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Tumor DNA: an emerging biomarker in head and neck cancer

Joseph A. Bellairs¹, Rifat Hasina², and Nishant Agrawal^{1,2}

¹Pritzker School of Medicine, University of Chicago, 5841 S. Maryland Avenue, MC 1035, Chicago, IL 60637, USA

²Section of Otolaryngology-Head and Neck Surgery, University of Chicago Medicine, Chicago, IL, USA

Abstract

Head and neck cancer (HNC) includes a diverse range of malignancies arising commonly from mucosal epithelia of the upper aerodigestive tract. Head and neck squamous cell carcinoma (HNSCC), the most common form of HNC, develops in the oral cavity, pharynx, and larynx and is associated with tobacco exposure, alcohol abuse, and infection with oncogenic viruses. Despite global advances in cancer care, HNSCC often presents with advanced disease and is associated with poor 5-year survival of ~50%. Genotyping tumor tissue to guide clinical decision-making is becoming commonplace in modern oncology, but in the management of HNSCC, tissue biopsies with cytopathology or histopathology remain the mainstay for diagnosis. Furthermore, conventional biopsies are temporally and spatially limited, often providing a brief snapshot of a single region of a heterogeneous tumor. In the absence of a useful biomarker, both primary and recurrent HNSCCs are diagnosed with conventional imaging and clinical examination. As a result, many patients are diagnosed with advanced disease. Tumor DNA is an emerging biomarker in HNSCC. DNA fragments are constantly being shed from tumors and metastatic lesions, and can therefore be detected in blood and other bodily fluids. Utilizing next-generation sequencing techniques, these tumor DNA can be characterized and quantified. This can serve as a minimally invasive liquid biopsy allowing for specific tumor profiling, dynamic tumor burden monitoring, and active surveillance for disease recurrences. In HNSCC, analysis of tumor DNA has the potential to enhance tumor profiling, aid in determining patient prognosis, and guide treatment decisions.

Keywords

Liquid biopsy; Cancer genetics; Circulating tumor DNA; Head and neck cancer

1 Introduction

Worldwide, head and neck cancer (HNC) accounts for greater than 800,000 new cancer diagnoses annually, with approximately 61,000 new cases and 13,000 deaths reported in the

Correspondence to: Nishant Agrawal.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

USA each year [1, 2]. HNC encompasses malignancies originating from multiple anatomical subsites, including the oral cavity, pharynx, larynx, salivary glands, nasal cavity, and paranasal sinuses. Despite the anatomical diversity of the head and neck, more than 90% of HNC is classified as head and neck squamous cell carcinoma (HNSCC) originating from the oral cavity, pharynx, and larynx [3]. Although significant advances have been made in cancer care over the past decades, HNSCCs remain associated with poor 5-year overall survival of approximately 50% [4]. Targeted therapies for HNSCC are limited, in part because most genetic mutations in HNSCC inactivate tumor suppressor genes [5–8]. To date, there has been no groundbreaking targeted therapy for HNSCC, with most clinical trials yielding modest response rates and insignificant clinical benefits [9–11]. There is also a paucity of disease-specific biomarkers, forcing clinicians to rely on conventional imaging and clinical examination to evaluate disease burden, response to therapy, and disease recurrence.

HNSCC can be classified anatomically into squamous cell carcinoma (SCC) of the oral cavity, oropharynx, hypopharynx, and larynx. Well-established risk factors for HNSCC include tobacco exposure, sustained alcohol use, and infection with oncogenic human papilloma virus (HPV) [12, 13]. Over the past decade, there has been an observed shift in the distribution of primary tumors. A decline in cancers of the larynx and hypopharynx has paralleled an overall decrease in cigarette smoking [14]. Conversely, an overall increase in oropharyngeal SCC (OPSCC) has accompanied the identification that infection with HPV-16 and HPV-18 is a HNSCC risk factor [14, 15]. This changing paradigm has led to a divergence in HNSCC taxonomy, with HPV status correlating to patient demographics, molecular phenotype, and clinical features. HPV-positive tumors tend to occur in middle-aged, non-smoking white men with a history of exposure to multiple sex partners, whereas HPV-negative tumors occur in patients over 60 with a significant tobacco and alcohol use history [16, 17]. Whole-genome sequencing has identified that HPV-positive tumors frequently carry mutations of *TRAF3* and *PIK3CA*, while HPV-negative tumors present with mutations to *TP53*, tumor survival pathways (*MTOR* and *EGFR*), tumor proliferation pathways (*CDKN2A*), and tumor differentiation pathways (*NOTCH1*) [5, 8, 18]. Clinically, HPV-positive tumors carry a much better prognosis than HPV-negative tumors, with 2-year overall survival rates of 94 and 58%, respectively [16]. Epstein-Barr virus (EBV) DNA was one of the earliest cell-free DNA biomarkers to be used in head and neck cancer to evaluate nasopharyngeal carcinoma [19]. Serological titers of EBV-related biomarkers have recently been shown to correlate to tumor burden and predict metastatic potential in nasopharyngeal carcinoma [20].

