


# Head and Neck Squamous Cell Carcinoma Detection and Surveillance: Advances of Liquid Biomarkers

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Head and neck squamous cell carcinomas are aggressive tumors that often present at advanced stage in difficult-to-biopsy regions of the head and neck. With the rapid move to analyze circulating tumor DNA (ctDNA) to either detect cancer or monitor disease progression and response to therapy, we have designed this article as a primer to understand the recent studies that support a transition to use these circulating biomarkers as a part of routine clinical care. Whereas some technical challenges still need to be overcome, the utility of ctDNA in cancer care is already evident from these early studies. Therefore, it is critical to understand recent advances in this area as well as emerging questions that need to be addressed as these biomarkers move closer to enhancing routine clinical care paradigms.

**Key Words:** ctDNA, HPV, biomarker, HRAS, ccfDNA.

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## INTRODUCTION

Head and neck cancer is one of the leading causes of cancer deaths worldwide.<sup>1</sup> Head and neck squamous cell carcinoma (HNSCC) comprises the vast array of tumors, including those arising from the nose, nasal cavity, paranasal sinuses, oral cavity, pharynx, and larynx. Within the last several decades, the rising incidence of oropharyngeal cancer, particularly in the younger population, has been attributed to high-risk human papillomavirus (HPV) subtypes<sup>2</sup> as well as potential hereditary genetic factors.<sup>3,4</sup> The detection of primary HNSCC and recurrence is challenging due to the nature of the anatomy involved. Diagnosis is often delayed and requires clinical examination, imaging, and microscopic tissue analysis, all of which can be significantly hindered by the location of the tumors. There is significant interest in the development of a biomarker surveillance tool that might allow for earlier diagnosis and bypass the need to undergo a surgical procedure. In this review article, we performed a systematic review of the literature, focusing on the development of serum-based biomarkers, particularly circulating tumor DNA (ctDNA), in HNSCC. We included a

number of current articles that we believe will influence the future research of liquid biomarkers in HNSCC. As such, our systematic literature search included articles published through September 9, 2018, and was performed using MEDLINE, EMBASE, and Google Scholar for relevant articles. Non-peer-reviewed articles, letters to the editor, commentaries, and editorials were excluded. All review authors independently screened titles and abstracts of potential studies to assess validity (Table I). Subsequently, the full text of all eligible studies was independently reviewed by three review authors (P.L.S., J.R.B., J.C.B.) who evaluated the potential articles for inclusion.

## Background to Serum-Based Biomarkers

As defined by the Biomarkers Definitions Working Group, a biomarker is “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.”<sup>5</sup> Biomarkers may offer insight into diseases in many contexts, including screening and diagnosis, yielding predictive information to alter therapy and therapy monitoring or serving as a prognostic tool by risk stratification. All of these roles point to the central role of validating a biomarker with a specific clinical endpoint,<sup>5</sup> which may be significantly different depending on the point in disease progression (Fig. 1).

The term *circulating biomarkers* encompasses an array of analytes including proteins, circulating tumor cells (CTCs), ctDNA, and tumor exosomes. These circulating biomarkers have been explored in numerous cancers and situations, including screening, prognostication, evaluating response to therapy, and predicting response to novel therapeutics. Determining prognosis was one of the initial areas in which circulating biomarkers were explored. In nonmetastatic colorectal cancer, for example,

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TABLE I.  
Highlighted Liquid Biomarker Studies.

Authors	Year	Number of Patients	Findings
Chuang et al	2008	59	Quantitative measurement of HPV-16 DNA in saliva can potentially assist in surveillance and early detection of recurrence. Patients with the presence of HPV-16 DNA in salivary rinses are at significant risk for recurrence.
Cheng et al	2009	15	ctDNA plasma levels in cancer patients during radiation therapy are dynamic throughout the course of treatment and may provide real-time assessments of responses to chemotherapy and radiotherapy
Ahn et al	2014	93	Combined pretreatment plasma and saliva can increase the utility of pretreatment HPV-16 as a tool for screening patients with HPV-16-positive oropharyngeal cancer.
Wang et al	2015	47	Sensitivity of HPV-16 DNA detection varies greatly depending on tumor location: saliva is more often enriched for tumor DNA from the oral cavity, but plasma is more often enriched for tumor DNA from the other sites.
Dahlstrom et al	2015	262	Pretreatment serum HPV DNA is associated with both higher N category and overall disease stage but it does not seem to serve as a marker for disease recurrence among patients with oropharyngeal cancer.

