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Current Trends in Precision Medicine and Next-Generation Sequencing in Head and Neck Cancer

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Introduction

Despite substantial advances in imaging, diagnostics, and treatment options for patients with head and neck squamous cell carcinoma (HNSCC), 5-year overall survival (OS) has only modestly improved in the last few decades [1]. In 2021, it is estimated that 14,620 deaths will occur in the USA due to HNSCC alone [2]. This disease has largely been an environmentally mediated malignancy with tobacco and alcohol being the most important drivers of carcinogenesis. Currently, the mainstay treatment for HNSCC remains nonselective therapies with a combination of surgery, radiotherapy, and cytotoxic chemotherapy options.

In recent years, new epidemiologic patterns have arisen in HNSCC patients. The human papillomavirus (HPV) has been implicated in the rise of incidence for oropharyngeal SCC, with more favorable survival outcomes compared to HPV-negative disease [3, 4]. Additionally, there has been a rise in incidence of oral cavity SCC in younger patients that lack the traditional risk factors demonstrating a possibly distinct clinical and histopathologic entity [5]. Despite using traditional factors to help predict outcomes (namely tumor stage), we are realizing that not all HNSCC behaves the same, with subsets of HNSCC having better or worse outcomes than predicted by current staging algorithms. Combined, these findings highlight the critical need for further analysis of factors contributing to HNSCC carcinogenesis and prognosis. Key among these is incorporating precision medicine into care, including the validation of prognostic biomarkers and development of patient-specific treatment regimens.

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Conflict of Interest

The authors declare no competing interests.

Next-generation sequencing (NGS) has unlocked the potential to explore the molecular aspects of HNSCC with an aim to enhance precision medicine [6, 7]. Included in these goals for NGS are to highlight prognostic molecular alterations as well as to identify actionable genomic alterations for a variety of cancers, including HNSCC [8]. While NGS has gained popularity among oncologists, it remains in its infancy, and currently there are no evidence-based guidelines to guide our use of NGS clinically [9].

In the field of head and neck cancer, historical success with precision medicine has been limited. Despite the growing interest and efforts in exploring novel therapeutics, cetuximab (an anti-EGFR antibody) remains the only FDA-approved targeted therapeutic agent for HNSCC [10]. The plentitude of mutations, heterogeneity, and variability in HNSCC pose challenges in designing a single “magic bullet” agent, and therefore, patients currently require multimodal therapy to achieve optimal outcomes. Herein, we review the current state of clinical investigations in precision medicine, targeted therapies, and NGS in HNSCC. Additionally, we discuss the most recent advances, the opportunities, and gaps in knowledge related to the emergence of precision medicine within the field.

Current state of understanding of genomics in HNSCC

When imatinib entered the market as a “miracle drug” for the treatment of chronic myelogenous leukemia, the world was excited for the untapped potential of precision medicine and targeted therapies in all cancers [11]. Within the field of head and neck cancer, there was early optimism as investigators identified potential genetic targets that were impacting oncologic prognosis such as *EGFR* [12] and *BCL2* [13]. While these discoveries were promising, NGS technology was in its infancy to validate findings in larger cohorts and to perform genetic screens in large scales. Two landmark papers, published sequentially in 2011, provided the framework for understanding the landscape of mutations in HNSCC through whole exome sequencing [14, 15]. In addition to the previously identified genes implicated in HNSCC (*TP53*, *CDKN2A*, *PIK3CA*), Stransky et al. discovered that at least 30% of cases harbored mutations that regulate squamous differentiation (*NOTCH1*, *IRF6*, and *TP63*) [14]. Agrawal et al. had similar findings in their cohort and also elucidated that 89% of HPV-negative tumors had mutations in tumor suppressor genes, with challenging implications on targeted therapy options (as oncogenes are more easily targetable) [15]. Consistent with epidemiologic studies suggestive of biologic differences, HPV-positive tumors had significantly less mutational burden overall [14, 15], and none harbored *TP53* mutations (while they were present in 78% of HPV-negative tumors) [15].