Currently, treatment decisions for HNSCC are made primarily based on radiographic disease staging, with clinical trials and protocols offered to de-intensify treatment for HPV-positive disease. In general, early-stage disease (stage I and II) is treated with surgery or radiation alone [12, 21, 22]. Patients with advanced-stage disease (stage III and IVA/B) are often treated with a combination of surgery, chemotherapy, and radiation therapy [12, 21, 22]. Metastatic disease (stage IV C) is treated with combination chemotherapy or single-agent chemotherapy, depending on the patient's overall performance status [12, 21, 22]. All of these therapies are associated with significant morbidities that may drastically affect post-treatment quality of life. With the emerging realization that HPV-positive and HPV-negative

HNSCC represent biologically and clinically distinct entities, there is ongoing work to risk stratify and treat HNSCC patients on the basis of HPV infection status [23, 24]. Since HPV-positive tumors tend to occur in younger, highly functional patients, preserving quality of life is of utmost importance. Clinical trials evaluating treatment de-intensification in low-risk patients (HPV-positive) with the overall goal of decreasing treatment-related morbidity and maintaining established survival outcomes are ongoing [25, 26].

Changing paradigms in HNSCC biology and treatment have prompted renewed focus on early and accurate diagnosis and molecular characterization. Modern diagnosis employs clinical examination, advanced imaging (CT, MRI, and PET), and histological evaluation of tumor biopsies [21, 22]. The anatomic challenges of the head and neck make this diagnostic procedure complex and highly specialized, thus leading to delays in treatment and negative effects on patient prognosis and survival [27–29]. Furthermore, single-site biopsies may provide an accurate histologic diagnosis, but tumor heterogeneity and sampling bias can impede accurate genetic and molecular characterization of tumors. Recent work in renal cell carcinoma demonstrated that 63 to 69% of detected somatic mutations are heterogeneous, and therefore not detectable in all sequenced regions of a tumor [30]. Single-site biopsy is likely to underestimate mutational burdens, but extensive multisite biopsies are impractical due to the risk of complications and associated costs. Our current practices are analogous to investing significant time and money to characterize a single “tree,” but we frequently fail to appreciate the overall diversity of the proverbial “forest.”

There is a clear need to develop improved biomarkers for the diagnosis of HNC, and specifically HNSCC. The idea that specific tumor mutations could be used for cancer biomarkers was first proposed more than two decades ago [31–33]. Since all cancers, including HNSCC, are defined by genetic mutations, a biomarker based on tumor-specific DNA would be exquisitely specific for neoplastic cells. Because tumors are highly vascular and frequently involve mucosal surfaces, DNA collected from blood and other bodily fluids provides a broader profile of the tumor compared to a single-site biopsy. Exploiting genetic alterations for tumor characterization has been hampered by the fact that the concentration of tumor DNA is often very low in most bodily fluids. However, advances in sequencing technology over the past decade have facilitated the detection of mutated DNA from multiple bodily fluids. In this review, we will discuss the advantages and challenges associated with using tumor DNA as biomarker. We will also pay close attention to the applications of tumor DNA in HNC.

2 Tumor DNA as a liquid biopsy

2.1 Tumor DNA

Initially reported in the mid-twentieth century, circulating cell-free DNA (cfDNA) has been successfully utilized in multiple fields of medicine [31–34]. Perhaps the most notable application is the use of circulating fetal DNA collected from maternal plasma to screen for germline mutations [35, 36]. Multiple studies have also demonstrated that pathologic conditions, such as myocardial infarction, stroke, and trauma result in cellular injury and increase levels of measurable cfDNA [37–40].

