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Genomic Sequencing and Precision Medicine in Head and Neck Cancers

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Abstract

Head and neck squamous cell carcinoma (HNSCC) remains a common and deadly disease. Historically, surgical and chemoradiation treatments have been met with modest success, and understanding of genetic drivers of HNSCC has been limited. With recent next generation sequencing studies focused on HNSCC, we are beginning to understand the genetic landscape of HNSCCs and are starting to identify and advance targeted options for patients. In this review, we describe current knowledge and recent advances in sequencing studies of HNSCC, discuss current limitations and future directions for further genomic analysis, and highlight the translational advances being undertaken to treat this important disease.

Keywords

head and neck cancer; HNSCC; genomics; precision medicine; personalized medicine

Introduction

Head and neck squamous cell carcinoma (HNSCC) remains a common and highly aggressive disease. It is the sixth most common cancer worldwide; in 2012 alone there were over 600,000 new cases and over 375,000 deaths attributed to HNSCC [1]. While tobacco and alcohol historically have been the most important etiologic factors in the development of HNSCC [2], high-risk serotypes of the human papilloma virus (HPV) have changed the epidemiology, especially of oropharynx cancer, in recent years [3,4]. Head and neck cancers arise via an accumulation of environmentally induced and inherent mutations in key signaling pathways, leading to immortalization, growth in the absence of growth signals,

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resistance to anti-growth signals, and the ability to avoid apoptosis, angiogenesis, invasion, and metastasis [5,6]. Our understanding of the genetic landscape of HNSCC has evolved over time and recently rapidly expanded with the results of recent genomic studies. As such, we are beginning to understand genetic drivers and accordingly develop precision medicine paradigms to treat this important and deadly disease. Here, we review key findings from genomic sequencing studies, identify gaps in data and need for further studies, and discuss the translational care potential from these studies.

Early Genetic Studies

Prior to the genomics era, early studies attempted to identify key pathways in which alteration was thought to contribute to carcinogenesis in HNSCC, namely cell cycle dysregulation and constitutive cell proliferation. Early on, investigators discovered that loss of cell cycle regulation was an important driver for HNSCCs. Key genes involved in these pathways, namely *TP53*, *RB*, *CCND1*, *CDKN2A*, and *CDK4/6*, were determined to be mutated or otherwise aberrantly expressed in HNSCC [6–12]. Indeed, mutations in *TP53* are found in the majority of HPV-negative HNSCC cases [8,9]. Similarly, *CDKN2A* was noted to be inactivated by mutation or methylation in a large portion of HPV-negative HNSCCs [11], and *CCND1* was found to be amplified in 80% of HNSCCs [12]. For HPV-positive HNSCC, the two virally encoded oncogenes, *E6* and *E7*, inactivate p53 and pRB, respectively, thus disrupting cell cycle regulation, and providing a mechanism for tumorigenesis [13]. A common thread between HPV-positive and HPV-negative tumors was the disruption of *TP53* function, either through mutation (HPV-negative tumors) or viral protein inactivation (HPV-positive tumors).

Similarly, early studies highlighted the importance of increased activation of cell growth and proliferation pathways in HNSCC. One of the earliest critical regulators of proliferation identified in HNSCC was *EGFR*, a transmembrane growth factor receptor that signals through the Ras–MAPK, PI3K–PTEN–AKT and phospholipase C pathways to promote cell proliferation (Figure 1). Furthermore, EGFR can translocate to the nucleus and act as a transcription factor or co-activator of other transcription factors, such as STAT, leading to additional mechanisms for cell growth [6,14]. Importantly, early studies identified EGFR to be overexpressed or activated in a majority of cases of HNSCC, suggesting a critical role for this gene that would lead to future targeted therapeutic discoveries [6,15]. Other early studies identified frequent mutations and expression changes in other cell growth pathway genes, including *PIK3CA*, *PTEN*, and other growth factor receptors or their ligands [16–21]. Despite these important findings, however, studies were limited to single-gene analyses until the incorporation of newer sequencing techniques rapidly expanded our knowledge of the mutational landscape of HNSCCs.