In 2006, the Cancer Genome Atlas (TCGA) project was initiated to further advance our understanding of cancer genomics. This program has amassed thousands of high-quality cancer samples that have been characterized and have allowed for the research community to develop novel cancer therapeutics [16]. In 2015, the TCGA published the most comprehensive integrative genomic analysis of HNSCC in 279 patients, which expanded our understanding of the mutational landscape of HNSCC [17]. HPV-positive tumors were characterized by a loss of *TRAF3*, activating mutations of *PIK3CA*, and amplification of *E2F1*, while HPV-negative tumors mainly had *TP53* mutations. In a subset of HPV-negative tumors that behaved more favorably, they expressed normal *TP53*, but mutations in *CASP8*

and *HRAS*. They also discovered *CCND1* to be amplified in about one-third of HNSCC [17]. Notably, there are some limitations with existing genomic studies, as outlined below.

Recently, a multicenter consortium characterized the somatic mutational landscape of 227 oral tongue SCCs and identified two additional novel driver genes (*ATXN*1 and *CDC42EP1*), in addition to validating previously identified mutations [18•]. Analysis of 107 patients that were early onset (< 50 years of age) revealed significantly fewer non-silent mutations independent of smoking status, with implications that early onset oral tongue SCC may be a distinct subtype of HNSCC.

Current state of targeted therapies in HNSCC

To date, despite several potential actionable mutations identified in HNSCC, cetuximab remains the only FDA-approved targeted therapy in HNSCC. Two landmark randomized controlled trials demonstrated cetuximab to improve OS in conjunction with radiation and cytotoxic chemotherapy [10, 19]. Following the initial results of cetuximab, and utilizing data from TCGA and other sequencing studies in HNSCC, multiple clinical trials investigating targeted agents in HNSCC have been completed or are ongoing (Table 1) [20]. Currently, the majority of these therapeutics are based on precision medicine paradigms, with valid biologic targets based on known genetic aberrations in HNSCC. Notably, many of these agents are currently approved for other cancers, with the benefit of having known toxicity and tolerability profiles. Given the increased understanding that multimodality therapy may give the best chance for response in aggressive HNSCCs, many of these trials are focused on combination therapeutic regimens, either with conventional surgery, radiotherapy and cytotoxic chemotherapy, or in novel precision medicine targeted or immunotherapy combinatorial regimens (Table 1).

Recently, the NCI-MATCH has been a groundbreaking precision medicine cancer clinical trial that has matched patients to treatments based on actionable mutations by utilizing NGS to screen a large cohort of patients with various cancer types [21]. In 5,954 patients with a variety of advanced refractory cancer, 37.6% were identified to have actionable mutations, with 11.9% having multiple tumor mutations. Although HNSCC qualifies as a solid tumor under NCI-MATCH, none of the patients in this initial cohort had HNSCC; however, other head and neck cancers were explored such as thyroid cancer [22]. Notably, the study also identified 71.3% of cancers possessing resistance conferring tumor mutations. These findings illustrate the need to investigate combination targeted therapy regimens given the complexity and the multiple actionable mutations found in various tumors. Recognizing the need, a successor clinical trial has recently been launched. NCI-ComboMATCH will test combinations of targeted therapeutics that were found to be successful in preclinical in vivo studies to overcome drug resistance that is common in single-agent therapies [23]. These trials demonstrate the sweeping efforts by government agencies to make progress in the field of precision medicine and provide access to FDA-approved drugs used in other cancers based on mutational profile matches. Importantly, HNSCC will need to be investigated rigorously in these umbrella cohorts as well.

Of note, there has been some diminishment in enthusiasm for the benefit of targeted therapy in place of traditional therapeutics, most notably in the recent RTOG 1016 trial.

