets) (Agilent Technologies) NGS Q3 (N = 278, 62.8%), or WES (Novaseq) (with TruSeq-47, NGS-592 or WES, n = 443) (N = 106, 23.9%). All variants were detected with $>99\%$ confidence based on allele frequency and amplicon coverage, with an average sequencing depth of coverage of >500 and an analytic sensitivity of 5%. Whole transcriptome sequencing (n = 228) was done using RNA isolated from FFPE samples (NovaSeq). Pathway alterations are determined by combining mutations and amplifications of genes compiled from various manuscripts.

HPV Detection

HPV16/18 (HPV+) was detected using the Caris pipeline which includes 39 unique baits to detect HPV16 and 50 unique baits to detect HPV18 out of a total of 2360 total pathogen baits. The threshold for positive is ≥ 100 reads for either HPV16 or HPV18.

Immunotherapy (IO)-related Biomarker Assessment

A combination of multiple test platforms was used to determine the microsatellite instability (MSI) or mismatch repair (MMR) status of the tumors profiled, including fragment analysis (Promega), IHC (MLH1, M1 antibody; MSH2, G2191129 antibody; MSH6, 44 anti-body; and PMS2, EPR3947 antibody; Ventana Medical Systems Inc.), and NGS (for tumors tested with NextSeq or WES, 7000 target microsatellite loci were examined and compared with the reference genome hg19 from the University of California). A tumor was determined MSI-high (MSI-H) by fragment analysis if 2 or more mononucleotides out of the 5 markers included in the assay were abnormal; a tumor was considered mismatch repair deficient (dMMR) by IHC if the complete absence of protein expression of any of the 4 proteins was observed; a tumor was considered MSI-H by NGS by a threshold of 46 or more altered loci per tumor. MSI or MMR status of the tumor was determined in the order of IHC, fragment analysis, and NGS.

Tumor mutational burden (TMB) was measured by counting all nonsynonymous missense mutations found per tumor 1.4 Mb sequenced/tumor). A cut-off of 10 mutations/Mb, based on the result of the

KEYNOTE-158 trial showing the clinical activity of pembrolizumab in tumors harboring a TMB ≥ 10 (TMB-H) across a variety of previously treated solid tumors (14).

PD-L1 expression was tested via IHC using SP142 antibody (Spring Biosciences) and 22c3 (Agilent) with a positive cut-off for $\geq 1\%$ staining, according to standard protocol.

Immune Microenvironment

The tumor-infiltrating immune cell landscape was analyzed by quanTIseq. QuanTIseq is a computational pipeline for the quantification of the tumor immune contexture from human RNA-seq data. QuanTIseq takes FASTQ files of RNA-seq reads from tumor samples or other cell mixtures and quantifies the proportions of 10 different immune cell types via deconvolution present in the heterogeneous sample.

Statistical Analysis

The molecular features of HPV+ and HPV− tumors were compared. Categorical data were assessed using a χ^2 or Fisher exact test, where appropriate. Immune cell abundance in the tumor micro-environment was estimated using the method described above (Fintello 2019, Genome Medicine) and significance was tested using a nonparametric Wilcoxon rank-sum test. Gene expression for immune checkpoint genes was normalized to the median gene expression in the control group and fold change was calculated; significance was tested using a nonparametric Wilcoxon rank-sum test. *P*-values were adjusted for multiple hypothesis testing by Bonferroni (continuous) or Benjamini-Hochberg (categorical). All statistical analyses were 2-sided at a significance level set to 0.05.

RESULTS

Entire Cohort

Four hundred forty-three VSC tumors were included in the analysis. Three hundred four (68.6%) tumors were from a local/regional site and 139 (31.4%) were from a distant (or non-GYN) site (Table 1). The median age was 66 yr old, and ranged from 30 to 90 yr old.

The top 10 most commonly mutated genes were *TP53* (238/433, 55%), *CDKN2A* (113/380, 29.7%), *TERT* promoter (45/239, 18.8%), *PIK3CA* (82/440, 18.6%), *FAT1* (16/105, 15.2%), *NOTCH1* (11.2%), *KMT2D* (38/366, 10.4%) *KMT2C* (29/313, 9.27%), *FBXW7* (29/416, 6.97%), and *HRAS* (23/431, 5.34%), as seen in Figures 1A and B.