Insights from Initial Next Generation Sequencing Studies

Given the complicated DNA landscape, multiple possible genetic alterations, and advancements in gene sequencing, researchers subsequently turned to next generation sequencing (NGS) tools to identify fundamental tumorigenic mechanisms for HNSCC (Table 1). Two key studies published in 2011 provided the initial mutational landscape of

HNSCC by whole exome sequencing. Stransky et al. analyzed 74 HNSCC tumors (with normal tissue comparison) [22], and Agrawal et al. performed whole exome sequencing and gene copy number analyses on 32 HNSCCs [23]. Combined, these studies identified high rates of mutations in *TP53*, *PIK3CA*, and *CDKN2A*, consistent with early investigations into the genetic drivers of HNSCC. Interestingly, a high rate of inactivating *NOTCH1* mutations was verified, suggesting a tumor suppressor role for this gene. This had biologic plausibility given the role of *NOTCH1* in squamous differentiation (Figure 1) and highlighted the utility of NGS tools in identifying previously unidentified genes associated with HNSCC. While limited in number, analysis of HPV positive patients suggested an lower overall mutational burden and different mutational spectrum in comparison to HPV negative tumors.

In a landmark study, The Cancer Genome Atlas (TCGA) profiled 279 HNSCCs with whole exome sequencing, copy number analysis, and RNA-Seq [24]. Mutations identified in early clinical studies and the initial Stransky et al. and Agrawal et al. cohorts were verified. Notably, loss of function aberrations in canonical cell cycle pathway genes (*TP53* mutations and *CDKN2A* deletions), and cell growth and proliferation genes (*EGFR* amplifications and *PIK3CA* aberrations) were identified at high rates, consistent with earlier studies. Additionally, mutations in *NOTCH1* were identified in 19% of patients, highlighting the potential importance of this gene in HNSCC development. Of note, mutations in the immune surveillance/recognition pathway were discovered, particularly *HLA-A/B*, suggesting a role for the importance of immune recognition in HNSCC development and potential treatment. Finally, while limited, a subset of HPV positive HNSCCs (36 tumors) demonstrated a different mutational profile from HPV negative patients on initial analysis.

Limitations of Early Next Generation Sequencing Studies

Despite recent progress in genomic sequencing in HNSCC with the previous studies, there remain significant gaps in data for specific patient populations and tumor subsets. Most of the TCGA patients are men with significant smoking or drinking histories. Nearly 90% of the patients for whom detailed genetic analyses were performed were white [24]. The remaining patients are predominantly black, with very few individuals of Asian or American Indian descent. Data on other etiologic factors (such as betel quid exposure), often more prevalent in other epidemiologic cohorts, are limited. The majority of tumors assessed in the earliest sequencing studies were also advanced staged tumors, with 79% [24], 92% [22], and 91% [23] of the TCGA, Stransky, and Agrawal cohort being from stage III/IV tumors. Finally, most of the included study populations were HPV negative, with just 12.9% [24], 14% [22], and 12.5% [23] of the TCGA, Stansky, and Agrawal study populations being HPV positive. Thus, many of the HPV positive specific mutations required further study and validation, particularly when facing the increasing rates of HPV positive HNSCC.

Young, low-risk, and early-stage cancer patients are also infrequent in this dataset (only 13/279 TCGA patients are less than 46 years old with less than one pack-year smoking history) [24]. These patients can offer unique opportunities to identify significant genetic features in HNSCC given the lack of environmental factors, increasing the likelihood of specific genetic drivers of tumorigenesis. Sequencing of early-stage tumors and carcinoma *in situ*/dysplasia will help differentiate early-stage mutations, which may be responsible for

the initial transition to carcinoma, and late-stage mutations, acquired only after the malignancy has developed and which may drive further aggressiveness and metastatic features. Furthermore, analyses of young patients without common risk factors for HNSCC will likely uncover a “cleaner” genetic profile and aid in the identification of “driver” mutations.

While the information provided by the TCGA dataset allows analyses of mutational status and copy number for hundreds of genes, it does not fully translate these results into functional relevance at the level of the transcriptome, proteome, and even epigenome. For certain genes, correlations were observed in TCGA. One such link was identified between copy number and expression level for let-7c-5p and miR-100-5p, and the deletion of these miRNAs was correlated further with expression of target genes including *CDK6*, *E2F1*, and *PLK1* [24]. However, gene expression levels for members of some of the most commonly dysregulated pathways in HNSCC, such as *EGFR*, *FGF19*, *CCND1*, and *CDKN2A*, show poor correlation with gene copy number [25]. Extension of DNA and RNA sequencing data to correlations with protein expression levels and methylation status is even more difficult. Nevertheless, the results published in TCGA did identify many significant relationships between gene mutation status, miRNA, and methylation subtypes, including a relationship between commonly altered *NOTCH1* with methylation subtype [24]. However, the currently available data is not clearly interpretable or generalizable and these findings require further validation.

