Genetic Landscape of Human Papillomavirus–Associated Head and Neck Cancer and Comparison to Tobacco-Related Tumors

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ABSTRACT

Head and neck cancer is the fifth most common cancer worldwide. It is often amenable to curative intent therapy when localized to the head and neck region, but it carries a poor prognosis when it is recurrent or metastatic. Therefore, initial treatment decisions are critical to improve patient survival. However, multimodality therapy used with curative intent is toxic. The balance between offering intensive versus tolerable and function-preserving therapy has been thrown into sharp relief with the recently described epidemic of human papillomavirus–associated head and neck squamous cell carcinomas characterized by improved clinical outcomes compared with smoking-associated head and neck tumors. Model systems and clinical trials have been slow to address the clinical questions that face the field to date. With this as a background, a host of translational studies have recently reported the somatic alterations in head and neck cancer and have highlighted the distinct genetic and biologic differences between viral and tobacco-associated tumors. This review seeks to summarize the main findings of studies, including The Cancer Genome Atlas, for the clinician scientist, with a goal of leveraging this new knowledge toward the betterment of patients with head and neck cancer.

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INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is the fifth most common nonskin cancer worldwide; the annual incidence is 600,000 cases, and 50,000 cases are diagnosed annually in the United States.^{1,2} Although HNSCC historically was viewed as a tobacco- and alcohol-related cancer, infection with high-risk human papillomaviruses (HPVs) during the past decade has emerged as a risk factor for a substantial fraction of HNSCCs.³ The so-called HPV epidemic is most prominent in patients of Northern European and North American origin, whereas other areas of the world still see few HPV-associated tumors.⁴⁻⁶ In studies that include an assessment of HPV status, a shift in the traditional risk factors is nearly universally noted: viral-associated tumors are seen in younger populations that have lymph node–positive oropharyngeal carcinomas and lower rates of smoking.⁷ Having said this, most patients with HPV-associated oropharyngeal carcinoma are still men in their 50s who have a smoking history, and true nonsmokers constitute only a minority.^{1,3,8-10} Unfortunately, a precise characterization of patients with HNSCC by HPV status is challenging. First, because HPV is not yet part of the staging system in HNSCC, tumor registries, such as

the Surveillance, Epidemiology, and End Results (SEER) program, do not routinely record it. Adding to the knowledge gap is a lack of consensus of diagnostic reagents to detect HPV. Variability in the assays used across studies limits generalizability and comparability of results.^{11,12}

Although morphologic characteristics, such as basal squamous histology, poor differentiation, and absent keratin formation, are associated with HPV infection, the diagnosis of HPV is made through molecular testing. A mystery of the disease is that no precursor lesion on which to screen patients during the long latency has been reported, despite the assumption that viral exposure occurs via sexual contact in young adulthood. Screening HPV molecular assays, including polymerase chain reaction–based techniques, risk false-positive detection of endemic viral infections not associated with cancer, whereas specific *in situ* hybridization (ISH) assays often have lower sensitivity.¹³ A useful proxy for HPV infection in tumors is by immunohistochemistry (IHC) assessment of p16, the protein product of the gene *CDKN2A*.¹⁴ As shown in [Figure 1](#), p16 is a repressor of the D cyclins that, in turn, partially phosphorylate the retinoblastoma tumor-suppressor protein (RB1). In the setting of viral infection, expression of the E7 viral oncoprotein mediates degradation of RB1, and

Fig 1. Role of E6 and E7 in the cell cycle pathways and gene alterations as a function of human papillomavirus (HPV) tumor status. Genes altered in HPV-positive or viral oncogenes (red) or in HPV-negative tumors (black). RB, retinoblastoma tumor-suppressor protein.

pathway feedback results in overexpression of the cell senescence pathways, including p16.¹⁵ The detection of p16 is a proxy for RB1 loss through any mechanism, including mutations of RB1 that are not mediated by E7 or HPV. Therefore, p16 detection can result in the false assumption that E7 is expressed and, by extension, that the tumor is HPV positive.¹⁶ For instances of high pretest probability for HPV, such as in squamous cell carcinomas of the oropharynx, the true-positive rate for p16 as an indication of HPV is high.¹⁴ When the pretest probability is lower, such as in the oral cavity, the true-positive rate decreases to 10%-41%, which renders p16 IHC an ineffective diagnostic tool. If it is assumed that 5% of nonoropharynx tumors are HPV positive, as many as 1,500 cases per year outside the oropharynx may be difficult to assess with p16. Ultimately, direct tests of E6 and E7 may prove superior, but these are not yet routine.¹⁷