In the field of oncology, cfDNA released from tumors is referred to as circulating tumor DNA (ctDNA). Because cancer is by definition a disease originating from a genetic abnormality, ctDNA can be distinguished from cfDNA on the basis of somatic mutations. While point mutations most commonly separate malignant cells from non-cancerous ones, aneuploidy, viral oncogenes, frameshifts, and amplifications can also trigger malignant transformations [41, 42]. Rapid growth and increased cell turnover are hallmarks of many cancers, but this process inevitably leads to an increased fraction of cells undergoing apoptosis and necrosis [43, 44]. Phagocytosis is impaired within a tumor, thus leading to an accumulation of cellular debris and subsequent release into surrounding tissues. Tumor DNA that enters the circulation becomes ctDNA, but depending on the anatomic location of the tumor, mutated DNA can also be retrieved from saliva, stool, urine, and Pap smear fluids [32, 34, 45–47]. The amount of DNA shed from a tumor is likely determined by its overall size, vascularity, invasion into mucosal surfaces, and location (Fig. 1).

Historically, applications of ctDNA have been limited because tumor DNA represents a small fraction of the total DNA found in bodily fluids. In most cancer patients, ctDNA represents < 1.0% of total cfDNA, making detection and quantification challenging and inconsistent with traditional sequencing techniques [48, 49]. The advent of digital genomic technologies has revitalized investigation of tumor DNA as a cancer biomarker. Digital polymerase chain reactions (dPCR); beads, emulsion, amplification, and magnetics (BEAMing); pyrophosphorolysis-activated polymerization (PAP); and Safe Sequencing System (Safe-SeqS) have proven to be highly sensitive at detecting ctDNA in cancer patients [42, 48, 50–53]. The ability to detect and quantify tumor DNA collected from blood and other bodily fluids opens the door to a wealth of clinical applications. There are PCR-based and next-generation sequencing (NGS)-based technologies that have been described for ctDNA detection which may or may not depend on prior knowledge of the somatic mutations from the primary tumor. The common goal is to apply an error reduction technology to identify low-frequency mutations.

The use of circulating tumor cells (CTCs), detection of whole cells circulating through the blood stream, has been studied in several cancers including HNSCC in an effort to utilize them as biomarkers. These studies have revealed that CTCs have limitations due to technical challenges including EpCAM expression and low cell yield [54]. The studies in HNSCC are early and suggest a potential role as a prognostic biomarker [55].

2.2 A liquid biopsy

Conventional tissue biopsies are essential in modern cancer care. In HNSCC, HPV infection status is screened with p16 immunohistochemical (IHC) analysis of biopsy specimens [12]. Many targeted therapies also require tumor tissue to identify driver mutations and allow patients to access personalized treatment protocols. However, from the patient perspective, biopsies are invasive, time-consuming, and costly. Biopsies, like all surgical procedures, also carry risks of complications. Perhaps the most important limitation for conventional biopsies is the growing realization that they are spatially and temporally insufficient to analyze dynamic tumors.

The discovery that tumors are genetically heterogeneous, where one region of a three-dimensional tumor may contain specific mutations but lack others, fundamentally restricts the utility of conventional biopsies [30]. Similarly, in metastatic disease there are observed genetic differences when a metastatic lesion is compared to the primary tumor and other metastatic lesions [30]. Intratumoral and intrametastatic heterogeneity could explain a tumor's propensity to develop resistance to targeted therapies despite reassuring genetic profiles obtained from pretreatment biopsies. Minor genetic mutations within tumor subpopulations have the propensity to become the dominant clones when appropriate selective pressure is applied. Conventional biopsies are poorly suited to account for tumor heterogeneity due to the costs and risks associated with extensive tumor and metastasis sampling. Analogously, serial biopsies to evaluate genetic changes in tumors over time and in response to treatment are not feasible from an economic and patient safety perspective.

As we progress into an era of personalized medicine, there is a clear need for a less invasive biopsy technique to accurately evaluate tumor mutations. A liquid biopsy utilizing tumor DNA found in blood and other bodily fluids could address many limitations of conventional biopsies, while also opening the door to new paradigms in cancer care. The minimally invasive nature of a liquid biopsy is vital to its clinical utility. Blood and other bodily fluids are usually accessible in a standard physician office visit, potentially allowing for rapid diagnosis and characterization of tumors. Furthermore, these minimally invasive techniques could provide tumor-specific information in patients with contraindications to conventional biopsies. Blood and bodily fluids can also be collected throughout a treatment course, thus opening the door for dynamic tumor monitoring. The advantages and applications of a liquid biopsy fall under two broad categories: genetic characterization and tumor quantification.