The most commonly amplified genes were *FGF3* (27/348, 7.76%), *FGF19* (27/357, 7.56%), *CCND1* (26/360, 7.22%), *EGFR* (22/361, 6.09%), *FGF4* (21/361, 5.82%), *NFIB* (18/357, 3.98%), *CD274* (14/360, 3.89%), *PDCD1LG2* (14/361, 3.88%), and *JAK2* (13/339, 3.83%), as seen in Figures 1A and B.

The 5 most commonly altered pathways, combining gene mutations and amplifications, were TP53 (239/439, 54.4%), cell cycle (130/440, 31.6%), RTK RAS (121/440, 27.5%), chromatin remodeling (CR; 103/440, 23.4%) and PI3K (101/440, 23%) (Fig. 1C).

When examining IO-related biomarkers, VSC tumors had low dMMR/MSI-H status (5/359, 1.39%). Eleven percent (11.2%) of VSC tumors had high TMB (42/374). VSC tumors had high PD-L1 positivity (352/427, 82.4%) (Fig. 1D).

Supplementary Table 1 (Supplemental Digital Content 1, http://links.lww.com/IJGP/A141) depicts the most common genomic differences between local/regional and distant VSC tumor samples. There were no significant differences in gene mutations, gene amplifications, pathways altered, or any of the biomarkers related to IO-therapy.

HPV+ Versus HPV−

Next, where available, we examined the molecular and immune landscape of VSC tumors by HPV 16/18 status (HPV+ or HPV−) and *TP53* mutation status (p53mt or p53wt). Thirty-nine of 105 tumor samples evaluated for HPV status were HPV+ (37.1%). No *TP53* mutations were seen in the HPV+ tumors (0 of 39). Among the 66 HPV− tumors, 52 (78.8%) were HPV−/p53mt and 14 (21.2%) were HPV−/p53wt.

Within this analysis, *CDKN2A* mutations were found to be unique to HPV− tumors and were seen more frequently in the p53mt compared with p53wt group (51.9% HPV−/p53mt, 14.3% HPV−/p53wt, 0.0% HPV+, *q*-value <0.001). HPV+ tumors had a significantly increased number of mutations in the *KMT2C* gene (1.9% HPV−/p53mt, 7.1% HPV−/p53wt, 25.6% HPV+, *q*-value <0.028), as well as more frequent alterations in the CR pathway (52.6% HPV+ vs. 28.6% HPV−/p53wt vs. 17.3% HPV−/p53mt, *q*-value 0.0386), but lower alterations in the telomerase maintenance pathway (7.9% HPV+ vs. 57.1% HPV−/p53wt vs. 65.4% HPV−/p53mt, *q*-value <0.001). Interestingly, the HPV−/p53wt cohort had a significantly higher rate of mutations in the *PI3KCA* gene (42.9% HPV−/p53wt vs. 26.32% HPV+ vs.

TABLE 1. *Patient demographics*

Characteristics	All	All with known HPV status	HR HPV+	HR HPV−/TP53mt	HR HPV−/TP53wt
N (%)	443	105 (100)	39 (37.1)	52 (49.5)	14 (13.3)
Age, median (range)	66 (30–90)	64 (30–90)	62 (30–83)	66 (34–90)	61.5 (48–84)
Biopsy site					
Local/regional, N (%)	304 (68.6)	76 (72.4)	29 (74.4)	37 (71.2)	10 (71.4)
Distal, N (%)	139 (31.4)	29 (27.6)	10 (25.6)	15 (28.8)	4 (28.6)

HPV indicates human papilloma virus.