Although the demographic shifts may be modest between patients who are HPV positive and HPV negative, clinical outcomes are dramatically different. Although patients with HPV commonly present with advanced nodal disease and clinical stage, prognosis is favorable compared with patients without HPV.³ Improved outcomes are seen across treatment modalities, including chemotherapy, radiation, chemoradiotherapy, and potentially also surgery, which have led to proposals to incorporate HPV status in staging.⁹ Although outcomes are better, the implications for selection of patient therapy by HPV status are not yet clear, because few existing clinical trials assessed HPV status.¹⁸⁻²⁰ Controversies are most pronounced in molecularly targeted therapy, for which little conclusive published data exist, although retrospective studies have been presented in scientific meetings. Two randomized, phase III studies that used anti-epidermal growth factor receptor (EGFR) therapy either combined with chemotherapy or as a single agent versus methotrexate suggest less activity of EGFR agents in p16-positive tumors.^{21,22} An additional phase II study indicated a 0% response rate for single-agent anti-EGFR therapy in HPV-associated HNSCC according to highly accurate E6/E7 RNA-based HPV testing.²³ Conversely, in the EXTREME (Erbix in First-Line Treatment of Recurrent or Metastatic Head and Neck Cancer) study, a nonsignificant trend toward benefit in patients who were positive by p16

and/or ISH was seen, although analysis was limited because of small numbers and the use of p16 IHC for nonoropharynx tumors.²⁴ The phase III trial of cetuximab in combination with radiation favored outcomes in patients with oropharynx tumors in the cetuximab treatment arm.¹⁸ In a retrospective subset analysis, patients with p16-positive and p16-negative oropharynx tumors benefited from cetuximab.²⁵ However, analysis that used HPV nucleic acid testing by ISH did not show a clear benefit, which suggests that p16 expression, rather than HPV status, may be important. In short, controversy remains as to whether the combination of cetuximab with radiation seems to benefit patients with HPV-positive oropharyngeal cancer. By contrast, the data are stronger that patients who are p16 positive for HPV may benefit, although such patients include some HPV-negative, yet p16-positive, tumors. At a molecular level, published studies suggest an anticorrelation between staining for the EGFR receptor and benefit of cetuximab.²⁰ HPV-positive tumors have generally shown low or absent levels of EGFR protein expression or *EGFR* gene amplification.^{1,10} Unfortunately, preclinical models fail to clarify the picture, because HPV-positive cell lines of HNSCC remain in early development. Ongoing trials, such as Radiation Therapy Oncology Group study RTOG 1016 (NCT01302834), may clarify some controversies about cetuximab. Other studies leverage the dramatically improved outcomes for patients with HPV-positive tumors by de-escalating therapy and potentially decreasing toxicity.²⁶

In parallel with lessons learned by clinical trials, rapidly emerging advances in tumor characterization, including DNA sequencing, RNA sequencing, miRNA characterization, epigenetic characterization, and proteomics, are in sight and have been widely reviewed elsewhere.^{1,27-34} Data from large cooperative endeavors, such as The Cancer Genome Atlas (TCGA), and individual efforts, such as the Chicago HNC Genomics cohorts, have illuminated the genome of HNSCC in a manner that reveals the most commonly altered genes and coordinated patterns of genome alteration, which could be leveraged to accelerate our understanding of the disease, improve personalization of existing therapies to increase effectiveness, and propose accelerated strategies for novel therapeutics.^{1,10}

Fig 2. Copy number alterations by chromosome position in human papilloma-virus (HPV) –positive and HPV-negative tumors.