Based on a subgroup analysis of patients with HPV-positive disease in the Bonner et al trial [10], cetuximab was shown to have an improvement in OS in recurrent/metastatic cohorts [24]. Despite this initial optimism for cetuximab, two recent multicenter randomized clinical trials of patients with HPV-positive oropharyngeal SCC comparing cetuximab with radiotherapy versus cisplatin with radiotherapy (RTOG1016 and De-ESCALaTE HPV) showed cetuximab to be inferior to current standard of care cisplatin (De-ESCALaTE showing 2-year OS 97.5 to 89.4% and 2-year recurrence 6 to 16.1%, RTOG 1016 showing 5-year progression-free survival 78.4 to 67.3%, and 5-year locoregional failure 9.9% versus 17.3% in favor of cisplatin) with no difference in toxicity [25••, 26••]. The results of the study highlight the need for further investigations and rigorous analysis of specific subgroups of HNSCC that may be best treated with precision medicine agents.

Next-generation sequencing platforms in HNSCC

NGS platforms have improved tremendously in recent years, streamlining a once costly and lengthy process into a usable tool for oncologists in general, including head and neck cancer providers. Even in its still-developmental era, NGS is increasingly being considered and incorporated into clinical care; in a recent nationally representative survey study of 1,281 oncologists, approximately 75% reported using NGS to guide patient care (despite a lack of clinical guidelines), with clinicians earlier in their careers being more likely to incorporate this technology into their practices [9].

Currently there are dozens of NGS platforms that are FDA-approved ranging from single-gene analyses (e.g., *EGFR* mutations for non-small cell lung cancer, *BRAF* mutations for melanoma) to several hundred gene panels (such as the FoundationOne CDx; Foundation Medicine, Cambridge, MA) (Table 2) [8, 27]. In HNSCC, multigene sequencing platforms are preferred to single-gene testing to allow for testing of multiple potential actionable targets and to avoid the need for repeated biopsies and potential treatment delays for patients, with the goal of directing patients to the most appropriate clinical trial if applicable [28]. Historically, costs for NGS panels have been prohibitory, but with increasing adoption of NGS in clinical treatment paradigms (particularly with recurrent/metastatic disease), these costs have been reducing overall and have been increasingly covered by insurance. Currently, multigene NGS platforms are most frequently being employed in the recurrent/metastatic setting for HNSCC and to identify patients that may be candidates for biomarker-driven and combinatorial clinical trials (Tables 1 and 2). Additionally, some authors are incorporating NGS in routine clinical care to correlate genomic alterations with oncologic outcomes [29].

It important for clinicians and scientists to recognize the limitations of NGS as more assays become readily available. Assay discordance has mainly been attributed to tumor heterogeneity, but technical variations may play an important role as well [30]. Interpretation and clinical translation of the hundreds of gigabytes of data produced from NGS poses a challenge. It is difficult to parse out clinically significant variants, tumor promoting mutations, or true “actionable” targets from passenger mutations, particularly in HNSCC [31]. More than half of oncologists report that NGS test results are challenging to interpret, indicating the uncertainty on how to clearly incorporate NGS results into clinical decision-

making [9]. Given that NGS assay results may be ambiguous, it is critical that we develop evidence-based clinical guidelines to assist clinicians in making sound decisions for patients with this growing technology.

Challenges in precision medicine in HNSCC

Cancer heterogeneity—There are limitations with the major HNSCC sequencing studies (namely TCGA), as most of the patients in these cohorts were Caucasian, elderly, male, HPV-negative, and smokers. Thus, extrapolating data to other subsets of patients (i.e., young, non-Caucasian, women, non-smoker populations) may not be advisable. Indeed, as noted above, we are seeing distinctly different mutational profiles in these other populations (HPV-positive disease, young patients, non-Caucasian patients), showing that HNSCC in these cohorts may have different genetic drivers and may respond differently to precision medicine and targeted therapeutics.