ctDNA = circulating tumor DNA; HPV = human papillomavirus.

both increased baseline ctDNA presurgery and persistently detectable ctDNA postoperatively have been associated with poor prognosis.<sup>6,7</sup> Enumeration of CTCs in breast cancer has been found to be an independent predictor of survival in metastatic breast cancer. Interestingly, the number of CTCs after 1 month of therapy has been validated to predict survival. Importantly, this is prior to when traditional radiographic imaging response would be expected.<sup>8</sup> Trials have not demonstrated a benefit to early change in therapy based on CTCs, emphasizing the dual importance of validated tumor biomarkers and effective therapeutics. In addition, research into the use of CTCs in HNSCC have been limited by small cohorts, heterogeneous cancer subtypes, and nonstandardized detection methods. Therefore, this requires further validation and study.<sup>9,10</sup> Ultimately, comparative work in metastatic breast cancer has suggested that ctDNA is a more sensitive biomarker than enumeration of CTCs, and their dynamics may better reflect tumor response to therapy.<sup>11</sup>

Circulating tumor biomarkers have begun to make a foray into the clinic with the era of precision oncology and targeted therapeutics. This has been most notable in lung adenocarcinoma; studies have demonstrated a ctDNA-based assay to detect targetable mutations and resistance pathways (i.e., EGFR) in the circulation, potentially avoiding biopsy.<sup>12-14</sup> Genotyping of plasma ctDNA has demonstrated a sensitivity of 74% to 86.3% (specificity 96.5%–100%) for EGFR exon 19/L858R mutants. Even more compelling is that the mutation most frequently responsible for the loss of EGFR-directed therapy, T790 M, was able to be accurately detected with a

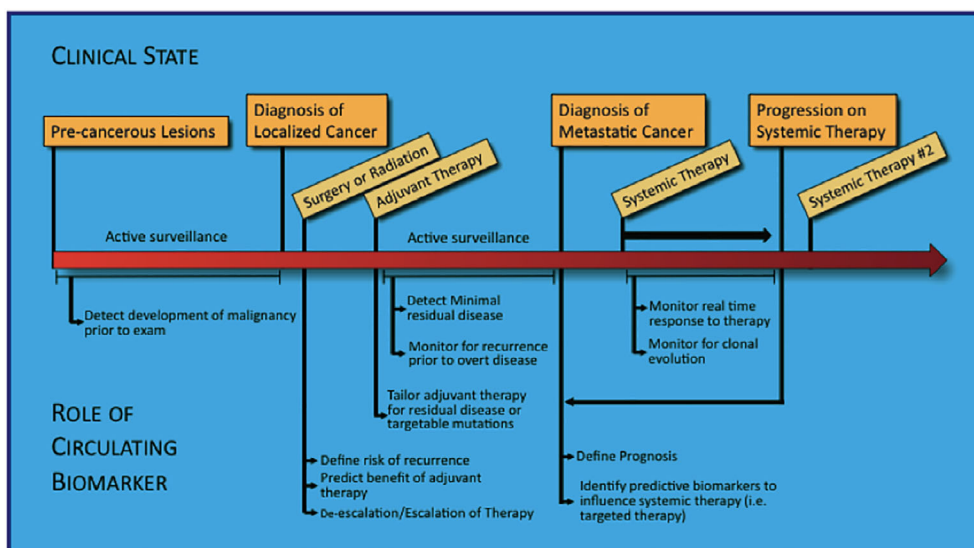


Fig. 1. Circulating biomarkers may have different goals depending on clinical course and intent of the assay. The schematic represents a standardized long-term clinical course for a patient with HNSCC. Treatment modalities are indicated on the top, whereas potential uses of circulating biomarkers are defined on the bottom. In the case of ctDNA assays, endpoints focused on active surveillance of premalignant patients may require a broad ability to detect diverse genetic alterations in order to detect cancers with unknown alterations, whereas active surveillance of patients with a sequenced primary tumor may only require monitoring of a few established alterations (e.g., patient-specific *TP53* mutations). Similarly, analysis of genetic changes during clonal outgrowth may also benefit from broad spectrum assays to detect unknown alterations, whereas assays monitoring for treatment-driven selection of therapy-resistant clones (e.g., therapy-driven mutations) may be able to focus on monitoring of highly recurrent mechanisms of resistance for a specific therapeutic. ctDNA = circulating tumor DNA; HNSCC = head and neck squamous cell carcinoma. [Color figure can be viewed in the online issue, which is available at [www.laryngoscope.com](http://www.laryngoscope.com).]