Much like conventional tissue biopsies, liquid biopsies can be used as a tool to probe the genetic profile of a lesion [50]. Tumor DNA obtained from bodily fluids will contain exact copies of the mutations present in the tumor, and thus can be used to guide targeted therapies. Because tumor DNA is shed throughout a primary tumor and from metastatic lesions, liquid biopsies will also account for tumor and metastasis heterogeneity. Information about a cancer's heterogeneous makeup could then be used to design therapeutic strategies targeting both primary mutations as well as minor subpopulation mutations that could confer treatment resistance. A better understanding of the subpopulations within a tumor could also provide valuable prognostic information and allow for intensification/de-intensification of therapy when indicated.

Paramount to the utility of this technique is the sensitivity of current sequencing techniques to detect tumor DNA in liquid biopsies. Because tumor stage correlates with overall tumor burden, staging correlates directly with sensitivity [45]. Amongst multiple different tumor types, tumor DNA sensitivity is approaching 100% for stage IV disease [48, 56]. Early disease and micrometastatic disease have lower levels of ctDNA and are therefore more likely to yield false negative results [56]. Theoretically, the sensitivity of these techniques is limited by the error rate of DNA polymerase, which is generally assumed to be 0.01% [57]. This translates to a detection threshold of 0.01%, and any result reporting a smaller fraction of mutant DNA can be considered negative. Next-generation sequencing approaches, such as digital droplet PCR, are under investigation and have shown promising detection thresholds

as low as 0.005% [58]. These detection limits are important to keep in mind, because false negative results could adversely delay diagnosis.

Because tumor burden directly correlates with the fraction of ctDNA, liquid biopsies can be used to monitor tumor burden. Traditional cancer biomarkers such as PSA, cancer antigen 19–9, and carcinoembryonic antigen can be used to monitor therapeutic response, but they are plagued by low specificity. Many conditions unrelated to tumor growth and progression can result in non-specific biomarker elevations. Additionally, these proteins are relatively stable in the circulation and can persist in circulation for weeks, making it possible to monitor the effects of interventions over weeks to months [59–61]. Conversely, tumor DNA is highly specific to malignant disease, has a short half-life, and is readily quantifiable. A half-life of approximately 2 h allows for changes in tumor burden to be monitored over hours as opposed to weeks [48]. Because tumor DNA levels accurately track with disease burden, they can also be used to track the clinical course of a disease. Multiple studies have demonstrated that tumor DNA levels rapidly increase with disease progression, and subsequently fall following successful therapeutic interventions [45, 48, 62].

Tumor DNA can also be used in post-treatment monitoring. In current practice, there is no effective way to screen patients and identify those that have residual disease following surgery or curative therapy. Instead, risk of residual disease, and therefore likely benefit of adjuvant chemotherapy, is evaluated with TNM staging [21, 22]. Genetic characterization is emerging as a tool to further risk stratify patients, but it falls short of detecting post-treatment disease [63]. A pilot study validated the use of ctDNA as a marker for disease recurrence in colorectal cancer. Patients underwent curative surgery, and tumor samples were analyzed for tumor-specific mutations. These mutations were used as personalized templates to identify ctDNA after surgery. Patients were followed post-operatively over the course of 2 to 5 years, and ctDNA was measured at different intervals. Of the 18 patients enrolled, all those with detectable ctDNA following therapy suffered a disease recurrence ($n = 13$), while those with no detectable post-operative ctDNA ($n = 5$) remained disease free [48].

3 Utility of tumor DNA in head and neck cancer

3.1 Current applications

The use of tumor DNA as a means to profile tumors and evaluate tumor burden has been reported in colorectal, lung, prostate, pancreatic, melanoma, and breast cancers [45, 48, 62, 64, 65]. Unfortunately, applications of tumor DNA in HNC are somewhat lagging behind these other malignancies and largely limited to HNSCC (Table 1). Early work was done primarily in late-stage (stage IV) HNSCC patients. In a study evaluating ctDNA in 640 patients with different tumor types, 10 HNSCC cancer patients were studied. Gene mutations were identified in biopsy samples, and tumor-specific ctDNA was isolated in 70% ($n = 7$) of patients. This same study also reinforced the principle that ctDNA is more readily detected in advanced disease. In the entire study cohort, ctDNA was isolated in 47, 55, 69, and 82% of patients with stage I–IV disease, respectively [45].