In addition to tumor heterogeneity between patients, intratumor heterogeneity is an important factor to consider, as it has been long established that individual cells or clonal cell populations within an individual tumor may display distinct molecular differences. Tumor heterogeneity is thought to be driven by Darwinian-like evolution with genetic instability as its main driver [32]. Over the past two decades, NGS has allowed investigators to better elucidate the importance that intratumor heterogeneity has on the efficacy of targeted therapies. A landmark study by Gerlinger et al. performed multiregion genetic analysis on multiple spatially separated samples of four patients with metastatic renal cell carcinoma [33]. They discovered extensive intratumor heterogeneity between the various samples taken, and a single tumor biopsy specimen revealed a minority of genetic aberrations that are present in the entire tumor [33]. Recently, Rasmussen et al. illustrated the importance of this concept by demonstrating that *PD-L1* positivity varies markedly within tumors of patients with HNSCC by taking six random core biopsies of each specimen. They discovered that by using a 1% cutoff, 36% of specimens were concordant with tumor proportion scores (TPS) and 52% with combined positive scores (CPS) [34]. Mroz et al., utilizing the HNSCC TCGA data, illustrate that higher intratumor heterogeneity is associated with worse OS [35]. More recently, single-cell sequencing platforms have further characterized distinct intratumor cellular subpopulations in HNSCC, demonstrating that tumors are heterogeneous with niche cell and mutational populations, with further implications on variations in tumor response to precision medicine [36]. These findings suggest that a single biopsy may underestimate the mutational burden or targetability of heterogeneous tumors and may explain why validating cancer biomarkers as predictors for treatment response is challenging.

Drug resistance—Despite some tumors displaying robust initial responses to targeted therapy, many are followed by relapse [37]. Authors suggest multiple biological determinants of resistance that coexist and interplay with each other including gross tumor burden, growth, heterogeneity, physical barriers, the immune system, undruggable genome, and therapeutic pressure [38]. Tumor burden/extent of disease is almost universally correlated with curability [39] and may predict the probability of drug-resistant clones [40]. Some cancer cells display plasticity with the ability to exhibit resistance to certain therapies

and adapting to form sensitive or resistant progeny, much like how multidrug-resistant bacteria develop [41]. Additionally, conventional chemotherapy and radiotherapy agents can augment genomic instability, potentially leading to an emergence of new mutations among surviving cancer cells [38]. Tumor suppression mutations such as *PTEN* can lead to resistance to targeted therapies [42]. Any single biological determinant of resistance can be the underpinning for treatment resistance. Resistance will continue to be a large challenge, as there are many underlying mechanisms that make cancer biologically complex.

"Actionable" mutations—Not all tumors may have actionable mutations, or a genetic aberration with a specific therapeutic that can target the altered gene/pathway. As illustrated in the NCI-MATCH cohort of nearly 6,000 patients, only about 38% of tumors had an "actionable" mutation [21]. Often, there may be several "actionable" mutations within a tumor, thus making it challenging to identify the potential main driver for the tumor. This raises the questions on whether all "actionable" mutations should be targeted, or if there is a hierarchy on which ones should be targeted. Additionally, it is important to highlight that the vast majority of HPV-negative HNSCC tumors exhibit mutations in tumor suppressor genes [15, 18•]. Unfortunately, our current therapeutics primarily target activated oncogenes, and there are limited options for clinical agents restoring lost tumor suppressor gene function, with historic trials (such as adenovirus-mediated restoration of *TP53*) showing at best modest results [43].

Precision immunotherapy challenges—Many patients do not ultimately benefit from immunotherapy due to primary resistance or relapse after a period of response due to acquired resistance [44]. While nivolumab and pembrolizumab have yielded improved response rates and OS benefits in recurrent/metastatic cases of HNSCC, only a subset of patients are responders [45, 46]. With immune checkpoint inhibitors, a key reason for primary resistance is a lack of recognition of tumor cells by T cells due to the absence of presented tumor antigens, as cancer cells can develop mechanisms to avoid antigen presentation and detection [47, 48]. A recent study demonstrated that primary resistance to PD-1-based immunotherapy may be due to abnormal gut microbiome composition, and broad spectrum antibiotics significantly compromised survival while being treated with immunotherapy [49]. Current areas of research on the molecular resistance mechanisms for PD-1/PD-L1-based immunotherapy agents in HNSCC include tumor cell adaptation, impaired T-cell function and proliferation, changes in the tumor micro-environments, and activation/dependence of alternative immune checkpoints [50]. There is a strong interest in identifying biomarkers that can better predict the response of PD-1/PD-L1-based immunotherapy, as the current regimens (CPS scores) can be far from reliable.