sensitivity of 70.3% to 77% (specificity 63%–100%).<sup>12,13</sup> Although this could detect roughly 70% of EGFR mutations, given the sensitivity, a tumor biopsy may still be warranted in patients with a negative result. Past studies have repeatedly documented that patients with targetable mutations treated with targeted therapies have superior PFS compared to those treated with conventional chemotherapy.<sup>15,16</sup> Hence, these ctDNA biomarkers are one step closer to achieving the ultimate goal of biomarker development: altering clinical endpoints and improving outcomes. Based on these data, the FDA approved the Cobras EGFR mutation test as first ctDNA biomarker assay for use in clinical practice. Use of similar liquid biopsies is being incorporated into societal guidelines for patients with advanced non-small cell lung cancer.<sup>17</sup> Each of the circulating tumor biomarkers (e.g., CTCs, ctDNA, exosomes, proteins, antibodies) is distinct with complimentary roles; for this review, we will focus on ctDNA and its potential application in HNSCC.

### **Introduction to ctDNA**

The concept of ccfDNA (circulating cell-free DNA) correlation with human disease was first introduced in 1948,<sup>18</sup> and increased concentrations of ccfDNA in the circulation of cancer patients was first noted in 1977.<sup>19</sup> Subsequently, evidence that cfDNA is released into the circulation by tumors (termed ctDNA) was found when tumor-specific aberrations were noted in cfDNA, including tumor suppressor and oncogene mutations, microsatellite instability, and DNA methylation.<sup>20–23</sup>

A variety of biological fluids, including blood, lymph, urine, saliva, and cerebrospinal fluid, contain ccfDNA. In the blood, ccfDNA is predominately short, double-stranded fragments of nuclear and mitochondrial DNA of approximately 160 to 180 base pairs length.<sup>24</sup> The primary mechanism of DNA release into circulation continues to be somewhat debated; however, the literature suggests that it is a result of apoptosis and possibly also necrosis and viable tumor cell secretion.<sup>25,26</sup> Lo et al. used whole genome sequencing of plasma DNA of pregnant women to demonstrate that plasma DNA molecules showed a predictable fragmentation pattern consistent with nuclease-cleaved nucleosomes, implicating apoptosis.<sup>27</sup> Size distribution of cfDNA in healthy individuals and cancer patients also confirms this, revealing an enrichment of size fragments of single or multiples of nucleoprotein complexes, and suggested that the main driver of release may be apoptosis.<sup>28</sup> Underhill et al. demonstrated that ctDNA fragments may be shorter (approximately 130 base pairs) than ccfDNA derived from noncancer cells.<sup>29</sup>

Through assessment of tumor genetic aberrations in ccfDNA, studies have estimated that the fraction of ctDNA within the total ccfDNA in cancer patients is approximately 10%.<sup>7,30</sup> Levels of ccfDNA can be influenced by increased production as well as stability to circulating nucleases and clearance in the kidney, liver, and spleen. Real-time assessment of a cancer may be possible from ctDNA because the half-life of ccfDNA ranges from about 15 minutes to several hours.<sup>31–36</sup> Some reports indicate that the growth rate of the tumor leads to a

higher degree of necrosis, corresponding to an increase in circulating tumor DNA. Diehl et al. suggested that DNA fragments found in the circulation are derived from necrotic neoplastic cells that had been engulfed by macrophages.<sup>37</sup>

