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Cancer Diagnostics and Molecular Pathology

Comprehensive Genomic Profiling of Advanced Penile Carcinoma Suggests a High Frequency of Clinically Relevant Genomic Alterations

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Penile cancer • Sequencing • Targeted therapy • Mutation • Genomic profiling • Human papillomavirus

Abstract

Background. Advanced penile squamous cell carcinoma (PSCC) is associated with poor survival due to the aggressiveness of the disease and lack of effective systemic therapies. Comprehensive genomic profiling (CGP) was performed to identify clinically relevant genomic alterations (CRGAs).

Materials and Methods. DNA was extracted from 40 μm of formalin-fixed, paraffin-embedded sections in patients with advanced PSCC. CGP was performed on hybridization-captured, adaptor ligation-based libraries to a mean coverage depth of 692× for 3,769 exons of 236 cancer-related genes plus 47 introns from 19 genes frequently rearranged in cancer. CRGAs were defined as genomic alterations (GAs) linked to targeted therapies on the market or under evaluation in mechanism-driven clinical trials.

Results. Twenty male patients with a median age of 60 years (range, 46–87 years) were assessed. Seventeen (85%) cases were stage IV and three cases (15%) were stage III. CGP revealed 109 GAs (5.45 per tumor), 44 of which were CRGAs (2.2 per tumor). At least one CRGA was detected in 19 (95%) cases, and the most common CRGAs were *CDKN2A* point mutations and homozygous deletion (40%), *NOTCH1* point mutations and rearrangements (25%), *PIK3CA* point mutations and amplification (25%), *EGFR* amplification (20%), *CCND1* amplification (20%), *BRCA2* insertions/deletions (10%), *RICTOR* amplifications (10%), and *FBXW7* point mutations (10%).

Conclusion. CGP identified CRGAs in patients with advanced PSCC, including *EGFR* amplification and *PIK3CA* alterations, which can lead to the rational administration of targeted therapy and subsequent benefit for these patients. ***The Oncologist*** 2016; 21:1–7

Implications for Practice: Few treatment options exist for patients with advanced penile squamous cell carcinoma (PSCC). Outcomes are dismal with platinum-based chemotherapy, with median survival estimated at 1 year or less across multiple series. Biological studies of patients with PSCC to date have principally focused on human papillomavirus status, but few studies have elucidated molecular drivers of the disease. To this end, we performed comprehensive genomic profiling in a cohort of 20 patients with advanced PSCC. Findings of frequent mutations in *CDKN2A*, *NOTCH1*, *PIK3CA*, and *EGFR* (all in excess of 20%) point to potential therapeutic avenues. Trials of targeted therapies directed toward these mutations should be explored.

Introduction

In 2015, the incidence of penile cancer in the United States is estimated to be 1,800, and approximately 320 deaths will occur because of the disease [1]. However, the annual incidence groups penile squamous cell carcinoma (PSCC) cases of different stages, for which survival varies markedly. In a large series, patients with stage I disease have an estimated 10-year survival of 89% [2]. In contrast, patients with penile cancer with metastatic disease have only an estimated survival of 21% at 2 years [2].

Despite the poor prognosis of patients with advanced PSCC, National Comprehensive Cancer Network guidelines are predicated largely on small prospective studies or

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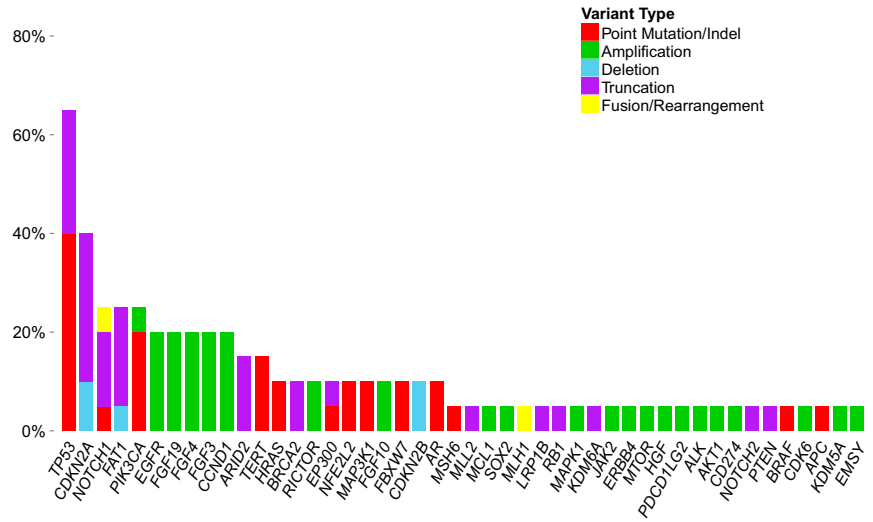