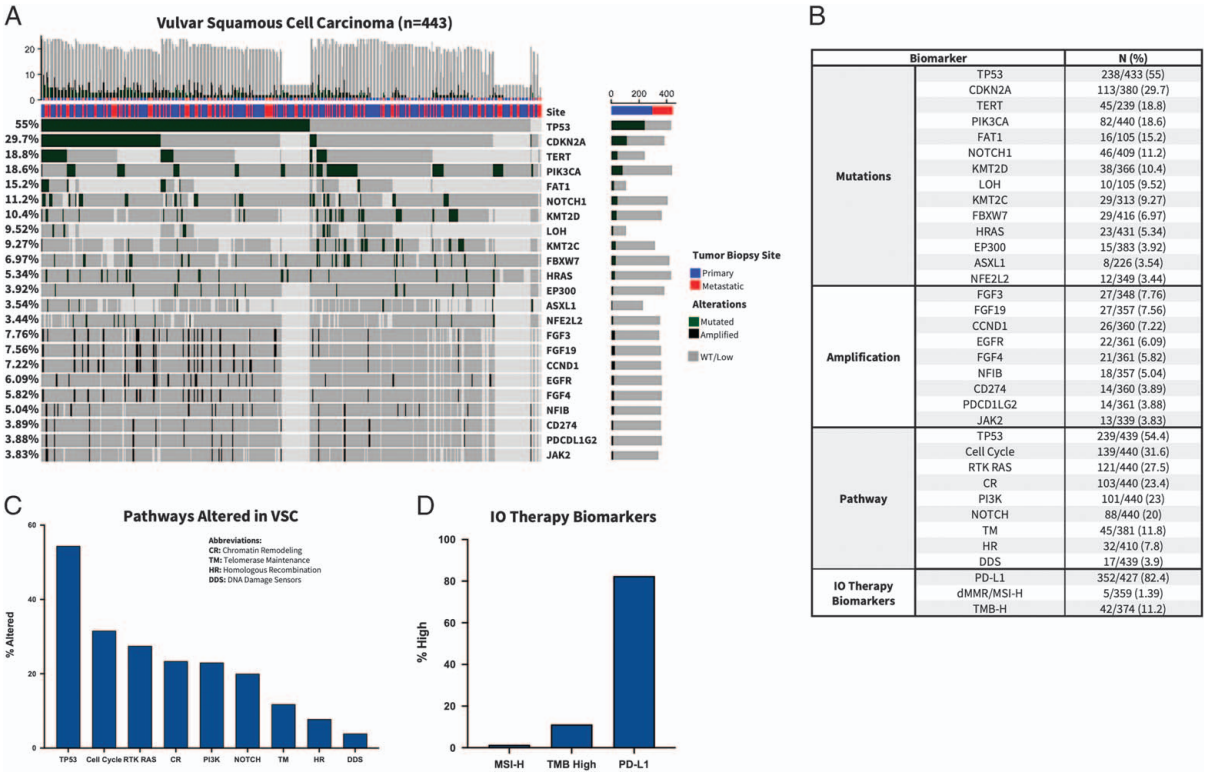
5.77% HPV−/p53mt, *q*-value 0.028) and alterations in the PI3K/AkT/mTOR pathway (57.1% HPV−/p53wt vs. 34.2% HPV+ vs. 7.7% HPV−/p53mt, *q*-value 0.0386) than the other 2 cohorts (Figs. 2A, B, D).

When comparing IO-therapy related biomarkers, there was no significant difference in dMMR/MSI-H, TMB, or PD-L1 positivity (Figs. 2C, D).