The anatomic and pathologic features of HNSCC allow for unique applications of tumor DNA. Because HNSCC is by definition a mucosal disease, all tumors are anatomically related to mucosal surfaces and bathed in secretions. This allows tumor DNA to be collected from the basal surface of a tumor *via* blood, and from the apex in saliva. Regarding its unique pathogenesis, HPV-positive HNSCC will contain viral oncogenes. This means that HPV-positive malignancies can be screened for HPV-specific DNA fragments in place of somatic mutation. A recent retrospective study evaluated the feasibility of using HPV-16 DNA in plasma and saliva as marker for OPSCC. Utilizing samples from 81 patients with HPV-positive tumors, analysis of pretreatment saliva was 52.8% sensitive in detecting disease, while pretreatment plasma was 67.3% sensitive. However, combined analysis of pretreatment plasma and saliva yielded a sensitivity of 76% and a specificity of 100%. Post-treatment samples were also analyzed for HPV-16 DNA, and a combined analysis was 69.5% sensitive and 90.7% specific in predicting disease recurrence in 3 years. These promising results demonstrate clinical utility of HPV-16 DNA in screening for OPSCC primary disease and as a predictive tool for recurrence [66].

While the previously mentioned studies have evaluated primarily advanced (stage III and IV) disease, a recent study highlighted the utility of tumor-specific DNA to detect early HNSCC in multiple anatomic subsites. Both plasma and saliva were screened in 47 HNSCC patients, and tumor DNA was found in 96% of patients. Subgroup analysis determined that tumor DNA was identified in 100% ($n = 10$) of early (stage I and II) disease and 95% ($n = 37$) of advanced (stage III and IV) disease. When segregated by primary tumor origin, tumor DNA was detected in all patients with lesions of the oral cavity ($n = 15$), larynx ($n = 7$), and hypopharynx ($n = 3$), and in 91% ($n = 22$) of patients with oropharyngeal tumors. This study also briefly examined the utility of tumor DNA for post-surgery surveillance. Post-treatment samples were collected from a total of eight patients and analyzed for tumor DNA. Tumor-specific DNA was found in four patients, three of whom ultimately developed recurrences. Post-treatment samples from the remaining five patients yielded no tumor DNA, and at a median follow up of 12 months no disease recurrences had been diagnosed [67].

These proof-of-principle studies demonstrate that tumor DNA, collected from either plasma or saliva, can be used as a diagnostic biomarker in HNSCC. Tumor DNA, as determined by the presence of somatic mutations (*TP53*, *PIK3CA*, *CDKN2A*, *NOTCH1*) and HPV viral genes (*E6*, *E7*) is highly specific for HNSCC when isolated from saliva and plasma samples [66, 67]. When both blood and saliva are examined in tandem, the sensitivity of these assays also increases. Tumor DNA can also be used to identify both early and advanced HNSCC across multiple anatomic subsites [67]. Interestingly, tumor DNA appears to be more detectable in early HNSCC than other early malignancies [45, 67]. It is unclear if this is a reflection of differing techniques between studies, or perhaps due to anatomical considerations of the head and neck, such as the increased vascularity of the region.

3.2 Future applications

Now that the utility of tumor DNA has been demonstrated in multiple cancers, including HNSCC, research can begin to focus on applications to improve patient care (Fig. 2). While still largely investigational, this technique represents a minimally invasive diagnostic

approach to HNC. The ability to detect somatic mutations and HPV DNA in blood and saliva could greatly speed the diagnosis of new HNSCC, and hopefully leads to improved outcomes. Furthermore, the ability to accurately characterize individual tumors will hopefully usher in a new generation of targeted and personalized therapeutic strategies. Improving techniques to quantify tumor DNA will also allow for better disease monitoring and surveillance. Accurate monitoring of overall tumor burden will allow clinicians to determine in near-real time which therapies are most efficacious. There are also promising signs tumor DNA could provide valuable information about disease recurrence months before clinical or radiographic evidence appears [66, 67].

These pilot studies set the stage for larger studies to explore the impacts of tumor DNA on patient care. As the costs associated with next-generation sequencing technology continue to decline, these larger studies will become more feasible. Furthermore, research can also take place in other HNCs that affect proportionally less patients than HNSCC, but can be equally as devastating from a mortality and morbidity perspective.