Figure 1. Frequency of genomic alterations in advanced penile cancer. Long-tail plot outlining all observed clinically relevant genomic alterations in a series of 20 patients with advanced penile cancer.

retrospective series rather than randomized trials because of the rarity of the disease [3]. Systemic therapy is typically chemotherapy; data from a single-arm phase II trial supports the use of paclitaxel, ifosfamide, and platinum (TIP) as neoadjuvant therapy for patients with clinical evidence of nodal involvement [4]. For metastatic disease, TIP is also the preferred first-line regimen, although historical regimens such as 5-fluorouracil with cisplatin may also be considered [5]. Cetuximab has been seen to be active in a subset of patients with PSCC; one patient in one non-epidermal growth factor receptor (EGFR)-stratified series had a response rate of 23.5% to cetuximab with chemotherapy backbone [6–8]. *EGFR* expression was assayed in four other patients, with a range from 1+ to 3+, but expression did not correlate well with response [6–8].

A challenge in developing novel systemic strategies for penile cancer is the limited understanding of the heterogeneity of the oncogenic drivers of disease, particularly human papillomavirus (HPV) infection. HPV has been associated with 50%–70% of cases of squamous penile cancer across multiple series, but these studies amalgamate early, and patients with late-stage PSCC who likely have distinct clinicopathologic features and biology [9–14]. Broader molecular studies of penile carcinoma have been performed in a limited series of patients, but interpretation is again confounded by heterogeneity of the stage of disease and diagnostic methodologies used [15, 16]. Herein, we report results from comprehensive genomic profiling (CGP) in a cohort of patients with advanced penile cancer.

Materials and Methods

Formalin-fixed, paraffin-embedded (FFPE) slides or blocks were obtained for a series of 20 patients with advanced squamous cell penile cancer. DNA was extracted with a minimum threshold of 20% of DNA derived from tumor. CGP based on targeted next-generation sequencing was performed using hybridization-captured, adaptor ligation-based libraries in a Clinical Laboratory Improvement Amendments-certified laboratory (Foundation Medicine, Inc., Cambridge, MA). DNA was extracted from 40 μM of FFPE sections in patients with advanced PSCC. Cases were sequenced with a median coverage exceeding 500× across 3,769 exons in 236 cancer-related genes and 47 introns derived from 19 genes frequently rearranged in cancer. Bayesian algorithms were used to detection substitutions. Comparison with healthy control specimens was used to ascertain copy number alterations. Finally, local assembly algorithms were used to detect insertions and deletions. The genomic alterations (GAs) linked to approved or investigational targeted agents were termed clinically relevant genomic alterations (CRGAs). Only deidentified data were used for the current analysis. Pathologic stage was determined by review of available medical records. Local site permissions were used for this study.

Results

Among the 20 male patients included in the analysis, the median age was 60 years (range, 46–87 years). Seventeen patients (85%) had stage IV disease, and the remaining three (15%) had stage III disease. CGP revealed 109 GAs, with an average of 5.45 GAs per patient, and of the 109 GAs, 44 GAs (40%) were CRGAs, occurring at a mean frequency of 2.2 per case. *TP53* was mutated in 13 cases (65%) and was the most common GA (Fig. 1). At least one CRGA was detected in 19 patients, and the most common CRGAs were *CDKN2A* (8 patients [40%]), *NOTCH1* (5 patients [25%]), *PIK3CA* (5 patients [25%]), *CCND1* (4 patients [20%]), *EGFR* amplification (4 patients [20%]), *BRCA2* insertions/deletions (2 patients [10%]),*RICTOR* amplifications (2 patients [10%]) and *FBXW7* point mutations (2 patients [10%]) (Table 1). Less frequent alterations in our series included FGF amplification and mutation of chromatin remodeling genes (e.g.,