### **Studies to Date of ctDNA in Head and Neck Cancer**

The use of ctDNA in patients with HNSCC is less established than in patients with other cancers such as colorectal, breast, and lung. Nevertheless, research has demonstrated that it is indeed viable, and the potential for applications is vast.<sup>32–36</sup> The most-studied ctDNA biomarker in HNSCC is HPV-related (HPV+) malignancies because HPV and its downstream protein effectors present as convenient biomarkers for detection in the circulation.<sup>38</sup> Using real-time polymerase chain reaction (RT-PCR) amplification, a minute amount of HPV-16 DNA has been shown to be detectable in saliva and plasma samples. This study in question included 93 patients with oropharyngeal or unknown primary squamous cell carcinoma with known HPV status and a complete set of pre- and posttreatment plasma or saliva samples.<sup>32</sup> One study of patients with various stages on HNSCC demonstrated that tumor DNA, as defined by either somatic mutations or HPV incorporation, is detectable in 96% of patients when both plasma and saliva are sampled.<sup>33</sup> When considered by body fluid type, the sensitivity of detection varied greatly depending on tumor location. ctDNA was detectable in the saliva samples of between 47% and 100% of patients, with the highest detection in tumors of the oral cavity (100%). Plasma sensitivity was similarly as variable, ranging from 80% to 100%, highest in the hypopharynx (100%) (Table II). These complimentary detection rates emphasize the importance of considering which body fluid(s) to evaluate in the development of tests. Interestingly, even when saliva and plasma were combined, tumor DNA was detected in just 86% of HPV+ patients.<sup>33</sup>

Preliminary research has also suggested that the presence or absence of HPV-16 DNA is a feasible means of surveilling disease posttreatment HPV-16 DNA status in saliva.<sup>32,34</sup> The detection of HPV-16 DNA in salivary rinses occurred on average over 3 months before clinical detection of recurrence.<sup>34</sup> In this particularly study, 59 patients were included who presented with histopathologically confirmed HNSCC, had one or more posttreatment salivary sample, and had been previously treated with curative intent. However, only four of these patients had recurrent HPV+ tumors, of which two had detectible HPV-16 in surveillance salivary rinses. A larger study demonstrated that, when combining plasma and salivary surveillance testing, persistence of HPV-16 DNA posttreatment was 69.5% sensitive and 90% specific for predicting recurrence within 3 years<sup>32</sup> (Table II). However, due to the excellent outcomes of HPV-related oropharyngeal cancer, these findings were based on a small number of recurrences. Not all studies have found ctDNA to have clinical utility in predicting disease recurrence in HNSCC.<sup>35</sup> A study with a different approach assessed the

TABLE II.  
Studies of Circulating Tumor DNA in Head and Neck Cancer.

ctDNA Detection (Wang et al. <sup>33</sup> )			
Site	Detected in Saliva	Detected in Plasma	Detected in Saliva or Plasma
Oral cavity	100%	80%	100%
Oropharynx	47%	91%	91%
Larynx	70%	86%	100%
Hypopharynx	67%	100%	100%
HPV+	40%	86%	86%
Total	76%	87%	96%

HPV-16 DNA as Marker of Recurrence in HPV+ HNSCC

	Sample Source	No. HPV + Patients	Sensitivity	Specificity
		Chuang et al., 2008*	Saliva	20
Ahn et al., 2014 <sup>†</sup>	Saliva	72	19%	97%
	Plasma	52	55%	96%
	Serum or plasma	46	70%	91%

ctDNA detection rates from three of the first HNSCC studies are summarized.

\*Testing characteristics for surveillance HPV DNA association with tumor recurrence.

<sup>†</sup>Testing characteristics for posttreatment HPV DNA association with tumor recurrence within 3 years.

ctDNA = circulating tumor DNA; HNSCC = head and neck squamous cell carcinoma; HPV+ = human papillomavirus-related.

use of pretreatment rather than posttreatment ctDNA as a predictor for disease recurrence in oropharyngeal cancer. Pretreatment HPV DNA in the serum was associated with a higher nodal category and overall stage; however, it was not associated with differences in progression-free survival.<sup>35</sup> This study had a recurrence rate of just 13% and thus may not have been powered to detect significant associations. Studies to date have lacked the statistical power to validate the role of ctDNA in the detection of recurrence. Confirmation and validation of a candidate biomarker is one of the most challenging issues in translational science. It is a time- and labor-intensive process requiring multiple patient cohorts. Significant literature has been published outlining processes for appropriate clinical biomarker discovery, including independent discovery and validation cohorts prior to clinical evaluation for utility. Clinical evaluation may take one of many designs, including prospective clinical trials in which the test may or may not direct management. Design of a prospective biomarker validation clinical trial requires close collaboration with colleagues in bioinformatics and statisticians, but the sample size is dependent in part on the role of the test. If the biomarker is designed to detect disease recurrence, an ample number of patients treated uniformly must be enrolled to detect an appropriate number of reoccurrences to make appropriate conclusions on the biomarker test characteristics. Even more challenging, in order to define the clinical utility of a proposed biomarker, a study (often as a follow-up) must be designed