STRUCTURAL ALTERATIONS

Several publications have reported the copy number landscape of HPV-positive versus HPV-negative HNSCC (Fig 2).^{1,27-35} Across much of the genome, the copy number alterations from HPV-positive and HPV-negative tumors are highly concordant, and shared amplifications are 1q, 3q, 5p, 8q, and others. Common deletions include 3p, 5q, 11q, and others. In some instances, comparison of altered regions across studies is challenging because of the small number of patients who are HPV positive. Although many regions are clearly shared, others are strikingly divergent. Recently, a region on chromosome 14q32 was reported showing deep deletions in HPV-positive tumors and few, if any, deletions in HPV-negative tumors.¹ Focal deletions, which include homozygous deletions, are relatively rare in a cancer genome and, when recurrent, are often suggestive of key novel tumor-suppressor genes. In combination with sequencing data, homozygous deletions noted in the TCGA study identified that the tumor-suppressor gene *TRAF3* was inactivated in approximately 20% of HPV-positive tumors; this inactivation had been previously unrecognized in HNSCC, although *TRAF3* loss had been seen in nasopharyngeal carcinoma.^{1,36} *TRAF3* is implicated in innate interferon and acquired antiviral responses, including in Epstein-Barr virus, HPV, and HIV.³⁷⁻⁴⁰ *TRAF3* also serves as a ubiquitin ligase and negative regulator for nuclear factor- κ B (NF- κ B) –inducing kinase, a critical signal component in activation of transcription factors, which are implicated in cell survival and other features of the malignant phenotype.³⁸

A second deletion that presents more prominently in patients with HPV-positive tumors occurs in chromosome 11q. Although the deletion occurs in a broad region, which makes a single gene target difficult to identify, several prominent tumor-suppressor genes are suggested, including *ATM1*. In terms of broad and focal amplification, perhaps the most prominent difference occurs on chromosome 7, where HPV-positive tumors exhibit no evidence of amplification. The prominent focal amplification seen at the locus of *EGFR* is likewise absent in HPV-positive tumors.¹ The importance of this distinction for anti-*EGFR* therapy remains obscure, but the lack of genomic targeting for amplification is evidence that HPV-positive tumors do not select for this gene at the clonal level. By contrast, HPV-

positive more so than HPV-negative tumors display a clear prominence of amplification of 3q at the locus for the squamous lineage transcription factors *SOX2*, *TP63*, and the oncogene *PIK3CA*. *PIK3CA* activation promotes activation of cell cycle, metabolism, and transcription factors that regulate diverse genes that promote the malignant phenotype.^{1,2,41}

When cell cycle signaling is considered (Fig 1), statistically significant copy number changes in at least three genes is remarkable. As expected, chromosome 9p, the locus for the gene *CDKN2A* which produces the protein product p16, is frequently lost in HPV-negative versus HPV-positive tumors. Similarly, HPV-negative tumors demonstrate prominent amplifications of *CCND1* (cyclin D), whereas HPV-positive tumors contain a striking focal amplification at 20q11, the location of *E2F1*. The *E2F1* gene, which encodes a key transcription factor that promotes genes that mediate cell cycle initiation from G0 to G1 and cell proliferation, is amplified in approximately 20% of HPV-positive HNSCCs but in only 2% of HPV-negative HNSCCs.¹ *E2F1* together with tumor-suppressor proteins TP53 and RB1, which are inactivated by the HPV E6 and E7 oncoproteins, critically deregulate cell cycle control and genomic stability in HPV-positive HNSCC. A fourth gene in the cell cycle pathway, *RB1*, although only rarely inactivated, is more often lost in HPV-positive tumors.

At this time, studies have detected only rare instances of activated fusion oncogenes based on analysis of DNA structural rearrangements. Most prominently, in a minority of HPV-positive samples, several authors have reported *FGFR3-TACC3* fusions that seem promising as a therapeutic target.⁴²⁻⁴⁴ In addition, there was evidence in single samples for structural alterations associated with *MET* activation. Finally, the most well-described alternative transcript of *EGFR*, the so-called vIII mutant, was only rarely observed, at a rate of less than 1%.¹

MUTATION SIGNATURE

It is perhaps somewhat surprising that tumors that presumably have disparate molecular origins as HPV-positive virally associated tumors and HPV-negative tobacco-associated tumors share as much homology in their genome landscape as the copy number data demonstrate. Other similarities between these subtypes include the generally similar mutation rate and number of copy number changes in each group. Other aspects of their molecular signature are, however, distinctly different.