A final gap in the TCGA dataset is the lack of recurrent and/or metastatic HNSCC patients. Interestingly, while this analysis is composed of primary tumors, most precision medicine trials enrolled recurrent and/or metastatic cancer patients. Thus, HNSCC sequencing results were analyzed and implemented very differently in the research lab as opposed to the clinic, since the patient populations being considered had genetic landscapes that are potentially wholly distinct. Sequencing of patients with recurrent or metastatic disease is critical to determining the key mediators of resistance, invasion, and metastasis. Genetic data collected at multiple cancer occurrences or anatomic sites for a single patient might also address important questions regarding tumor heterogeneity.

Subsequent Sequencing Studies

These early studies greatly expanded the field’s understanding of the genetic mutations underlying HNSCC and led to the development of several targeted therapies. However, as discussed above, there were many patient populations and disease states requiring further evaluation. Since completion of these initial analyses, several other studies were performed in order to better delineate the specific mutational drivers of particular patient cohorts. Evaluation of the genetic drivers of recurrent and metastatic patient tumors is particularly valuable, as survival rates are dismal in this cohort, and they have limited current treatment options [26,27]. Hedberg et al. [28] performed whole exome sequencing on patient matched tumor pairs from HNSCC patients with either nodal metastasis or disease recurrence. This study found that the index tumors contained similar mutations to those discovered previously. Interestingly, mutations in *C17orf104*, inositol 1,4,5-tri-sphosphate receptor, type 3 (*ITPR3*), and discoidin domain receptor tyrosine kinase (*DDR2*) were found in the

metastatic or recurrent tumors but not in the original tumors. Although this study did provide greater information about the genomic mutations behind recurrent or metastatic disease, as in previous studies, the patient cohort again consisted mainly of male Caucasians with a significant smoking history (87%). Additionally, only 1 patient had HPV positive disease, limiting the study's applicability to HPV positive tumors.

As HPV related tumors have increased in prevalence, understanding the genetic drivers behind HPV positive HNSCC has become increasingly important. A study by Morris et al. [29] used targeted NGS to evaluate the genetic mutations in 151 recurrent and metastatic HNSCC patients. This revealed enrichment in the presence of *TERT* mutations in HPV negative tumors, which were not noted with previous studies. *TP53*, *TERT* promoter, and *CDKN2A* were found to be more frequently altered in the HPV negative tumors. Comparing primary HPV positive tumors with recurrent or metastatic HPV positive tumors, the mutational profile of the recurrent or metastatic HPV positive tumors more closely aligned with HPV negative tumors (higher rates of *TP53* mutations and lower rates of *PIK3CA* mutations). Although this study was limited by a small sample size (21 HPV positive patients compared with 30 HPV negative patients), it provides important direction for further study and valuable information on the management of recurrent HPV positive tumors, which may behave more like HPV negative HNSCC.

A study by Seiwert et al. [30] evaluated HPV positive HNSCC identified genetically distinct mutational patterns from HPV negative tumors. In sequencing 51 HPV positive and 69 HPV negative tumors, the authors found HPV positive tumors had unique mutations in *DDX3X, CYLD*, and *FGFR*. They were also uniquely enriched for *PIK3CA* pathway mutations, and alterations in the DNA damage pathway and immunologic genes appeared to favor HPV positive tumors. Moreover, HPV positive HNSCC also tended to lack *EGFR* mutations. These genetically distinct mutations may provide an explanation for the more favorable prognosis typically associated with HPV positive tumors. Additionally, it offers potential targets for drug development, and stratification of targeted therapeutic options based on HPV status.

Epidemiologic data indicates an increasing incidence of oral tongue squamous cell carcinoma in young patients, often with no other risk factors [31–33]. While the TCGA dataset largely overlooks this patient population, some recent studies have considered the genetic differences between young, low-risk HNSCC patients and traditional HNSCC patients (older smokers and drinkers). In one such study, amplification of *FGFR1* was identified as a possible driver alteration in a young, low-risk patient. Further investigation revealed aberration of *FGF/FGFR* family members in over one third of HNSCC patients in the TCGA dataset [34]. Pickering et al. [35] performed whole-exome sequencing and copy number analysis on a cohort of 16 young oral tongue cancers and 28 old tongue cancers treated at MD Anderson. When their results were combined with young and old tongue cancer groups from the TCGA dataset, there was a trend toward decreased frequency of *FAT1*, *TP53*, and *PIK3CA* mutations in young tongue patients, although none of these relationships reached statistical significance. Additionally, both MD Anderson and TCGA young tongue cohorts had a lower median number of mutations compared to older patients. No differences in copy number or smoking signature were observed between patient groups

in either cohort, further suggesting that there might be other important and unidentified mutational drivers in young, low-risk HNSCC patients.