Table 1. Clinicopathologic features and genomic alterations in 20 patients with penis squamous cell carcinoma

Patient	Tissue of origin	Age	HPV status	Median exon depth	Known somatic short variants	Likely somatic short variants	Known CNAs	Likely rearrangements
1	Lymph node	62	No	1,103	None	*BRCA2*:NM_000059:c.227C>G_p.S76*(0.09,1252)	None	NOTCH1_INPP5E_truncation_106
2	Penis	46	No	736	*CDKN2A*:NM_000077:c.238C>T_p.R80*(0.51,394), *HRAS*:NM_005343:c.38G>T_p.G13V(0.47,725), *PIK3CA*:NM_006218:c.3140A>G_p.H1047R(0.27,757)	*ARID2*:NM_152641:c.2340_2364delAGGACAGATCCCTTCAGGCACTCCT_p.G781fs*3(0.38,723), *ARID2*:NM_152641:c.5371_5374delAAGA_p.K1791fs*9(0.31,588)	None	
3	Lymph node	64	No	744	*AR*:NM_000044:c.865G>A_p.E289K(1.0,342), *MLL2*:NM_003482:c.11962C>T_p.Q3988*(0.21,723), *PIK3CA*:NM_006218:c.1633G>C_p.E545Q(0.26,618)	*ARID2*:NM_152641:c.3926C>G_p.S1309*(0.22,1034), *CASP8*:NM_001228:c.1356-1G>A_p.splice(0.35,761), *NCOR1*:NM_006311:c.161C>G_p.S54*(0.25,752), *RB1*:NM_000321:c.277C>T_p.Q93*(0.15,470)	None	
4	Lymph node	75	No	510	*CASP8*:NM_001228:c.1066C>T_p.Q356*(0.04,584), TP53:NM_000546:c.646G>A_p.V216M(0.1,390)	*ARID2*:NM_152641:c.5305C>T_p.R1769*(0.05,697), *NOTCH1*:NM_017617:c.4052_4061delCCTGCGGCAG_p.G1353fs*89(0.05,159)	None	
5	Urethra	70	No	731	*MSH6*:NM_000179:c.1168G>A_p.D390N(0.49,946), *NOTCH1*:NM_017617:c.1057C>T_p.R353C(0.12,393), *HRAS*:NM_005343:c.35G>A_p.G12D(0.26,460)	*FBXW7*:NM_033632:c.1627A>G_p.R543G(0.15,780), *FBXW7*:NM_033632:c.502-1G>A_p.splice(0.14,845), *NOTCH1*:NM_017617:c.3120_3121insCGGCTGC_p.G1041fs*26(0.1,207), NOTCH2:NM_024408:c.2563_2564delGA_p.S856fs*17(0.11,777)	None	
6	Skin	46	Yes, HPV-16	663	None	*KDM6A*:NM_021140:c.2041G>T_p.G681*(0.69,412), *BRCA2*:NM_000059:c.1310_1354>TTTTCTTACTTCAGAGAATTCTTTGCC_p.K437_L452>DFLTSENSLP(0.26,729)	FGF4_amplification(10,exons 3 of 3), CCND1_amplification(10,exons 5 of 5), FGF3_amplification(10,exons 3 of 3), FGF19_amplification(10,exons 3 of 3), MCL1_amplification(10,exons 5 of 5)	
7	Lymph node	50	No	656	*FBXW7*:NM_033632:c.1514G>T_p.R505L(0.3,636), *AR*:NM_000044:c.865G>A_p.E289K(1.0,598), *PIK3CA*:NM_006218:c.1633G>A_p.E545K(0.15,505)	None	None	
8	Penis	87	No	725	*CDKN2A*:NM_000077:c.238C>T_p.R80*(0.52,376), *TP53*:NM_000546:c.548C>G_p.S183*(0.43,495), *NFE2L2*:NM_001145412:c.44G>C_p.G15A(0.19,1087)	None	RICTOR_amplification(7,exons 39 of 39), FGF10_amplification(7,exons 3 of 3)	
9	Lymph node	48	No	863	*TP53*:NM_000546:c.586C>T_p.R196*(0.22,1020)	None	FGF4_amplification(11,exons 3 of 3), FGF3_amplification(11,exons 3 of 3), CCND1_amplification(12,exons 5 of 5), FGF19_amplification(11,exons 3 of 3)	
10	Penis	46	No	728	*TP53*:NM_000546:c.404G>T_p.C135F(0.1,686)	*LRP1B*:NM_018557:c.5115-2A>T_p.splice(0.21,733), *TP53*:NM_000546:c.1020_1021insTTCC_p.R342fs*6(0.38,621)	EGFR_amplification(46,exons 30 of 30), FGF3_amplification(21,exons 3 of 3), CCND1_amplification(21,exons 5 of 5), FGF4_amplification(21,exons 3 of 3), FGF19_amplification(21,exons 3 of 3)	
11	Skin	47	No	704	*TP53*:NM_000546:c.659A>G_p.Y220C(0.29,689), *EP300*:NM_001429:c.2773C>A_p.P925T(0.66,540)	None	ALK_amplification(7,exons 29 of 29), SOX2_amplification(15,exons 5 of 5), KDM5A_amplification(13,exons 28 of 28), CDKN2B_loss(0,exons 5 of 5), CDKN2A_loss(0,exons 5 of 5), ERBB4_amplification(8,exons 27 of 28), AKT1_amplification(7,exons 13 of 13), PIK3CA_amplification(15,exons 20 of 20)	MLH1_MLH1_duplication_10