As expected, the transversions associated with smoking in many tumors (ie, mutations that change a purine nucleotide to another purine (A $\leftrightarrow$ G) or a pyrimidine nucleotide to another pyrimidine (C $\leftrightarrow$ T) at CpG sites) were more frequent in HPV-negative tumors.¹ By contrast, a more unique signature has recently been reported in HPV-positive tumors and in some other tumors in which a predominance of T $\rightarrow$ C mutations is noted. These cytosine-to-thymidine C $\rightarrow$ T mutations have recently been shown most prominently in virally transformed cancers, in which there is increased cytosine deaminase mutagenesis that is characteristic of the apolipoprotein B mRNA editing enzyme catalytic (APOBEC) family of enzymes.⁴⁵ APOBEC cytosine deaminase activity is induced and has emerged as a potential mutagenic factor in virally induced human cancers. Indeed, sequence data from HPV-positive HNSCCs and other human tumors reveal

genome-wide cytosine mutations consistent with APOBEC deamination and edits to the cellular DNA.^{46,47} The importance of this particular mutation signature is that it may explain, in part, a striking pattern seen in the distribution of mutations of one of the key oncogenes of HNSCC, *PIK3CA*, between HPV-positive and HPV-negative tumors. Although *PIK3CA* is altered in tumors without regard to viral association, two specific C>T mutations in viral-associated tumors occur predominantly in two hotspots within the helical domain. These result in E542K and E545K amino acid substitutions that are implicated in *PIK3CA* kinase and oncogene activation.^{1,27,45} The specificity of *PIK3CA* targeting emphasizes even more strongly the importance of the 3q locus described in HPV-positive tumors described in the Structural Alterations section. In smoking-related tumors, *PIK3CA* mutations, including variants of unknown significance on signaling, are seen throughout the gene and are seen much less commonly in the hot spots. The signature APOBEC mutation of *PIK3CA* in HPV-positive HNSCC evokes the well-known transversion mutation of *KRAS* (G12C and G12V) in smoking-related lung adenocarcinoma.⁴⁸ Strikingly, although squamous cell carcinoma of any site rarely demonstrates *KRAS* mutations, they are reported in HPV-positive HNSCC.²⁷ Although the data are sparse, the interaction between smoking and *KRAS* mutation suggests a mechanism through which tobacco might augment risk in HPV-positive HNSCC.

HPV VIRAL INTEGRATION

In addition to the distinct mutational signature (APOBEC *v* smoking pattern), HPV-positive tumors are characterized by a second distinct structural alteration compared with HPV-negative tumors: the integration of viral DNA. Although the true rate of HPV integration into the human genome is unknown, existing data suggest that at least two thirds of patients with high expression of E6 and E7 by RNA quantification have detectable integration sites in the genome.⁴⁹ The exact nature of the integration is the subject of ongoing investigation. Most tumors seem to have a single primary integration site, although the integration event itself may be complex at sites of gene amplification.⁵⁰ Less common are tumors with multiple insertion sites on different chromosomes. Most viral integrations occur in or near genes, although the data do not yet support a recurrent site of integration to identify selective pressure on a specific location in the genome. As with most structural rearrangements in cancer, the predicted impact of integration is generally to silence the gene.¹ Several tumor-suppressor genes have documented viral integration sites, such as *RAD51* and *ETS2*. Whether the integration event resulted in tumor initiation or progression has not been definitively shown versus the alternative possibility that the gene disruption may be a passenger event. In the case of a passenger event, the targeting of a gene may be nonspecific and may result from a stochastic event that occurred at any open chromatin location. The lack of recurrent events gives more weight to the lower relevance of the integration site to tumor initiation and progression.