P53 Wild Type Versus P53 Mutant

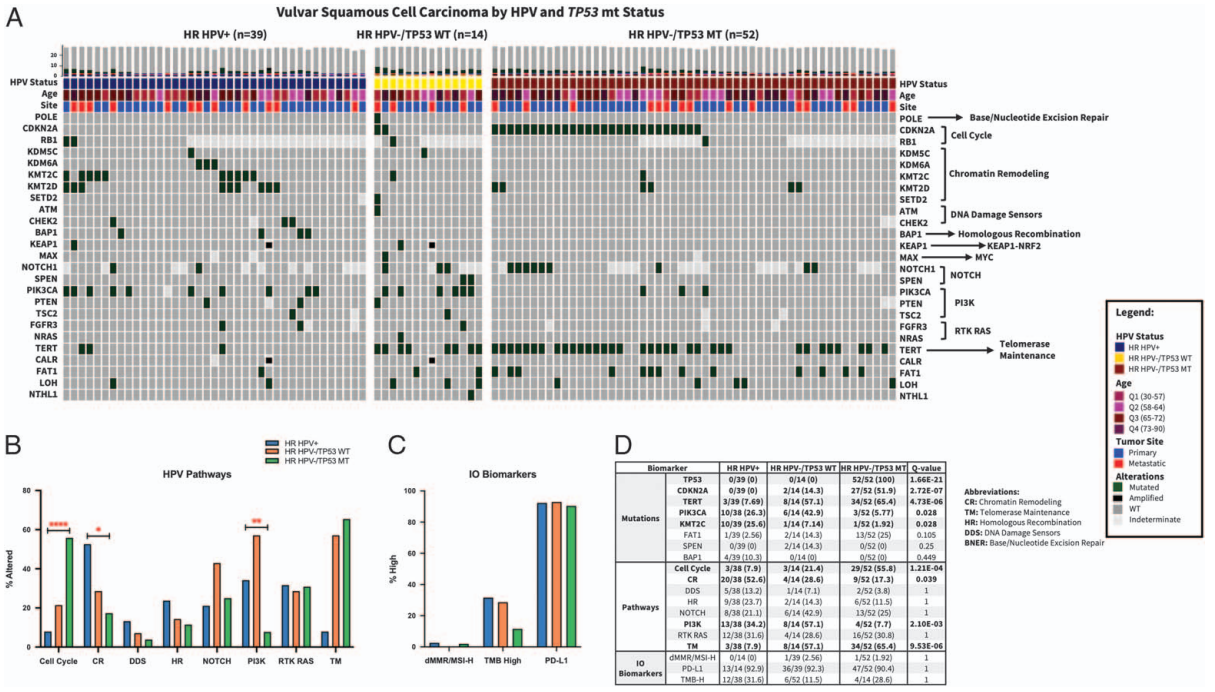
Given the proposed prognostic significance related to tumor p53 status in VSC and the mutual exclusivity of *TP53* mutation status to HR HPV− tumors, we performed a subanalysis stratified by p53 mutation status (without IHC), regardless of HPV status (Supplemental Table 2, Supplemental Digital Content 1, http://links.lww.com/IJGP/A141).

CDKN2A was significantly more frequently mutated in p53mt samples compared with p53wt tumors (50.4% vs. 3.7%, $q < 0.001$). Tumors with p53wt had a significantly higher rate of mutations in *KMT2C*, *PIK3CA*, *KMT2D*, *BAP1*, and *FGFR3* than in p53mt samples. *CCND1* (11.5% vs. 1.36%, $q = 0.007$) and FGF19 (11.1% vs. 2.76%, $q = 0.070$) were more often



Biomarker		N (%)
Mutations	TP53	238/433 (55)
	CDKN2A	113/380 (29.7)
	TERT	45/239 (18.8)
	PIK3CA	82/440 (18.6)
	FAT1	16/105 (15.2)
	NOTCH1	46/409 (11.2)
	KMT2D	38/366 (10.4)
	LOH	10/105 (9.52)
	KMT2C	29/313 (9.27)
	FBXW7	29/416 (6.97)
	HRAS	23/431 (5.34)
	EP300	15/383 (3.92)
	ASXL1	8/226 (3.54)
	NFE2L2	12/349 (3.44)
Amplification	FGF3	27/348 (7.76)
	FGF19	27/357 (7.56)
	CCND1	26/360 (7.22)
	EGFR	22/361 (6.09)
	FGF4	21/361 (5.82)
	NFIB	18/357 (5.04)
	CD274	14/360 (3.89)
	PDCD1LG2	14/361 (3.88)
	JAK2	13/339 (3.83)
Pathway	TP53	239/439 (54.4)
	Cell Cycle	139/440 (31.6)
	RTK RAS	121/440 (27.5)
	CR	103/440 (23.4)
	PI3K	101/440 (23)
	NOTCH	88/440 (20)
	TM	45/381 (11.8)
	HR	32/410 (7.8)
	DDS	17/439 (3.9)
IO Therapy Biomarkers	PD-L1	352/427 (82.4)
	dMMR/MSI-H	5/359 (1.39)
	TMB-H	42/374 (11.2)

FIG. 1. Molecular landscape of VSC. (A) Oncoprint showing the most common gene mutations and gene amplifications in VSC. (B) Table showing the mutation, gene amplification, and pathway alterations prevalence in VSC (N altered/total, % altered). (C) Most commonly altered pathways (by mutation and gene amplification) in VSC. (D) IO therapy-related biomarkers in VSC. dMMR/MSI-H was calculated by IHC, fragment analysis, and NGS. TMB high was determined by a cut-off of ≥10 mutations per Mb. PD-L1 was tested by IHC using clones 22c3 and SP142 (cut-off ≥1%). dMMR indicates mismatch repair deficient; MSI-H, microsatellite instability-high; NGS, next-generation sequencing; TMB, tumor mutational burden; VSC, vulvar squamous cell carcinoma.