An increasing number of sequencing studies for other head and neck cancers have also been performed in the last decade but leave many questions unanswered. Studies on salivary gland and thyroid tumors, for example, have contributed to an increasing body of knowledge on these histologically diverse tumors [36–38]. Among other gene fusions, some of the most noteworthy in salivary gland cancer include *PLAG1*- and *HMGA2*- fusions in pleomorphic adenomas [39], *MAML2-CRTC1/3* fusions in mucoepidermoid carcinoma [40], and *MYB-NFIB* fusions in adenoid cystic carcinoma [41]. Other alterations frequently described affect genes in DNA damage repair and kinase signaling pathways; these alterations include mutations in *NOTCH1/2* and *PIK3CA* in adenoid cystic carcinomas and amplification of *EGFR* and *ERBB2* in mucoepidermoid carcinomas, adenoid cystic carcinomas, and other adenocarcinomas [42,43]. TCGA data on papillary thyroid carcinoma suggests that gene fusions involving *BRAF* and *RET* as well as mutations in *BRAF, RAS*, *EIF1AX*, *PPM1D*, *CHEK2*, and the *TERT* promoter are frequent and likely significant oncogenic events in this cancer type [37]. In more aggressive poorly differentiated and anaplastic thyroid carcinomas, the mutation and gene fusion landscapes are similar to those in papillary thyroid carcinoma but more frequently involve genes associated with the *PIK3CA-PTEN-AKT-mTOR* pathway, *SWI-SNF* complex, histomethyltransferases, and DNA mismatch repair[38]. These sequencing studies in other head and neck cancers may provide insight into additional plausible drivers for HNSCC, although early results suggest that different mutational drivers exist in these different tumor subtypes.

Additional work has begun to dissect the genetic profile of HNSCCs from patients of various epidemiologic cohorts. Aside from the TCGA dataset, which is composed primarily of white patients, large sequencing analyses have been performed on Asian patient groups from India and Singapore [44–46]. Comparing the alteration frequencies in these populations has indicated that there may be significant differences in the mutation rates in *CDKN2A*, *NOTCH1*, and *PIK3CA* [47]. Smaller, targeted sequencing studies of other commonly altered genes in various epidemiologic cohorts also display wide variation; for example, *TP53* mutation rates of 10.6% were reported in an Asian tongue cancer study as compared to the 80.6% frequency observed in TCGA patients with oral cavity cancer [24,48]. A recent genome-wide association study used data from North American, European, and South American patients noted differences in HNSCC risk for seven novel loci as well as the previous studied *ADH1B* gene. *ADH1B* and other alcohol related genes have been studied in various ethnic cohorts; based on the data presented here and elsewhere, the protective effects of mutant ADH1B*1 and ADH1C*1 alleles are less in North American and European populations than in Asian or South American ones [49,50]. Conversely, HLA haplotype HLA-DRB1*1301–HLA-DQA1*0103–HLA-DQB1*0603 is protective in North American and European head and neck cancer, particularly in HPV positive oropharyngeal tumors. HLA haplotype is less critical in South American cohorts although this may be influenced by the infrequency of HPV positive oropharyngeal cancer in this region [49]. These initial findings suggest the need for further investigation into population- and epidemiologic-specific cohorts.