MUTATIONS AND PATHWAYS

Among the most anticipated results from recent tumor-profiling projects was the accounting of driver gene somatic mutations. A driver

gene is defined as a gene that, when mutated, is responsible for either initiation or progression of a cancer. In the context of cancer sequencing projects in which mutations cannot be individually tested for their biologic function, driver mutation status is defined as a mutation that occurs more often than expected by chance. Many other incidental mutations, called passenger mutations, occur in the tumor, but these are attributed to the inherent instability of the cancer genome. Because the methods to rigorously define driver mutations continue to evolve, additional factors beyond statistical significance are considered when the most likely candidates for driver gene status are selected; factors include whether the gene is mutated at a specific functional position, the gene has been reported in other cancers, and mutations occur in conjunction with copy number or other structural alterations. Several reports with significant numbers of patients sequenced suggest that most common driver mutations from the overall population of HNSCC (mutations with a frequency > 5% to 10%) have been detected (Table 1).^{1,27-31} Data remain limited in subpopulations, including in HPV-positive tumors, for which the cohorts remain relatively underpowered for the most rigorous statistical approaches.

Many mutations, along with previously described regions of copy number and structural alteration, fall into a few cancer pathways. Most notably, almost every tumor had at least one mutation in the cell cycle and survival pathway; mutations included *CDKN2A* (p16) mutations, deletions, and hypermethylation; *RB1* mutations and deletions; *E2F1* amplification; *CCND1* amplification; *MYC* amplification; and *TP53*. The gene *CCND1* bears special mention, because it is among the most frequently altered oncogenes in HNSCC, although this occurs almost entirely in HPV-negative tumors. By the canonical pathway representation (Fig 1), it is immediately upstream of *CDK4/CDK6*, which is a target of palbociclib, an inhibitor that was recently approved in breast cancer.⁵¹ Trials in HNSCC are ongoing. Interestingly, nearly every HPV-negative tumor has inactivation of *CDKN2A*, and most have inactivation of *TP53*. One notable exception was a subset of oral cavity cancers that had p16 loss but wild-type *TP53*. Tumors in these patients have a strikingly high rate of *CASP8* inactivation and concurrent *HRAS* activating mutations, which suggests a distinct clinical entity that, in at least one data set, was also associated with favorable clinical outcomes.¹ Other investigators have reported similar favorable outcomes associated with wild-type *TP53* in combination with other genomic alterations.⁵² The weight of the evidence, including the low rate of *TP53* in patients with HPV-positive tumors who also benefit from favorable outcomes, suggests that wild-type *TP53* status might serve as a universal marker of favorable outcome without regard to HPV status.

A second pathway clearly targeted by multiple driver mutations is the receptor tyrosine kinase pathway, which includes the *RAS*, *PIK3CA*, and *PTEN* branches. Numerous targets are altered, including activating mutations of *PIK3CA*, *HRAS*, *FGFR3*, *KRAS*, *FGFR2*, *EGFR*, and *KRAS* (Fig 3). The *EGFR* mutations are worthy of special note, because these are generally not the canonical activating mutations seen in lung cancer in the kinase domain of the gene. Mutations are more often in the juxtamembrane region of the gene and include a number that have been documented to be functional in other cancer types, including glioblastoma. Additional kinase pathway mutations include loss of function from repressor elements in the pathway; examples include *PTEN*, *PIK3R1*, and *NF1*.

Relevant to the recent enthusiasm in immune-based therapy in solid tumors, which includes the recent approval of nivolumab