(continued)

Table 1. (continued)

Patient	Tissue of origin	Age	HPV status	Median exon depth	Known somatic short variants	Likely somatic short variants	Known CNAs	Likely rearrangements
12	Penis	55	Yes, HPV-6	519	*CDKN2A*:NM_000077:c.238C>T_p.R80*(0.57,418), *TP53*:NM_000546:c.488A>G_p.Y163C(0.26,543), *TP53*:NM_000546:c.514G>T_p.V172F(0.02,510)	*NOTCH3*:NM_000435:c.1416_1417delGT_p.C473fs*3(0.23,443), *NOTCH3*:NM_000435:c.1378+2_1378+3TG>GT_p.splice site 1378+2_1378+3TG>GT(0.05,277), *NOTCH1*:NM_017617:c.1057_1058insGTGT_p.A355fs*29(0.07,221), *EP300*:NM_001429:c.3671+1G>T_p.splice site 3671+1G>T(0.26,559)	None	
13	Penis	50	No	369	*TERT*:NM_198253:c.-124C>T_p.promoter -124C>T(0.24,38), *CDKN2A*:NM_000077:c.238C>T_p.R80*(0.29,131), *TP53*:NM_000546:c.438G>A_p.W146*(0.31,339)	*CDKN2A*:NM_000077:c.218_236delCCGACCCCGCCACTCTCAC_p.A76fs*64(0.44,205), *TP53*:NM_000546:c.821_844delTTTGTGCCTGTCCTGGGAGAGACC_p.V274_R282>G(0.14,558)	EGFR_amplification(19,exons 30 of 30), FAT1_loss(0,exons 26 of 26)	
14	Lymph node	79	No	452	*TERT*:NM_198253:c.-146C>T_p.promoter -146C>T(0.27,15), *BRAF*:NM_004333:c.1390G>A_p.G464R(0.19,663), *TP53*:NM_000546:c.817C>T_p.R273C(0.23,546), *NFE2L2*:NM_001145412:c.37G>T_p.D13Y(0.27,713), *PIK3CA*:NM_006218:c.1633G>A_p.E545K(0.4,632)	*FAT1*:NM_005245:c.4686_4686delC_p.F1562fs*23(0.2,414)	CDKN2B_loss(0,exons 5 of 5), MTOR_amplification(8,exons 57 of 57), CDKN2A_loss(0,exons 5 of 5)	
15	Lymph node	51	No	445	None	*CDKN2A*:NM_000077:c.234_256del23_p.T79fs*33(0.16,337), *CDKN2A*:NM_000077:c.303_307delGGCGC_p.R103fs*15(0.13,423), *TP53*:NM_000546:c.367_368insCTTG_p.T125fs*25(0.28,382), *PTEN*:NM_000314:c.415_437del23_p.L139fs*33(0.16,484)	MAPK1_amplification(10,exons 8 of 8)	NOTCH1_NOTCH1_deletion_25
16	Penis	73	Yes, HPV-16	447	*TP53*:NM_000546:c.509C>T_p.T170M(0.29,453)	None	EGFR_amplification(9,exons 30 of 30)	
17	Soft tissue	62	No	563	*TERT*:NM_198253:c.-124C>T_p.promoter -124C>T(0.22,36), *MAP3K1*:NM_005921:c.580A>G_p.M194V(0.74,1572), *APC*:NM_000038:c.4901C>T_p.P1634L(0.65,628), *TP53*:NM_000546:c.482C>A_p.A161D(0.27,576)	*FAT1*:NM_005245:c.8205C>A_p.Y2735*(0.3,824)	EGFR_amplification(19,exons 30 of 30)	
18	Penis	62	No	487	*TP53*:NM_000546:c.91G>A_p.V31I(0.49,382), *CDKN2A*:NM_000077:c.238C>T_p.R80*(0.29,179), *MAP3K1*:NM_005921:c.917G>A_p.R306H(0.53,567)	*TP53*:NM_000546:c.641_642delAT_p.H214fs*7(0.14,416), *FAT1*:NM_005245:c.11876_11877insG_p.T3961fs*11(0.12,478), *TP53*:NM_000546:c.672+2T>C_p.splice site 672+2T>C(0.12,381)	None	
19	Penis	68	No	485	*TP53*:NM_000546:c.800G>C_p.R267P(0.27,520)	*FAT1*:NM_005245:c.10679C>G_p.S3560*(0.13,670)	FGF19_amplification(7,exons 3 of 3), EMSY_amplification(6,exons 20 of 20), FGF3_amplification(7,exons 3 of 3), FGF4_amplification(7,exons 3 of 3), CCND1_amplification(7,exons 5 of 5)	
20	Lymph node	60	No	621	None	None	None	