Next Steps for Genomic Sequencing in HNSCC

While significant advances have been made in recent sequencing studies of HNSCC, there remain multiple issues to address. Intra-tumor heterogeneity has been noted to contribute to tumor evolution, metastasis, and resistance to chemotherapy and radiation treatments [51]. In analyzing HNSCC cases in the TCGA database, Mroz et al. [52] found that high intra-tumor heterogeneity correlated with decreased overall survival, where the relation could not be explained by patient age, tumor grade, or other molecular characteristics such as HPV or *TP53* status. However, despite its potential clinical relevance, estimating heterogeneity from a single biopsy currently has several limitations. Importantly, this type of analysis requires a large dataset with both exome and whole genome sequencing. Sequencing studies have predominantly chosen exome sequencing for cost efficiency and confidence in calling somatic mutations [53]. Additionally, estimating heterogeneity from a single biopsy is sensitive to changes in sample processing and background noise from sequencing. Intra-tumor heterogeneity can be more robustly measured on an individual basis, where multiple samples are sequenced from different spatial regions of the tumor [54]. This method can identify clonal tumor populations, though again both exome and whole genome sequencing data is needed. For instance, in one case multiple spatial biopsies from an HPV positive oropharyngeal squamous cell carcinoma case identified separate clones allowing for phylogenetic reconstruction of the tumor development. Of note, one clonal population displayed more genetic similarity to the metastatic lymph node samples [55]. The common practice of obtaining a single biopsy for use in sequencing may cause clinicians and research scientists to miss important genetic events that would indicate metastasis or resistance to therapy. While a common core of mutations can be identified regardless of spatial region [56], future genetic sequencing studies of HNSCC will need to acknowledge the effects of intra-tumor heterogeneity.

The integration of genomic sequencing data with transcriptome, proteomic, or epigenetic expression profiles remains a daunting task, but could provide a wealth of information about the tumor biology of HNSCC. A preliminary attempt to correlate copy number variation and microarray data identified that highly enriched genes in antigen presentation and integrin signaling pathways were consistent with copy number changes [57]. In contrast, Mazumdar et al. [58] demonstrated that despite the frequency of *PIK3CA* amplification, there is not necessarily a correlative increase in signaling. The combination of genome and transcriptome data might distinguish HNSCC profiles [59], thereby resulting in more robust analyses for tumor signatures and biomarkers [60]. While protein expression and DNA methylation changes are available for many of the HNSCC cases in TCGA database [24], thorough investigation of the alterations, and integrations with genetics, have yet to be tackled. As individual approaches, proteomic and epigenetic profiling have already identified alterations in HNSCC cases on a large scale and nominated potential therapeutic targets [61–63]. Integrating this information with genetics remains a difficult barrier from a data processing and informatics standpoint. However, the construction of a cancer network from multiple approaches could yield novel insights into the tumor progression and therapeutic targeting of HNSCC.

So far, the focus on genetic sequencing data has been on non-synonymous mutations, or genetic changes that result in alterations in the amino acid sequence. However, there is evidence to suggest that synonymous mutations, those that don't result in a amino acid change and are "silent", have functional relevance. The most influential synonymous mutations affect gene splicing; for example, some have noted inactivating splice sites in *TP53* [64], which result in loss of the functional p53 protein. Additionally, impacts on mRNA stability and protein folding, or in certain cases miRNA binding and regulation [65], have been noted to play a possible role in cancer progression through changes in expression [66]. These mutations tend to cluster in the untranslated regions, and possibly in promoters such as mentioned above with mutations in the *TERT* promoter. The impact of recurrent synonymous mutations in HNSCC, however, have yet to be specifically evaluated.

A final critical step in ongoing sequencing analyses of HNSCCs is increasing the number of patients studied, particularly in currently underrepresented patient groups. Research to expand the current knowledge of potential genetic drivers in epidemiologically diverse, recurrent and/or metastatic, HPV positive, and young and low-risk HNSCC patients is warranted. Genetic sequencing of other head and neck cancers, of dysplasias and early stage carcinomas, and of particularly aggressive tumors will help further identify critical alterations in the development and progression of HNSCC. As a whole, this work will contribute to an improved understanding of HNSCC in specific patient sub-groups and inform clinical decisions on a more individual basis.

Translating Studies into Treatment

Ultimately, the goal of these genomic sequencing studies in HNSCC are to translate findings directly to patient care, whether through greater prognostic understanding of disease biomarkers, or to implement new targeted therapeutic agents. To date, however, only three new agents have been FDA-approved for HNSCC in the last 20 years: cetuximab (Erbitux) and just recently pembrolizumab (Keytruda) and nivolumab (Opdivo; Figure 1). Historically, the importance of EGFR in HNSCC was highlighted by numerous studies, as highlighted above. Thus, researchers used rational development of an antibody targeting EGFR (cetuximab) to inhibit this key pathway in HNSCCs. Landmark studies demonstrated its clinical utility as an adjuvant to RT [67,68], and in cisplatin-resistant disease [69], for which it currently has indicated use by national cancer treatment guidelines [70]. Additionally, it is used as standard of care in combination therapies for recurrent or persistent disease [70,71]. Importantly, these initial studies demonstrated less systemic toxicity for cetuximab in comparison to traditional chemotherapy agents, suggesting the potential for increased efficacy with a milder side-effect profile. More recently, ongoing studies are evaluating cetuximab's efficacy as a replacement for platinum-based therapy in combination with radiation in advanced HNSCC, with the allure for reduced toxicities with equivalent cure rates. Interestingly, initial data from some trials suggests a higher rate of toxicity with cetuximab than previously reported [72], highlighting a further need to investigate its efficacy and toxicity in a wide variety of scenarios.