wherein the results of the biomarker change clinical management.<sup>39–41</sup> In the situation of HPV-related HNSCC, the recurrence rate is so low that a large cohort is necessary to statistically determine the test characteristics of HPV ctDNA (sensitivity, specificity, area under the curve [AUC]). Furthermore, to solidify its role in clinical practice, a study would need to be designed in which observation would be changed based on detection of HPV ctDNA. For example, if ctDNA was detected, posttreatment patients would be randomized to more intensive surveillance or adjuvant chemotherapy and monitored for improvement in survival. Therefore, although ctDNA may indeed be a potential adjunct to provide less invasive monitoring for recurrent HNSCC, it requires further large prospective studies.

Another application of ctDNA in HNSCC is in assessing real-time responses to chemotherapy and radiotherapy. A study looking at the ctDNA plasma levels in cancer patients during radiation therapy found that concentrations were dynamic throughout the course of treatment. The patients studied had various types of cancer, but more than half of these were localized to the head and neck. In most of the patients, the levels of ctDNA underwent an initial, transient rise at the initiation of therapy, after which they declined.<sup>42</sup> However, this study was limited both by the variety of malignancies included and by the numerous different treatment modalities. Other studies have demonstrated similar results in patients undergoing radiation and chemotherapy in which there is an initial rise in concentration followed by a slow decline back to pretreatment level.<sup>43–46</sup> Further research may build upon this foundation to determine whether these dynamic levels of ctDNA correlate with treatment efficacy.

The final application of ctDNA that is currently under investigation in HNSCC is its use in metastatic disease. As aforementioned, research in other malignancies has demonstrated the ability to utilize ctDNA to predict response to chemotherapeutic agents.<sup>11</sup> It allows for a much less invasive means to select patients who these therapies may benefit. Similarly in lung adenocarcinoma, ctDNA has been able to identify targetable mutations (EGFR, ALK, ROS-1, BRAF) in which targeted therapies are beneficial.<sup>14–17</sup> Unfortunately, unlike lung adenocarcinoma, to date no driver mutations have been identified in HNSCC, and no mutations have been isolated predictive of response to systemic therapy. Given data regarding the association of tumor mutational burden as assessed by whole exome sequencing in tumor samples and response to immunotherapy,<sup>47</sup> ongoing research is aiming to define whether this information may be assessed from ctDNA. For example, given the recent emergence of immunotherapies in HNSCC,<sup>48</sup> one recent exploratory study using a commercial next generation sequencing (NGS) ctDNA assay investigated patients with unresectable or metastatic disease who were undergoing checkpoint inhibitor-based immunotherapy. Thirteen percent of the patients in this study had HNSCC. This study suggested that higher alteration number on ctDNA liquid biopsy is associated with a significantly improved response to checkpoint