Table 1. Genes Commonly Mutated in Head and Neck Squamous Cell Carcinoma

Tumor Group	Mutated Genes	
	Gene Symbol	Mutation Rate (%) [*]
All tumors	<i>CDKN2A</i>	15-22
	<i>FAT1</i>	5-23
	<i>TP53</i>	41-72
	<i>CASP8</i>	5-9
	<i>AJUBA</i>	
	<i>PIK3CA</i>	8-21
	<i>NOTCH1</i>	10-21
	<i>KMT2D</i> (ie, <i>MLL2</i>)	5-18
	<i>NSD1</i>	4-10
	<i>HLA-A</i>	
	<i>TGFBR2</i>	
	<i>HRAS</i>	
	<i>TPRX1</i>	
	<i>FLG</i>	
	<i>DDX3X</i>	
	<i>RPIK4</i>	
	<i>CUL3</i>	
	<i>PTEN</i>	
	<i>SYNE1</i>	
	<i>MED1</i>	
	<i>FGFR3</i> †	
	<i>NFE2L2</i> †	
	<i>PIK3R1</i> †	
	<i>RB1</i> †	
	<i>FBXW7</i> †	
	<i>SCN9A</i> †	
	<i>CHEK2</i> †	
	<i>PTCH1</i> †	
	<i>KRAS</i> †	
	<i>MLL3</i> †	
	<i>FGFR2</i> †	
	<i>ZNF217</i> †	
	<i>RIMS2</i> †	
	<i>EGFR</i> †	
	<i>NOTCH2</i> †	
	<i>NOTCH3</i> †	
HPV-positive subgroup	<i>TRAF3</i> †	
	<i>PIK3CA</i>	22-56
	<i>B2M</i> †	
	<i>ZNF750</i> †	
	<i>IFNGR1</i> †	
	<i>MLL3</i> †	
	<i>DDX3X</i> †	
	<i>FGFR2/3</i> †	11-14
	<i>NOTCH1</i> †	
	<i>NF1</i> †	
	<i>KRAS</i> †	
	<i>FBXW7</i> †	

^{*}Data are presented for selected genes with mutation rates > 10% in at least one series.

†Indicates that the gene did not meet strict statistical criteria for significance of q score < 0.1 in at least one study. The gene is reported because the sum of evidence supports its status as a driver.

dependence on the NF- κ B pathway. Mutations in *HLA-A/HLA-B* and *B2M* are also seen in squamous lung cancer and, although these have not clearly been functionally validated, represent important candidate driver mutations. The immune system remains an important area of investigation through integrated data analysis as well. Investigators with TCGA demonstrated dramatic differences in immune signatures across the molecular subtypes of HNSCC identified by gene expression patterns, most notably with a complete absence of immune infiltration in the classic subtype and an activated T-cell signature in the mesenchymal subtype.^{1,54,55}

Perhaps more difficult to target with current therapeutics, but nonetheless frequently altered, are three additional pathways: squamous differentiation, oxidative stress, and WNT signaling. Many tumors demonstrated loss-of-function mutations in *NOTCH* genes, almost always with amplifications of chromosome 3q, where the well-known squamous transcription factor *TP63* is located. Perhaps less well appreciated is the increasingly recognized interaction between this differentiation pathway and two frequently lost genes of the WNT pathway, *FAT1* and *AJUBA*.^{56,57} Several direct interactors with *TP63* and *NOTCH* seem affected by loss of function, including *ZNF750*⁵⁸ and, most important as the master regulator of oxidative stress, *NFE2L2*.⁵⁹ It may be fair to say, if early reports are validated, that the *NFE2L2* axis, with its repressors *KEAP1* and *CUL3*, may represent one of the early potential therapeutic biomarkers for clinical use. Alterations of oxidative stress are seen across the spectrum of smoking-related cancers and are tightly associated with the classic gene expression subtype, as has been validated in numerous studies.^{1,60} Early translational data and preclinical models suggest that activation of this pathway may be associated with resistance to radiation, which is a cornerstone of therapy in HNSCC.^{61,62}

Although there are many other mutations, one in particular bears special attention: the gene for nuclear receptor binding SET domain protein 1 (*NSD1*), which is altered in approximately 10% of tumors. The *NSD1* gene is a histone 3 Lys 36 (H3K36) methyltransferase, similar to *SETD2*, a gene most associated with alterations in the clear cell subtype of renal cell carcinoma. Just as with *SETD2* in clear cell carcinoma, mutations of *NSD1* were associated with a dramatic, genome-wide hypomethylation phenotype in HNSCC.^{1,63} Interestingly, *NSD1* seems relevant in relation to head and neck development in nonmalignant disease, as do germline inactivating mutations, which are associated with craniofacial abnormalities (Sotos syndrome).