Forecasting future advances in precision medicine in HNSCC

Advances in precision surgery—As precision medicine gains traction within HNSCC through novel therapeutics, "precision head and neck surgery" has also evolved with the advent of new digital technologies [51]. Since transoral robotic surgery (TORS) was introduced in 2006 [52], it has now been included in standard of care algorithms for patients undergoing definitive surgery for oropharyngeal SCC, with low complications and high effectiveness at achieving negative margins (~95%) [53].

Coupling standard of care surgery with real-time intraoperative tumor imaging has the potential to further advance precision surgery, particularly in HNSCC, where it is often challenging to distinguish cancer from normal surrounding mucosa. Recently, van Keulen et al. demonstrated in a case series the clinical utility of panitumumab (an anti-*EGFR* antibody) conjugated to a fluorescent probe to help identify margins in HNSCC more precisely with 100% effectiveness, showing the value of precision targeted agents in surgical guidance (Table 1) [54]. At our institution, we are actively investigating autofluorescence augmented reality by exploiting the inherent autofluorescence signature of cancerous tissue compared to normal mucosa with the use of a prototype time-resolved fluorescence spectroscopy integrated synergistically during surgery for HNSCC [55]. We have demonstrated in HNSCC surgical cases that label-free fluorescence lifetime imaging has the potential for clinical application for margin assessment, and distinction between healthy and cancerous tissue [56]. Further studies incorporating targeted therapeutics to help guide surgical resections, as well as to provide anti-cancer effects (so-called “theranostics”), will be of high interest for our field. Furthermore, future incorporation of machine learning and artificial intelligence, both for pre-treatment planning and intraoperative integration, may have additional benefits in developing precision surgery paradigms [57].

Developments in precision immunotherapy—Immunotherapy in HNSCC has gained fast traction over the last two decades, as there has been a better understanding of tumor immune microenvironments and the immune system’s critical role in regulating tumor cell behavior [58]. The approval of pembrolizumab and nivolumab (monoclonal antibodies targeting PD-1) for recurrent/metastatic HNSCC marked a promising development in the treatment of HNSCC and has since led to an increase in trials designed to assess the therapeutic implications of immunotherapy in combination with other treatments (Table 1). In addition, several scoring systems and gene mutations have been developed to predict treatment responses and stratify treatment approaches for personalized immunotherapy more accurately. Based on emerging data, two scoring methods designed to assess the expression of PD-L1, TPS, and CPS, have been developed and have been shown to predict response of tumors to anti-PD-1 therapy [59, 60], with similar predictive qualities [61]. Currently, implementation of CPS score is reproducible and is routinely used to stratify immunotherapy approaches, thus highlighting the importance of tailoring approaches for individual patients and their unique tumors [59]. As noted above, current active areas of research to enhance the precision of immunotherapy include interrogation of tumor cell adaptations, impairment of T cell functions, alterations of the immune microenvironment, and activation of alternative immune checkpoints [50]. Additional analyses of the immune microenvironment in HNSCC are identifying potential biomarkers for disease prognosis, and response to therapy (including Treg/$CD8^+$ T cell ratio and NK cell infiltration), with major implications in precision medicine and patient treatment paradigms [62, 63].

Of recent increased interest for precision immunotherapy in HNSCC are combinatorial regimens, including regimens with dual immune checkpoint agents, as well as combinations which may affect tumor cells and the immune microenvironment to more favorably respond to existing immune checkpoint agents. There are multiple ongoing clinical trials investigating these precision medicine paradigms (Table 1). Future trials examining these

effects should remain an integral aspect in the development of drug targets and designing treatment regimens harnessing the power of precision medicine.