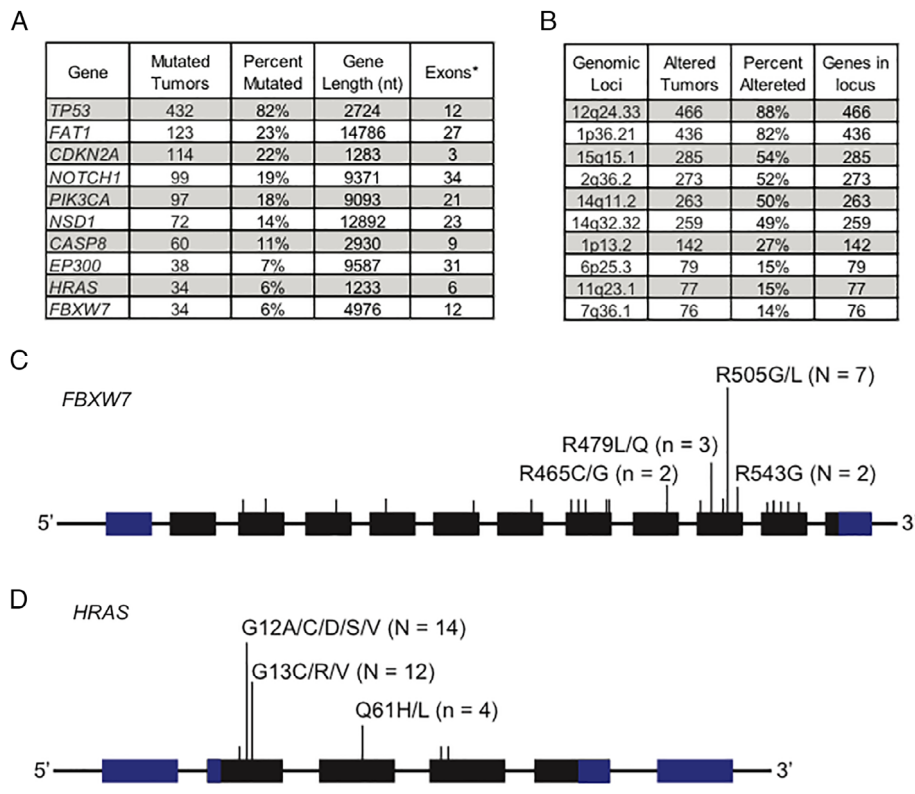


Fig. 2. Challenges in optimizing a ctDNA panel based on the diversity of HNSCC molecular alterations. (A) Top 10 most recurrent molecular alterations in primary untreated disease as defined by the HNSCC TCGA sequencing set (N = 530). Several of these genes are tumor suppressors from long, multi-exon genes. (B) Top 10 most recurrent copy number alterations from HNSCC TCGA data and the total genes in each region are indicated. (C) Representation of regions that are required to be covered if ctDNA analysis focuses on tumor suppressors using *FBXW7* schematic as an example (all exons would need coverage based on the low recurrence of hot spot alterations). Boxes represent exons, with blue boxes representing untranslated regions and black regions indicating coding sequences. Vertical lines indicate site of called nonsynonymous single nucleotide variant, and only the recurrent mutations were annotated. (D) As in (C), with representative regions of interest in the *HRAS* oncogene highlighting recurrent mutation sites presented in schematic as shown, suggesting the majority of *HRAS* mutant patients would be detected with probes covering G12, G13, and Q61. ctDNA = circulating tumor DNA; HNSCC = head and neck squamous cell carcinoma; HPV = human papillomavirus. [Color figure can be viewed in the online issue, which is available at [www.laryngoscope.com](http://www.laryngoscope.com).]

inhibitor-based immunotherapy.<sup>49</sup> This is consistent with findings from primary tissue biopsies. Despite the limitations inherent to a study with a smaller HNSCC population, the literature suggests ctDNA may have a role in predicting those metastatic and recurrent patients for whom the prospective therapeutic benefit of immunotherapy is greatest. With further validation, ctDNA could assist in limiting the number of patients who are unnecessarily exposed to the adverse effects of these therapies.

In addition to its use in HPV+ malignancies, biomarkers have also been studied in Epstein-Barr virus (EBV) related nasopharyngeal carcinoma (NPC) as a means to assess treatment response, prognosticate outcome, and screen. Several studies have shown that circulating EBV DNA is detectable in both the plasma and the serum of patients with NPC.<sup>50,51</sup> In one study, 107 patients with stage IIB-IV NPC had their plasma EBV DNA load tested before, at the midpoint of, and after the culmination of chemoradiation. Of these, there were 35 patients who failed therapy in whom there was detectable midpoint DNA in 74%. Detectable midpoint EBV DNA was found to be prognostic of treatment failure