INTEGRATED ANALYSIS

In addition to gene sequencing and copy number studies, there are mounting data from additional genomics platforms relevant to the understanding of HNSCC. The most mature of these data are gene expression studies that include microarrays and RNA sequencing from TCGA. It is now clear that reproducible subtypes of HNSCC can be defined by expression data, including the classic subtype associated with activated oxidative stress, the atypical subtype associated with lack of chromosome 7 amplification (including most HPV-positive cases), the mesenchymal subtype characterized by an activated T-cell signature, and the basal subtype associated with wild-type *TP53*. Molecular subtypes are an attractive way to classify tumors in some instances for the purposes of consideration of model systems and

in squamous lung cancer, several known or suspected driver mutations in the immune and cell death pathways are demonstrated in HNSCC.⁵³ Amplifications of *FADD* and *BIRC2*, along with loss-of-function mutations and deletions of *CASP8* and *TRAF3*, highlight a

Fig 3. Frequent genetic aberrations in selected pathways altered in HPV-positive (A) and HPV-negative (B) head and neck squamous cell carcinoma. Overview of key genetic aberrations for HPV-positive and HPV-negative head and neck cancers. Shades of blue indicate frequency of inactivating changes in presumed tumor suppressor genes. amp, amplification; del, deletion; HLA, human leukocyte antigen; mut, mutation; fus, fusion/translocation; wt, wild type.

potentially of therapeutic groups, although the clinical utility of this remains unproved.¹ Others have reported predictive gene expression signatures associated with prognosis, response to therapy, and HPV status; other phenotypes have been reported but have not generally been validated in clinical trials. The Chicago HNC Genomics cohort has recently reported a striking finding within HPV-positive HNSCC of two subgroups defined as prominent inflamed (inflamed mesenchymal HPV-positive subtype) or noninflamed (classic HPV-positive subtype) with potential implications for immunotherapy.^{1,10} Similar work has been reported with other biomolecule platforms that target epigenetics, miRNA, and proteomics.

OUTLOOK AND FUTURE CLINICAL IMPLICATIONS

As a first step, accurate HPV diagnostics are needed beyond the interim proxy biomarker of p16. With confidence in the diagnosis of virally associated tumors will come a definitive answer about the effectiveness of cetuximab as a function of HPV status and confidence in making HPV-related treatment decisions in the future (eg, de-escalation). Furthermore, the increased breadth of genomic data on *EGFR* and related pathway alterations invites a re-evaluation of predictive biomarkers of *EGFR*-directed therapies. Extension of the molecular profiling beyond HPV status to target the most common molecular alterations for both therapeutic and prognostic indications will become possible if profiling is done routinely (eg, associated with clinical studies). Two immediately obvious candidates include the *PIK3CA* and *CDK4/CDK6* pathways because of the high prevalence of *PIK3CA* mutations and

CCND1 amplifications and the availability of existing therapeutics. Beyond targeted therapies, preclinical data already suggest that adherent signaling in the *NFE2L2* pathway may predict differential phenotypes with respect to standard chemotherapy and radiation in ways that have clinical relevance, such as the selection of primary treatment modality. Other broad signatures, such as molecular subtypes predicted by expression profiling, might similarly influence the selection of therapies, such as immune-based treatment, and new technologies will allow reliable and reproducible determination of expression signatures as part of clinical trials. The discovery of molecular signatures and shared mutations across tumor types, such as squamous lung cancer and esophageal cancer, offers the potential to unify treatment paradigms across cancers that have historically been isolated by anatomic boundaries. Among the barriers that face clinicians and researchers in achievement of the promise of molecular characterization of HNSCC is uncertainty in the regulatory and reimbursement environment for molecular diagnostics. The pace of progress may be determined, in large part, by the ability of researchers and clinicians to order (and justify to payers or trial sponsors) the relevant clinical grade diagnostic tests to fuel molecularly targeted clinical trials and population studies.

In conclusion, head and neck cancer is a common and often fatal cancer that is typically treated with curative intent at the time of diagnosis. Many therapeutic questions, including the choice of optimal treatment modality and intensity and implications of HPV status on therapy, remain. By using the data provided by the first wave of comprehensive genome characterization, studies should focus and accelerate progress in this disease by highlighting the

frequent gene alterations in the disease. Researchers will be able to leverage this knowledge in the design of clinical trials, the development of model systems, and the assessment of patient outcomes.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Genetic Landscape of Human Papillomavirus–Associated Head and Neck Cancer and Comparison to Tobacco-Related Tumors

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