Biomarker		HR HPV+	HR HPV-/TP53 WT	HR HPV-/TP53 MT	Q-value
Mutations	TP53	0/39 (0)	0/14 (0)	52/52 (100)	1.66E-21
	CDKN2A	0/39 (0)	2/14 (14.3)	27/52 (51.9)	2.72E-07
	TERT	3/39 (7.69)	8/14 (57.1)	34/52 (65.4)	4.73E-06
	PIK3CA	10/38 (26.3)	6/14 (42.9)	3/52 (5.77)	0.028
	KMT2C	10/39 (25.6)	1/14 (7.14)	1/52 (1.92)	0.028
	FAT1	1/39 (2.56)	2/14 (14.3)	13/52 (25)	0.105
	SPEN	0/39 (0)	2/14 (14.3)	0/52 (0)	0.25
	BAP1	4/39 (10.3)	0/14 (0)	0/52 (0)	0.449
Pathways	Cell Cycle	3/38 (7.9)	3/14 (21.4)	29/52 (55.8)	1.21E-04
	CR	20/38 (52.6)	4/14 (28.6)	9/52 (17.3)	0.039
	DDS	5/38 (13.2)	1/14 (7.1)	2/52 (3.8)	1
	HR	9/38 (23.7)	2/14 (14.3)	6/52 (11.5)	1
	NOTCH	8/38 (21.1)	6/14 (42.9)	13/52 (25)	1
	PI3K	13/38 (34.2)	8/14 (57.1)	4/52 (7.7)	2.10E-03
	RTK RAS	12/38 (31.6)	4/14 (28.6)	16/52 (30.8)	1
	TM	3/38 (7.9)	8/14 (57.1)	34/52 (65.4)	9.53E-06
IO Biomarkers	dMMR/MSI-H	0/14 (0)	1/39 (2.56)	1/52 (1.92)	1
	PD-L1	13/14 (92.9)	36/39 (92.3)	47/52 (90.4)	1
	TMB-H	12/38 (31.6)	6/52 (11.5)	4/14 (28.6)	1

FIG. 2. Molecular landscape of HR HPV+/− vulvar squamous cell carcinoma (VSC). (A) Oncoprint showing the most commonly altered genes, organized by a pathway in VSC, stratified by HR HPV and TP53 mutation status. (B) Most common pathway alterations in VSC are stratified by HR HPV and TP53 mutation status. (C) IO therapy-related biomarker high % composition in VSC, stratified by HR HPV and TP53 mutation status. HPV indicates human papillomavirus; IO, immunotherapy.