Variants present in the Catalogue of Somatic Mutations in Cancer (COSMIC) that have been previously confirmed somatic are reported as known somatic alterations, whereas nonsynonymous variants in known oncogenes not found in the Single Nucleotide Polymorphism Database or COSMIC are reported as likely somatic variants.
Abbreviations: CNA, circulating nucleic acids; HPV, human papillomavirus.

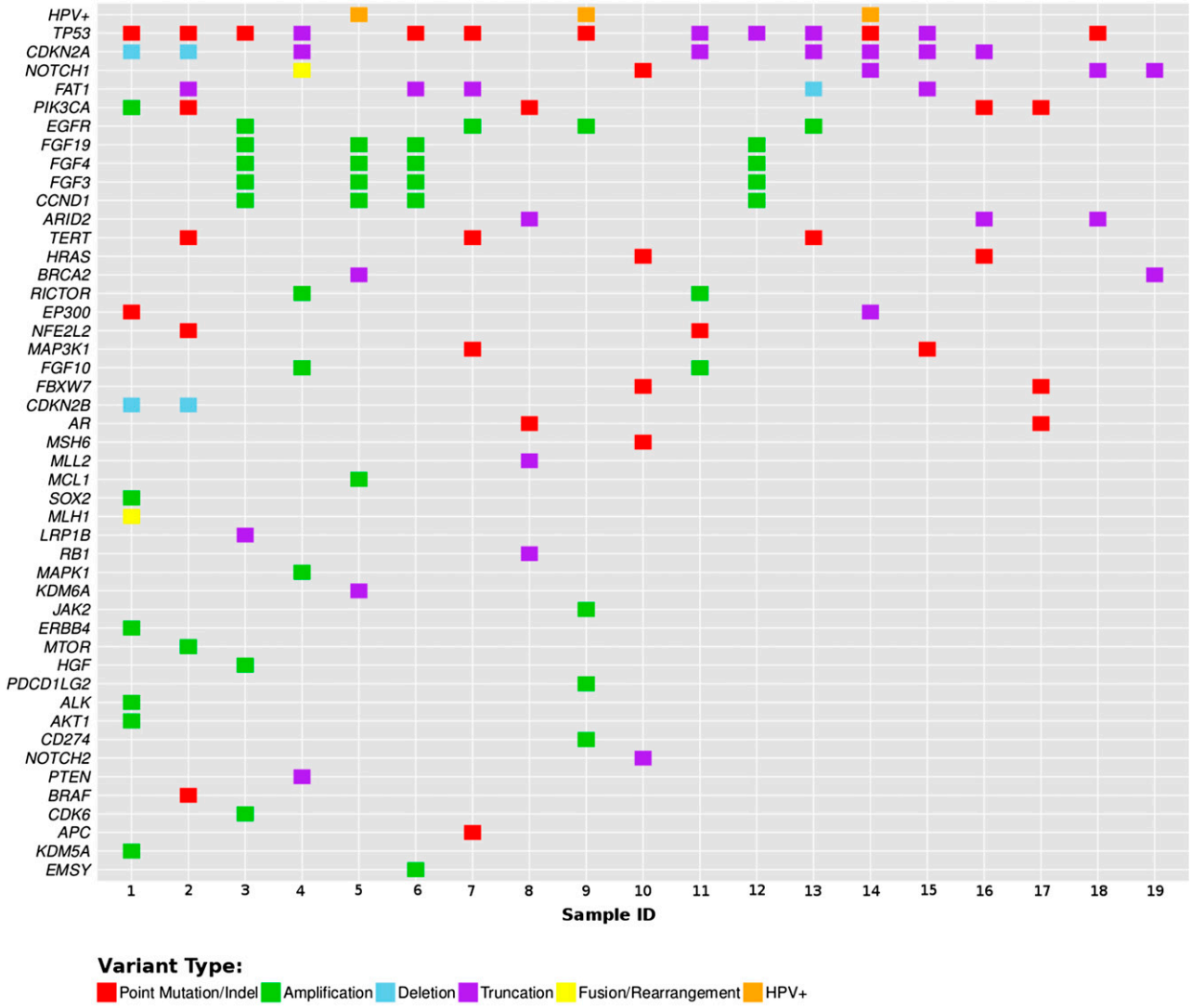

Figure 2. Genomic alterations in patients with advanced penile cancer. Plot detailing all clinically relevant genomic alterations for each patient in a series of 20 patients with advanced penile cancer.

Abbreviations: HPV, human papillomavirus; ID, identifier; PSCC, penile squamous cell carcinoma.

EP300). The 4 patients harboring *EGFR* amplification harbored between 9 and 46 copies, and they did not harbor any alterations of RAS (*HRAS*, *KRAS*, *NRAS*) family members. Alterations in RAS family members were identified in 2 patients (10%) and were *HRAS* G12D and *HRAS* G13D; HPV-16 was detected in 2 patients (10%), HPV-6 was detected in 1 patient (5%), and HPV-18 was not detected in any patients (Table 1; Fig. 2). Of the three patients who were HPV-positive, the patient who was HPV-6 positive (case 9) harbored a *TP53* alteration, and patient 14 harbored a *TP53* alteration and HPV-16 (Fig. 2).

Conclusion

Currently, outcomes for advanced penile cancer are poor, with a 2-year survival of only 21%, and little data exists to guide treatment beyond small nonrandomized studies using platinum-based chemotherapy [2]. The current series of 20 cases represents the largest ever cohort of patients with advanced PSCC assessed with CGP. To that end, CRGAs that could guide the rational use of targeted therapy were observed in all but one case.