Improvement in predictive biomarkers in HNSCC—Several biomarkers have been implicated in progression and survival in HNSCC. While the association of p16 and HPV status with survival has been well-described for many years, it has only now been incorporated into our staging manual for prognostic guidance [64]. There is a greater need for tumor biomarkers in HNSCC, both as prognostic guides and as treatment targets, in a similar fashion to the utility of *EGFR* mutations in specific lung cancers [65], or *BRAF* mutations in melanoma [66] or specific thyroid cancers [67]. A number of novel emerging prognostic biomarkers have been identified in recent years for HNSCC [68], with NGS offering the ability for enhanced characterization of tumors on the molecular level (Table 2), with several mutations such as *NOTCH*$_1$, *CDKN2A*, and *TP53* associated with poor survival [69]. An exciting on-going attempt to integrate mutational status with treatment stratification is an ongoing trial using high-risk mutations in *TP53* (almost ubiquitously mutated in HPV-negative HNSCC) to guide the extent of adjuvant therapy (radiation versus cisplatin with radiation; NCT02734537) [70]. Further prospective clinical trials which stratify care intensity on the basis of other mutational signatures or biomarkers will be critical to better develop precision medicine pathways for HNSCC.

Tumor-infiltrating lymphocytes (TIL) and other markers of the immune microenvironment are increasingly recognized as an important biomarker in HNSCC. In a prospective, epidemiologic study of 464 previously untreated patients with HNSCC, higher levels of TIL were associated with improved OS and disease-specific survival, after controlling for numerous clinicopathologic factors [71]. In a cohort of 76 patients with advanced laryngeal SCC, CD8 TIL counts were significantly associated with degree of clinical response to chemotherapy and predictive of disease-specific survival, suggesting that TIL assessment has a potential for a bioselection treatment approach [72]. A recent systematic review and meta-analysis of immune markers in HNSCC confirmed prior findings and showed that $CD163^+$ and M2 macrophages in addition to $CD57^+$ NK cells are the most significant predictors of survival among patients with oral cavity SCC [73]. While larger studies examining the prognostic relevance of immune microenvironment signatures are required (both for prognosis and response to immunotherapy), these findings suggest that some form of TIL grading may potentially be used to further personalize immunotherapy regimens and incorporated into future iterations of clinicopathologic staging and prognostic models for patients with HNSCC. Future studies implementing NGS technologies will certainly aid in identifying molecular targets for HNSCC patients and serve a role in prognostication moving forward.

Conclusion

Significant progress has been made in precision medicine and NGS in HNSCC; however, precision medicine remains in its early stages in HNSCC, with many opportunities for improvement and incorporation into standard of care. Initial studies and recent focusing on targeted HNSCC treatments have had a positive impact on advancing the development of novel therapy approaches and precision medicine regimens. Further incorporation of NGS

into HNSCC treatment algorithms and identification of prognostic and targetable molecular aberrations and immunologic signatures will be critical next steps for our field. Building on our existing knowledge, and increasing progress in precision medicine and NGS in HNSCC, there will be a critical need for future prospective studies and clinical trials to validate prognostic and stratifying biomarkers and combinatorial novel therapeutic trials.

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Opinion statement

As the field of oncology enters the era of precision medicine and targeted therapies, we have come to realize that there may be no single “magic bullet” for patients with head and neck cancer. While immune check point inhibitors and some targeted therapeutics have shown great promise in improving oncologic outcomes, the current standard of care in most patients with head and neck squamous cell carcinoma (HNSCC) remains a combination of surgery, radiation, and/or cytotoxic chemotherapy. Nevertheless, advances in precision medicine, next-generation sequencing (NGS), and targeted therapies have a potential future in the treatment of HNSCC. These roles include increased patient treatment stratification based on predictive biomarkers or targetable mutations and novel combinatorial regimens with existing HNSCC treatments. There remain challenges to precision medicine and NGS in HNSCC, including intertumor and intratumor heterogeneity, challenging targets, and need for further trials validating the utility of NGS and precision medicine. Additionally, there is a need for evidence-based practice guidelines to assist clinicians on how to appropriately incorporate NGS in care for HNSCC. In this review, we describe the current state of precision medicine and NGS in HNSCC and opportunities for future advances in this challenging but important field.

Table 1.