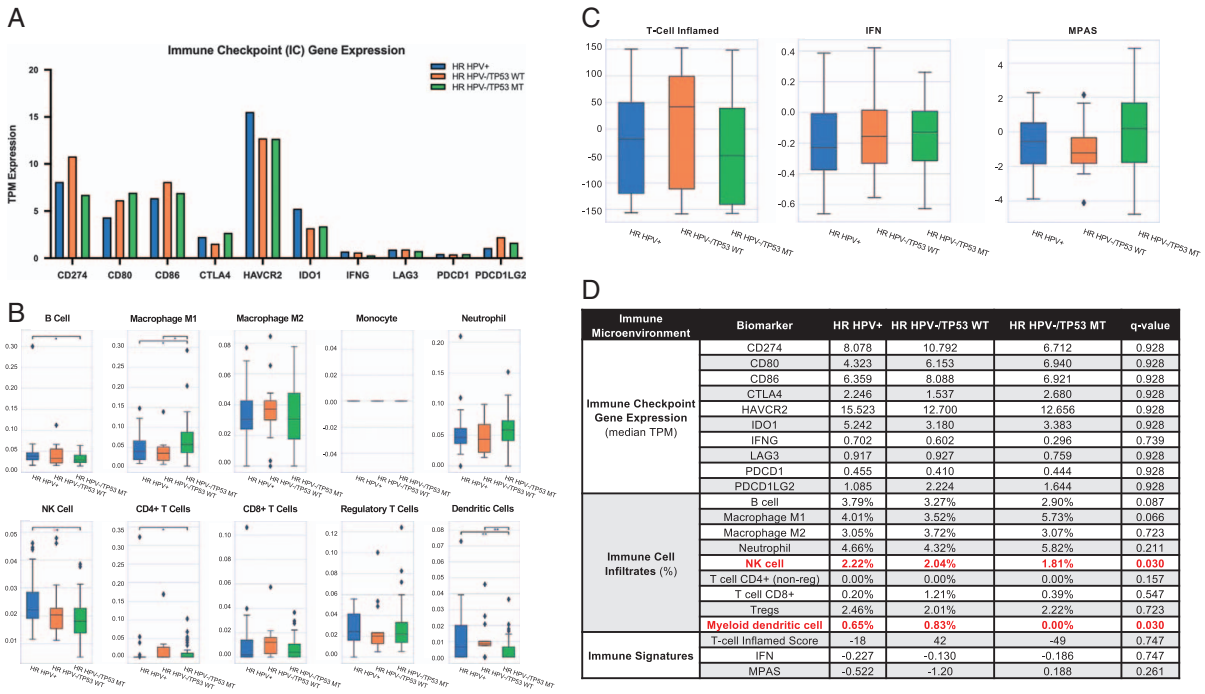
amplified in *p53*mt compared with *p53*wt groups. Lastly, the CR (35.6% vs. 13.9%, $q<0.001$), and PI3K/AkT/mTOR pathways (34% vs. 14.3%, $q<0.001$) were more often altered in the p53wt group compared with p53mt group.

Immune Cell Landscape

Ninety-eight VSC tumors with known HPV status underwent transcriptomic analysis and the immune deconvolution method, quanTIseq, to elucidate types of the immune cells identified within the tissue. When comparing HPV+ to HPV−/p53mt, and HPV −/p53wt, there were no significant differences in the relative abundance of B cells, macrophages M1/M2, neutrophils, CD4+ T cells, CD8+ T cells, and regulatory T cells, but there was a significant increase in natural killer (NK) cells (2.22% vs. 2.04% vs. 1.81%) and myeloid dendritic cells (0.65% vs. 0.83% vs. 0%) in HPV+ tumors compared with HPV− tumors regardless of p53 status (Fig. 3A). There were no significant differences in immune checkpoint gene expression of *CD274*, *CD80*, *CD86, CTLA4, HAVCR2/TIM3, IDO1, IFNG, LAG3, PDCD1*, or *PDCD1LG2* between HPV+, HPV− and p53wt, HPV − and p53mt VSC tumors (Figs. 3B, D). In addition, between the 3 groups, there were no significant differences in T-cell inflamed score, IFN score, or MAPK activation score (Figs. 3C, D).

DISCUSSION

The classification of VSC has rapidly evolved beyond histology-based descriptors and is pivoting toward pathogenic molecular-based identifiers, paralleling the advances seen in other gynecologic cancers. Traditionally, VSC has been described as either HPV-associated or HPV-independent. Only recently have analyses shown that there are likely at least 3 clinically meaningful subtypes: HPV-associated, p53-associated, and other (15). We set out to further characterize the largely unexplored non-HPV and non-P53 mutant VSC tumors using NGS, and found striking differences in the exome of VSC tumors when stratified by HPV and p53 status. In the HPV− VSC, we validated previous findings that these tumors usually contain more genomic alterations, especially in the CDK2NA gene, as well as with TERT, p53, and FAT1 genes when compared with HPV+ tumors (9,16). Our study also identified a novel association between HPV+ tumors and genetic alterations in *KMT2C*. *KMT2C* is involved in epigenetic