Previously, anecdotal reports have described striking clinical benefit with cetuximab in patients with PSCC. For these patients, pretreatment testing of *EGFR* expression was performed with immunohistochemistry, which is qualitative at best, and it is also likely biased by the nonreporting of failure of cetuximab treatment in PSCC [6–8]. The frequency of 20% of patients with PSCC in this series harboring *EGFR* high-level amplification suggests that the basis of occasional benefit from cetuximab is indeed linked to *EGFR* amplification. Moreover, if an analogy is made to the contraindication for anti-EGFR inhibitor treatment in *RAS*-mutated colorectal carcinoma, only 10% of patients with PSCC here harbored RAS family alterations [17].

Twenty-five percent of patients with advanced PSCC harbor alterations in *PIK3CA*, which suggests possible response to inhibitors of the mammalian target of rapamycin

(mTOR)/phosphatidylinositol 3-kinase pathway, such as rapamycin analogs, as observed in other tumor types [18]. One patient also harbored a *FBXW7* alteration, but it was wild type for *PI3KCA*, which also predicts sensitivity to rapamycin analogs such as temsirolimus [19]. Importantly, these patients harboring *PIK3CA* and/or *FBXW7* alterations (30%) did not also harbor *EGFR* amplifications (25%) because these groups of alterations occurred in a mutually exclusive fashion.