PD-1 inhibitors pembrolizumab and nivolumab were FDA approved in 2016 for the treatment of recurrent and metastatic HNSCC refractory to platinum-based therapy. The

Keynote-012 study evaluated the role of pembrolizumab in the treatment of previously treated recurrent or metastatic HNSCC. Notably, the patients studied in these trials had advanced, refractory disease, having failed previous standard of care. In this study, Chow et al. [73] found an overall response rate of 20%, with a response rate of 32% in HPV positive tumors and 14% in HPV negative tumors. Of those that responded, the progression free survival rate at 6 months was 37% in HPV positive tumors, as compared with 20% in HPV negative tumors. Finally, six month overall survival was 70% in HPV positive as compared with 56% in HPV negative HNSCC patients. In a study by Ferris et al. [74], nivolumab showed similar efficacy with an increase in median overall survival from 5.1 months in the standard therapy group as compared to 7.5 months in the treatment group. These agents were remarkably well-tolerated, particularly in comparison to traditional cytotoxic palliative chemotherapy.

While the overall cohort showed improvement in response to these immunotherapies, it is important to note that response rates for individual patients varied, with some patients having dramatic responses to therapy, while others did not see much benefit. As a result, genomic studies delineating the genetic markers in tumors that responded versus those that did not could prove extremely helpful in better delineating which patients would respond to these therapies. Chow et al. [73] did examine the role of PD-L1 status in predicting response: when considered alone, the presence of PD-L1 on tumor cells did not adequately predict response, but when immune cells were also evaluated for PD-L1 status, 22% of PD-L1 positive patients responded to treatment as compared with 4% of patients that were PD-L1 negative. However, prediction of response still remains low overall and other genetic markers will need to be investigated to allow for further targeted, personalized treatment of HNSCC. Additionally, further investigation with additional patients and evaluating PD-L1 status with additional methodologies may provide further insight into this issue. Finally, longer follow-up and toxicity evaluation are limited to date.

With the advancement and adoption of cetuximab, nivolumab and pembrolizumab into treatment regimens, the current lack of any established predictive or stratifying biomarker for patients remains a limitation. Despite being a targeted agent against EGFR, there is no correlation between *EGFR* mutation status, *EGFR* expression, and response to cetuximab [25]. Similarly, for pembrolizumab and nivolumab as mentioned above, the validity of PD-L1 as a biomarker for response remains uncertain. As a result, patients currently are not being stratified for treatment with cetuximab, pembrolizumab or nivolumab based on any genetic, histologic, or other biomarkers, bringing into question the potential utility of biomarkers for stratifying treatment. What is the best methodology to identify a biomarker (mutation status, RNA expression level, protein expression, or another biomarker)? Are there yet undiscovered biomarkers that may have prognostic or treatment-directing roles for our current FDA approved agents? What does this mean for additional targeted agents, and our correlative studies into HNSCC genomics?

The lack of correlative biomarkers for cetuximab, nivolumab and pembrolizumab should not dissuade us from advancing targets from genetic studies of HNSCC. Indeed, *EGFR* and *PD-1* were identified as viable targets from early genetic studies, and a large amount of basic scientific data corroborated these findings. Thus, many agents currently in clinical trials may

still have significant clinical utility. To this effect, precision medicine tumor boards are rapidly becoming a key component of medical decision making for HNSCC, and particularly for recurrent and metastatic HNSCC [29,75].

One avenue for early adoption of targeted agents into HNSCC, particularly via precision medicine tumor boards, is the use of FDA-approved agents for other cancers. As these agents have gone through rigorous validation of their efficacy and toxicities in other cancers with specific mutations, implementation in HNSCCs with similar genetic aberrations may achieve significant benefit. This has been a driving rationale behind the ongoing NCI-MATCH and SHIVA targeted therapy trials [75,76], which have demonstrated some initial encouraging results. Indeed, there have been reports of remarkable response of agents used in precision medicine paradigms that have not been traditionally used in a specific cancer type [77]. Early investigations into genetic aberrations in HNSCC suggest that multiple agents FDA-approved in other cancers may have potential in HNSCCs, such as using trastuzumab (currently approved for breast and gastrointestinal cancer) in *ERBB2* positive HNSCCS [78].