D

Immune Microenvironment	Biomarker	HR HPV+	HR HPV-/TP53 WT	HR HPV-/TP53 MT	q-value
Immune Checkpoint Gene Expression (median TPM)	CD274	8.078	10.792	6.712	0.928
	CD80	4.323	6.153	6.940	0.928
	CD86	6.359	8.088	6.921	0.928
	CTLA4	2.246	1.537	2.680	0.928
	HAVCR2	15.523	12.700	12.656	0.928
	IDO1	5.242	3.180	3.383	0.928
	IFNG	0.702	0.602	0.296	0.739
	LAG3	0.917	0.927	0.759	0.928
	PDCD1	0.455	0.410	0.444	0.928
	PDCD1LG2	1.085	2.224	1.644	0.928
Immune Cell Infiltrates (%)	B cell	3.79%	3.27%	2.90%	0.087
	Macrophage M1	4.01%	3.52%	5.73%	0.066
	Macrophage M2	3.05%	3.72%	3.07%	0.723
	Neutrophil	4.66%	4.32%	5.82%	0.211
	NK cell	**2.22%**	**2.04%**	**1.81%**	**0.030**
	T cell CD4+ (non-reg)	0.00%	0.00%	0.00%	0.157
	T cell CD8+	0.20%	1.21%	0.39%	0.547
	Tregs	2.46%	2.01%	2.22%	0.723
	Myeloid dendritic cell	**0.65%**	**0.83%**	**0.00%**	**0.030**
Immune Signatures	T-cell Inflamed Score	-18	42	-49	0.747
	IFN	-0.227	-0.130	-0.186	0.747
	MPAS	-0.522	-1.20	0.188	0.261

FIG. 3. Immune microenvironment of vulvar squamous cell carcinoma (VSC), stratified by HR HPV and TP53 mutation status. (A) Median immune checkpoint gene expression in VSC, stratified by HR HPV and TP53 mutation status. (B) Immune cell infiltrates in VSC, stratified by HR HPV and TP53 mutation status. (C) Immune signatures in VSC, stratified by HR HPV and TP53 mutation status. HPV indicates human papillomavirus.

changes through histone modification and is most thoroughly described in hematologic malignancies. Overall, our exome analysis illustrated genomic features separating the following 3 cohorts: HPV+, HPV −/p53wt, and HPV−/p53mt.

Mutations in *PIK3CA* genes, and alterations to the PI3K/AkT/mTOR pathway, were found to be most strongly associated with HPV−/p53wt associated tumors, which has been inconsistently described among prior genomic evaluations of VSC (6,13, 17–20). The PI3k/AkT/mTOR pathway is well established in its role in carcinogenesis and tumor progression of cervical, endometrial, and ovarian cancers, but this role had not been demonstrated in VSC (21). This finding may present a unique opportunity to address the significant proportion of VSC tumors that are HPV− and p53wt.

Standard treatment for locally advanced VSC is surgery and/or radiation with or without cytotoxic chemotherapy, usually regardless of histology or molecular subtypes (2,22). This is an important area of research given the significant morbidity and disfiguration associated with the surgical management of vulvar cancers, especially in locally advanced diseases, which may be mitigated by an improvement in drug therapies (23).

VSC has been described as the "forgotten woman's cancer" when it comes to precision medicine, as most clinical trials of targeted therapies have not included meaningful enrollment of patients with VSC (17). Our study adds to emerging data supporting the theoretical benefits of targeted therapy in VSC, particularly with mTOR inhibitors (6,24). Although there has been limited benefit of mTOR inhibitor used in cervical cancer treatments, our findings support the hypothesis that vulvar cancer is unique in its genomic alterations, especially when not associated with HPV (25). Furthermore, there are several studies showing prognosis of VSC is highly dependent on HPV and p53 status, with p53mt tumors having a poorer prognosis than p53wt tumors (15). However, within this p53wt group, our findings demonstrate a significantly more heterogenous genomic makeup than previously thought, a finding that could be explored and exploited.

The relationship between PD-1/PD-L1 expression, TMB, and MSI is unclear (14,26,27); each has been identified as a possible independent predictor of response to IOs across many cancer types (28–30). Although vulvar cancers are often included in many of the IO "basket" studies, they are usually too few in number to draw meaningful conclusions related to

vulvar cancer specifically. PD-L1 expression has been found to be highly prevalent in vulvar cancers while maintaining a low overall TMB (26). The current study found 82.4% of all vulvar cancers, regardless of HPV status, were PD-L1 positive. This is in stark contrast to the recent study by Williams et al. (25) that found PD-L1 status was greatly increased in HPV− VSC tumors (34%) compared with HPV+ tumors (9%). The differences in prevalence seen may be attributed to the limited number of samples in their cohort with PD-L1 status, n = 21, compared with ours, n = 427. There were an overall low number of MSI/dMMR (1.39%) and TMB-high (11.2%) tumors.