4 Conclusions

Tumor DNA is a promising biomarker that can be applied in HNC. Advanced next-generation sequencing techniques have made it possible to interrogate blood and other bodily fluids for tumor-specific genetic mutations. Because these mutations are the foundation of a patient's cancer, this minimally invasive technique offers improved specificity when compared to traditional cancer biomarkers. Sensitivity is determined by the next-generation sequencing technologies available, and as research in this field progresses, sensitivity will continue to increase. The true utility of tumor DNA, however, is that it allows for both tumor characterization and quantification. A minimally invasive liquid biopsy will address tumor heterogeneity and allow for dynamic tumor profiling. This level of tumor characterization can be utilized to identify therapeutic targets, track tumor evolution, and design the next generation of personalized therapies. In terms of quantification, serial analysis of tumor DNA allows for dynamic monitoring of tumor burden before and during treatment, and surveillance for disease recurrence post-treatment. In HNSCC, foundational studies have validated that tumor DNA can be identified in blood and saliva. Larger studies are needed to better characterize the utility of tumor DNA for cancer screening and patient prognosis.

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Fig. 1.

Schematic showing the shedding of tumor DNA from head and neck cancers into the saliva or plasma. Adapted from Bettegowda et al. (2014) [45]. Reprinted with permission from AAAS. Tumors from various anatomic locations shed DNA fragments containing tumor-specific mutations and HPV DNA into the saliva or the circulation

Fig. 2. Proposed applications of tumor DNA as a biomarker for HNSCC. ctDNA collected from patient plasma and saliva could aid clinicians in earlier diagnosis of HNSCC and serve as a valuable marker of tumor burden to monitor for response to treatment and disease recurrence

Table 1

Summary of published tumor DNA applications in HNSCC

Reference	Study population	Tumor DNA source	Mutational analysis	Results	Significance
Bettegowda et al. 2014 [45]	640 patients with different cancers, including 10 HNSCC patients with advanced disease (stage IV).	Plasma ($n = 10$).	Targeted sequencing, exomic sequencing, or whole-genome sequencing was used to identify a single tumor-specific mutation.	Tumor DNA was identified in 70% ($n = 7$) HNSCC patients.	ctDNA can be found in plasma of HNSCC patients.
Ahn et al. 2014 [66]	93 OPSCC patients, 87% ($n = 81$) with HPV+ positive tumors.	Pre- and post-treatment saliva ($n = 83$) and plasma ($n = 61$).	Quantitative PCR was used to probe for HPV-16 E6 and E7 viral oncogenes.	Amongst patients with HPV+ tumors, tumor DNA was detected in pretreatment saliva and plasma samples in 53% ($n = 38$) and 67% ($n = 35$) of patients, respectively. When combined, pretreatment saliva and plasma were 76% sensitive and 100% specific. Post-treatment saliva and plasma were 70% sensitive and 91% specific for disease recurrence.	In OPSCC, HPV viral oncogenes can be found in pretreatment saliva and plasma. Post-treatment viral oncogenes were sensitive and specific for disease recurrence.
Wang et al. 2015 [67]	93 HNSCC patients, 22% ($n = 20$) with early disease (stage I and II) and 78% ($n = 73$) with advanced disease (stage III and IV).	Pretreatment saliva ($n = 93$) and plasma ($n = 47$), in addition to post-treatment saliva ($n = 9$).	Tumors profiled for mutations in tiered fashion: first assessed for presence of HPV oncogenes, second assessed for common HNSCC mutations (<i>TP53</i> , <i>PIK3CA</i> , <i>CDKN2A</i> , <i>FBXW7</i> , <i>HRAS</i> , and <i>NRAS</i>), and finally whole exome sequencing was performed. One selected mutation was used per patient to query saliva and plasma samples.	Combined plasma and saliva testing detected tumor DNA in 96% ($n = 45$) of patients. Tumor DNA was detected in 100% ($n = 10$) and 95% ($n = 37$) of early- and advanced-stage disease, respectively. When segregated by site, tumor DNA was detected in 100% ($n = 15$), 91% ($n = 22$), 100% ($n = 7$), and 100% ($n = 3$) of patients with tumors of the oral cavity, oropharynx, larynx, and hypopharynx, respectively. Tumor DNA was detected in the post-treatment saliva of 3 patients that developed disease, but not in the 5 patients that remained disease free.	Tiered approach can be used to identify tumor-specific mutations in HNSCC. Tumor DNA was detected in early- and late-stage HNSCC. Tumor DNA was detectable in multiple different HNSCC subsites.

ctDNA circulating tumor DNA, HNSCC head and neck squamous cell carcinoma, OPSCC oropharyngeal squamous cell carcinoma, HPV human papilloma virus