Many other agents are actively being investigated and advanced in a variety of cancers simultaneously. Indeed, many early clinical trials are inclusive of all solid tumors, often stratifying based on genetic mutation status rather than tumor site. The NCI-MATCH trial, for example, has proposed a new classification system, grouping tumors by their genetic signatures (e.g. *ERBB2* mutated tumors versus non-*ERBB2* mutant tumors) as opposed to their body site (e.g. breast cancer versus HNSCC). As such, it will be interesting to follow which agents currently in clinical trials are approved for which tumors initially, and how rapidly they may adopted in other tumor types. Additionally, as these agents are increasingly being studied simultaneously across all tumors, perhaps approval for use in the future will be primarily based on mutation status rather than tumor site of origin. As a caveat, as we are well aware of the potential for lack of correlation between gene mutation or expression status and response to targeted therapy (e.g. cetuximab in HNSCC), there is potential in the future for other agents to have benefit in HNSCC regardless of mutation status. For example, novel PI3K inhibitors are rapidly being advanced in early clinical trials. This is a critical pathway in HNSCCs, and may have benefit in all HNSCCs, with or without genetic aberrations in the *PIK3CA* pathway. Thus, there will be need for further clarification and investigation into the applicability of novel targeted agents across various mutational profiles in HNSCC.

Conclusion

While a great amount of data recently has been generated from recent genetic sequencing studies of HNSCCs, we still have hurdles to overcome in order to fully characterize genetic drivers of this disease and implement these findings for clinical use. Continued sequencing studies are warranted to further evaluate different subpopulations of patients who develop HNSCC. While we have substantial data on advanced primary tumors from Caucasian patients with a smoking history, other demographics are underrepresented in our sequencing data. Specifically, further evaluation of young patients with no risk factors, patients with HPV positive HNSCC, early stage tumors, and specific histologic subtypes, and

stratification of unique mutations across specific HNSCC subsites may provide insight into novel genetic drivers. Additionally, since most of the initial sequencing studies were whole exome analyses of tumors, we have limited insight into non-coding genomic and epigenetic factors that may regulate gene expression and tumorigenesis. Thus, further evaluation of HNSCCs with these rapidly developing genomic, transcriptomic and proteomic techniques have shed further light into additional non-coding factors of HNSCC initiation and behavior. Finally, while we have made progress in recent years in advancing targeted agents into clinical use and establishing precision medicine trials, treatment of HNSCC still trails behind other cancers. Nevertheless, understanding of genetic drivers of HNSCC and correlative precision medicine implications is advancing at an exponential pace. Additional targeted agents, either in combination (with traditional therapies or other targeted agents) or as monotherapies, will undoubtedly progress in upcoming years. Along with the implementation of new targeted agents, identification of predictive biomarkers will increasingly become important in order to stratify treatment options, monitor response, and afford prognostic guidance. Overall, genomic study and translational treatment of HNSCC has greatly advanced over recent years and continues to evolve, with increased knowledge of genetic drivers of HNSCC and implementation of targeted therapy paradigms occurring at an ever-increasing pace.