Previous studies have shown an association between HPV+ tumor status, tumor immune microenvironment, and the positive response to IOs (31). There is evidence that the immune microenvironment is a prognosticator in solid cancers and may portend a good response to immunomodulators (31–33). Here, we analyzed the available VSC tumors with immune cell data stratified by HPV and p53 status. Of the 98 tumors with RNAseq data, there was a small but significant variation among the proportion of NK cells and myeloid dendritic cells. NK cells are a part of the innate immune system, which plays a major role in the regulation of oncogenesis, especially in the early stages (34). NK modulators are currently being explored in a variety of solid cancers, although none are FDA approved for gynecologic cancers at this time. Otherwise, we found the immune cell makeup was fairly similar across the subtypes of VSC defined in this paper.

The prevalence of HPV in our cohort with known HPV status was 37.1%, which falls within the wide range reported by recent systematic reviews (35). Ultimately, the true prevalence of HPV and its specific strains is not known in VSC tumors. Gargano et al. (36) analyzed 176 patients for 37 strains of HPV and found HPV-16 was the dominant type in VSC, consisting of 48% of cases. This prevalence seems low when compared to the study by Williams et al. (25) that found HPV-16 was found in 86% of cases of HPV-associated VSC. The prevalence of HPV-16 and 18 has decreased in cervical screening following the expansion of HPV vaccination programs, corroborating the recent findings that high-grade vulvar precancer events have also decreased with the implementation of HPV vaccination (37,38). Ostensibly, this may translate into a decrease in HPV+ VSC in the future. With a possible decrease in HPV+ VSC, HPV-independent VSC tumors may become the more prevalent type. Our study lays the groundwork for identifying possible targetable mutations in this emerging era of VSC.

Strengths and Limitations

VSC is a rare disease and usually requires multi-institutional collaboration to gather enough samples for any robust analysis. Our project has one of the largest cohorts to undergo NGS and includes samples from as many as 171 institutions. This may explain some differences between our study and other contemporary genomic analyses done on VSC, which are usually single-institution. A recent analysis of the MSK-IMPACT database found *PIK3CA* mutations to be strongly associated with HPV+ tumors, a finding discordant with our study. However, their cohort included combined vulvar and vaginal cases and only contained 4 HPV+ vulvar cancers (16). Another strength of our study is the use of WES. This allows the mapping of nearly all of the RNA-producing DNA at greater depth and coverage than whole genome sequencing.

There are 2 significant limitations to our study that should be addressed. First, HPV status was not available for the entire cohort and our data only includes HPV types 16 and 18. Although these are the agreed-upon dominant strains of HPV-driven VSC, there is a lack of concrete knowledge of the prevalence of other HPV strains by type in VSC. Therefore, some rare non-16/18 HPV+ tumors may have been included in our HPV− cohorts (32,39,40). Lastly, tumor samples undergoing NGS are usually recurrent or advanced stage, and sometimes with prior treatment exposure, which may decrease external validity.

CONCLUSION

Analysis of VSC WES suggests that there are (at least) 3 types of genetically distinct tumors: HPV+/p53wt, HPV −/p53mt, and HPV−/p53wt. TP53 and *CDKN2A* mutations in VSC appear limited to HPV− tumors. Comparing these 3 groups of VSC, HPV−/p53wt has significantly higher PI3K/AkT/mTOR pathway activity, while HPV−/TP53mt has a significantly higher activity of telomerase maintenance and cell cycle pathways. Patients with HPV− and p53wt VSC may benefit from enrollment in clinical trials assessing the efficacy of mTOR inhibitors. Although we eagerly await the ongoing trials assessing IOs in vulvar cancer, future prospective studies of VSC should take into account general genomic descriptors as well as HPV, p53, PD-L1, TMB, MSI, and the immune cell landscape status.

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