Examples of active precision medicine trial designs in HNSCC

Trial design	Population	Agent(s)	Gene/pathway	Phase in HNSCC (clinical trial ID)
Targeted therapy				
Targeted therapy, biomarker guided	R/M with *HRAS* mutation	Tipifarnib	*FNTA/HRAS*	II (NCT03719690)
	R/M after failed initial therapy, with alterations in *CDKN2A*,*CCND1*, or *CDK6*	Abemaciclib	*CDK4/6*	II (NCT03356223)
Targeted therapy + surgery	Neoadjuvant prior to surgery	Ruxolitinib	*JAK1/2*	II (NCT03153982)
	Patients undergoing surgery (for intraoperative guidance)	Panitumumab	*EGFR*	II (NCT04511078)
Targeted therapy + radiation	Advanced HNSCC, cannot tolerate cisplatin	Peposertib	*DNA-PK*	I (NCT04533750)
Targeted therapy + targeted therapy	R/M after failed initial therapy	Cetuximab Afatinib	*EGFR* *EGFR/HER2*	II (NCT02979977)
	R/M	NT219 Cetuximab	*STAT3* *EGFR*	I/II (NCT04534205)
Targeted therapy + chemotherapy + radiation	Adjuvant after surgery (one arm of trial)	Cetuximab Docetaxel	*EGFR*	II/III (NCT01810913)
Targeted therapy + immunomodulator	Prior to surgery or biopsy (one arm of trial)	Cetuximab TAK-981	*EGFR* *SUMO*	I (NCT04065555)
Targeted therapy + immunotherapy	R/M	Cetuximab Monalizumab	*EGFR* *NKG2A*	III (NCT04590963)
Immunotherapy				
Immunotherapy + surgery	Neoadjuvant/adjuvant prior to and after surgery	Pembrolizumab	*PD-1*	II (NCT02296684)
Immunotherapy + radiation	R/M after failed immunotherapy monotherapy	Pembrolizumab	*PD-1*	II (NCT03085719)
	Adjuvant after surgery (one arm of trial)	Atezolizumab	*PD-L1*	II/III (NCT01810913)
Immunotherapy + chemotherapy	R/M after failed immunotherapy monotherapy	Durvalumab Decitabine	*PD-L1*	II (NCT03019003)
Immunotherapy + immunotherapy	R/M	Enoblituzumab Retifanlimab Tebotelimab	*B7-H3* *PD-1* *LAG-3*	II (NCT04634825)
	R/M	Pembrolizumab Bapotulimab	*PD-1* *ILDR2*	I (NCT03666273)
Immunotherapy + immunomodulator	R/M	Pembrolizumab ADP-A2M4 T cells	*PD-1* *MAGE-A4*	II (NCT04408898)
Immunotherapy + immunomodulator	Prior to surgery or biopsy	Avelumab TAK-981	*PD-L1* *SUMO*	I (NCT04065555)
Immunotherapy + immunomodulator	R/M, HPV16+	Pembrolizumab BNT113	*PD-1* HPV virus	II (NCT04534205)

R/M recurrent/metastatic HNSCC2

Table 2.

NGS platforms and targetable/prognostic genes for HNSCC

Platform	Total genes tested	HNSCC biomarker genes tested	HNSCC targetable genes tested	Approval
FoundationOne CDx	324 (SNVs, indels, CNAs, rearrangements, MSI, TMB, PD-L1)	Yes	Yes	2017
MSK-IMPACT	468 (SNVs, indels, MSI)	Yes	Yes	2017
NantHealth Omics Core	468 (SNVs, indels, TMB)	Yes	Yes	2019
Illumina TruSight Oncology 500	523 (SNVs, indels, MSI, TMB)	Yes	Yes	2019
Tempus xT Oncology	648 (SNVs, indels, CAN, rearrangements, MSI, TMB, RNA transcriptome)	Yes	Yes	Pending

SNV single nucleotide variants, *Indels* insertions/deletions, *CNA* copy number alterations, *MSI* microsatellite instability, *TMB* tumor mutational burden