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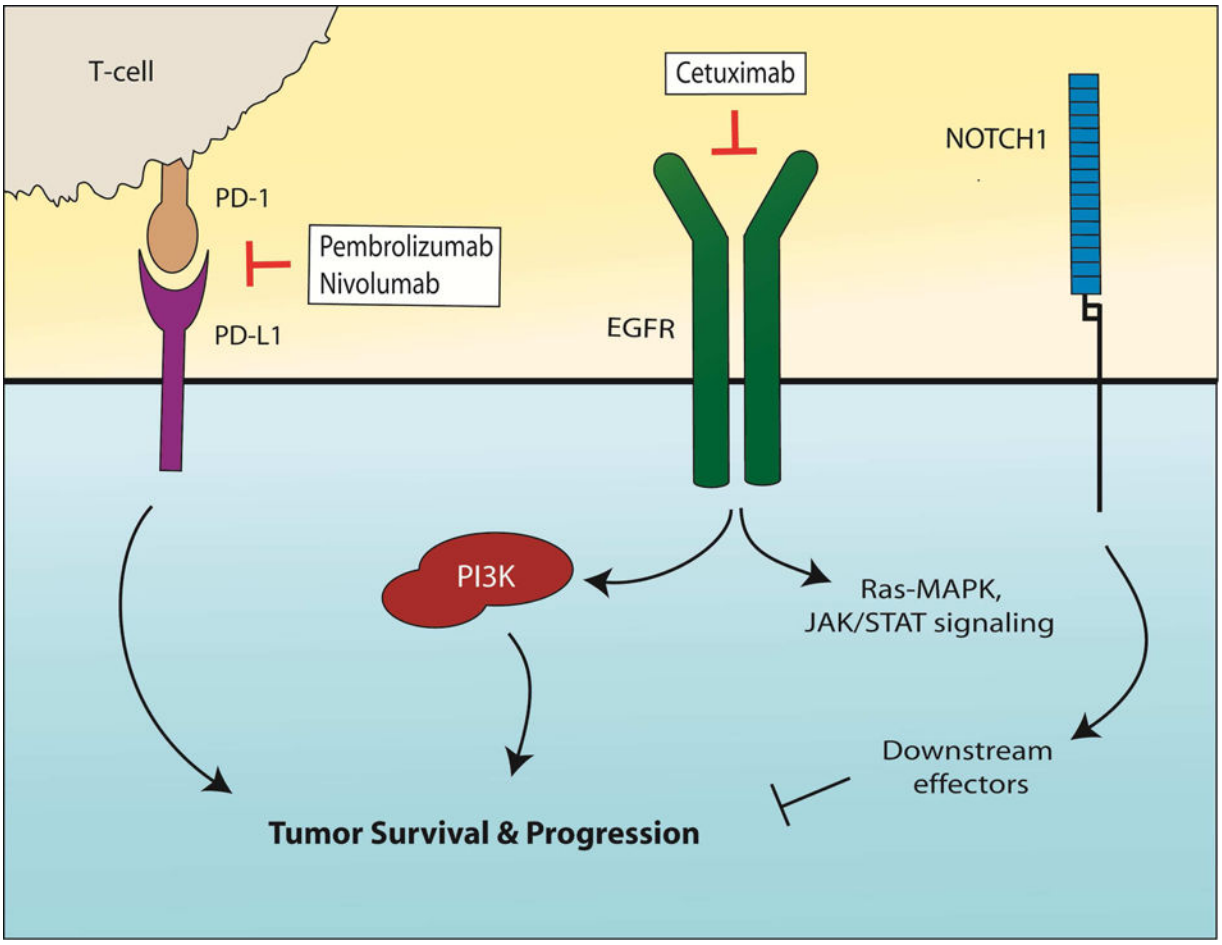

Figure 1. Targetable Pathways in HNSCC

Currently FDA-approved targeted agents are limited to cetuximab (EGFR antibody), pembrolizumab and nivolumab (PD-1 antibodies). Additional targetable and commonly mutated pathways include other members of growth factor receptor families, Ras-MAPK and JAK/STAT downstream signaling pathways, PI3K, and downstream effectors of Notch1.

Table I
Mutation Prevalence Across Various Epidemiologic HNSCC Cohorts

Mutation rates in commonly mutated genes from recent genomic sequencing studies. Variability exists across the different epidemiologic cohorts.

	HPV Positive	HPV Negative*	Young/Low Risk	Recurrent/Metastatic
EGFR	6%[24]	12%[30] – 15%[24]	–	–
FGFR1	0%[30]	2%[30] – 10%[24]	–	–
FGFR3	11%[24] – 12%[30]	1%[30] – 2%[24]	–	–
PIK3CA	22%[30] – 56%[24]	8%[22] – 34%[24,30,35]	0%[35]	–
HRAS	0%[24]	3%[30] – 5%[22,24]	–	–
PTEN	6%[24]	4%[30] – 12%[22,24]	–	–
NOTCH1	17%[24]	11%[22] – 26%[24,35]	25%[35]	–
TP53	3%[24]	63%[22] – 84%[24]	93.8%[35]	(72%)[29]
TERT	0%[29]	–	–	(54%)[29] (55%)[29]
TRAF3	22%[24]	1%[24]	–	–
E2F1	19%[24]	2%[24]	–	–
FAT1	0%[24]	25%*[35]	6.3%[35]	–
CDKN2A	0%[24]	25%[22] – 57%[24]	6.3%[35]	(37%)[29]