Alterations of *CDKN2A*, which encodes p16, occur in 40% of cases, and *CCND1* amplifications occur in 20% of cases [20]. Aberrations in p16 appear to increase sensitization to the novel CDK4/6 inhibitor palbociclib in preclinical models, but this finding has not yet been well borne out in clinical studies [21–24]. Cyclin D1 coactivates CDK4, but a small phase II trial in breast carcinoma did not demonstrate correlation between either p16 status or CCND1 alteration and response to palbociclib [25]. It remains to seen in PSCC whether either of these alterations confer sensitivity to cell cycle inhibitors.

HPV positivity and *TP53* mutation occur in a largely mutually exclusive fashion in squamous cell carcinomas driven by HPV [26–28]. The frequency of high-risk HPV infection in this PSCC series is much lower than cervical carcinoma (95%+) and anal carcinoma (90%+) (Fig. 2), which is consistent with the high frequency (65%) of *TP53* alteration. *PIK3CA* alterations are enriched in squamous carcinomas driven by high-risk HPV, and interestingly are also somewhat enriched in this PSCC series, although occurring solely in patients with a negative HPV test result.

High-risk HPV viruses (16, 18, 6, and 11) are members of the α-HPV family and exclusively infect nonkeratinized squamous mucosa, such as in the cervix, anus, and oropharyngeal tract, but they are currently thought to not infect cutaneous keratinizing squamous epithelium of the skin. PSCC of the foreskin, which has keratinized outer and nonkeratinized inner squamous epithelium, is classically HPV positive with pre-existing small or giant condylomas. This series is likely primarily HPV-negative PSCC of the glans or shaft, given that these are Western patients who have likely undergone circumcision [29]. Understanding the diversity of PSCC genomic profiles and possible correlation to clinicopathologic characterization may add insight into the open question of the role of HPV infection in the oncogenesis of PSCC, particularly in the context of a circumcised versus uncircumcised population.

Whether in the setting of practice or clinical trials, CGP offers the possibility of guiding the rational use of targeted therapy for individuals with advanced PSCC, who have a very poor prognosis under standard care regimens at present. In particular, 55% of patients in this small study harbor either *EGFR* amplification or *PIK3CA*/*FBXW7* alterations, suggesting possible clinical benefit from treatment with cetuximab or mTOR inhibitors, respectively. Ideally, larger cohorts can be assembled to validate the findings of the current study. Furthermore, prospective trials should be designed to determine whether the alterations specified herein predict activity of corresponding targeted therapies.

Acknowledgments

This study was published in conjunction with the 2015 American Society of Clinical Oncology Annual Meeting, Chicago, IL (abstract e15628).

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Disclosures

Siraj M. Ali: Foundation Medicine, Inc. (E); **Sumanta K. Pal:** Novartis, Pfizer, Genentech, Astellas (C/A), Genentech (H); **Kai Wang:** Foundation Medicine, Inc. (E, OI); **Norma A. Palma:** Foundation Medicine, Inc. (E, OI); **Eric Sanford:** Foundation Medicine, Inc. (E, OI); **Mark Bailey:** Foundation Medicine, Inc. (E, OI); **Jie He:** Foundation Medicine, Inc. (E, OI); **Julia A. Elvin:** Foundation Medicine, Inc. (E, OI); **Juliann Chmielecki:** Foundation Medicine, Inc. (E, OI); **Rachel Squillace:** Foundation Medicine, Inc. (E, OI); **Edward Dow:** Baxalta (E), Foundation Medicine, Inc. (E, OI); **Deborah Morosini:** Foundation Medicine, Inc. (E, OI); **Jamie Buell:** Foundation Medicine, Inc. (E, OI); **Roman Yelensky:** Foundation Medicine, Inc. (E, OI); **Doron Lipson:** Foundation Medicine, Inc. (E, OI); **Garret M. Frampton:** Foundation Medicine, Inc. (E, OI); **Jeffrey S. Ross:** Foundation Medicine, Inc. (E, RF, OI); **Philip J. Stephens:** Foundation Medicine, Inc. (E, OI); **Vincent A. Miller:** Foundation Medicine, Inc. (E, OI). The other author indicated no financial relationships.

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board

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