and was more predictive of outcomes than was tumor stage.<sup>46</sup> Another prospective study found that patients with NPC who recurred or metastasized had higher pretreatment plasma or serum EBV DNA concentrations than those who did not. Within the first year after treatment, quantification of plasma EBV DNA was a better adverse prognosticator than disease stage.<sup>52</sup> Finally, EBV has been studied as a means to screen 20 thousand asymptomatic, ethnically Chinese, middle-aged male patients for NPC. Of these, 309 tested persistently positive, and 34 went on to have confirmed NPC; the sensitivity and specificity of the presence of EBV DNA in plasma was found to be 97.1% and 98.6%, respectively. These patients were detected at earlier stages had better progression-free survival than those in previously studied cohorts.<sup>53</sup> This study is limited insofar that it had a short follow-up time and thus was unable to evaluate the impact on long-term outcomes, particularly in the setting of the high rate of survival in NPC.<sup>54</sup> Additionally, the high number needed to screen, and the expenses associated with screening necessitate further evaluation of its cost-effectiveness. Despite these potentially promising applications, the

clinical utility of EBV DNA as a prognostic biomarker still needs to be validated in prospective clinical trials.

### **Techniques for Analyzing ctDNA**

Recent advancements in single molecule-based NGS technology, coupled with improvements in bioinformatics approaches, have enabled the ability to genotype cancer patients in real time from minimal amounts of ctDNA. In fact, several different techniques have recently been developed to detect and quantify ctDNA in a variety of cancer settings, which can be broadly divided into three main types of assay based on the intent to detect either early stage cancer, changes to molecular pathways driving tumor evolution, and response to therapy or evidence for early recurrence of an already genotyped cancer.

Early detection assays that attempt to discover unidentified cancers are perhaps the most technically challenging because they require highly sensitive methods that assess broad spectrums of the most probable pathogenic alterations. The primary challenge of these assays is the ability to differentiate between somatic events and alterations from either the normal germline or those that occur during hematopoietic cell proliferation,<sup>55</sup> which are generally thought to require a high depth of sequencing coverage and comprehensive databases of germline and hematopoietic alterations. Due to current limitations in the number of genes that can be cost-effectively sequenced to appropriate depth for high confidence annotation, early detection ctDNA assays tend to rely on panel-based approaches to identify 50 to 100 common genetic alterations found in either an individual cancer or a small set of genetically related cancers.<sup>36,56,57</sup>

For cancers driven by a relatively small set of consensus drivers, early detection assays have the advantage of having to sequence smaller gene sets; in contrast, for cancers such as HNSCC, which are known to have a large and diverse number of oncogenic drivers.<sup>58–60</sup> The detection of early diagnosis panels becomes slightly more challenging because the number of alterations observed across large multi-exon genes, such as *TP53* or *NOTCH1*, requires significantly more probes and sequencing coverage than oncogenes with “hotspot” molecular alterations occurring at only a few nucleotides in genes, such as *HRAS* and *PIK3CA* (Fig. 2).

To overcome these types of challenges, Phallen et al. recently developed a technique called targeted error correction-SEQ, which examines 58 cancer-related genes and uses optimized sequencing and bioinformatics methods to reduce the overall potential of amplification, sequencing, and contamination errors.<sup>6</sup> Using this method, the authors were able to detect stage 1 or 2 disease in 59% to 71% of patients with colorectal, breast, lung, or ovarian cancers representing a large improvement in the ability to detect malignancies. Although this method has not been applied to a large population of patients, it will be interesting to determine the predictive value of the assay for each population.

In contrast to these complex assays to detect early disease or genetic evolution, the assays developed to detect early recurrence have mostly focused on the

tracing individual lesions. The rationale for this approach is based on heterogeneity lineage tracing experiments from several different cancer types, suggesting that once a driver lesion occurs in a tumor, it can often be traced in subclones recurring either locally or distantly.<sup>61–65</sup> In the case of HPV+ HNSCC or mucoepidermoid carcinoma, the development of methods to individually monitor high-risk HPV DNA or CRTC1/3-MAML2 gene fusions, respectively, represents a straightforward approach to develop assays with high specificity for detection.<sup>66,67</sup> Consequently, once the tumor specific primary lesion has been characterized and likely driver alterations are identified, individualized assays can be developed to trace likely driver lesions. Several examples have emerged in literature that leverage quantitative PCR, RT-PCR, digital droplet PCR, and/or NGS.<sup>68–70</sup> This method has been especially useful for monitoring recurrence in cancers that are defined by highly recurrent “hotspot” mutations such as BRAF V600E in melanoma; however, even these studies have also shown limits in sensitivity suggesting that monitoring for additional loci may provide an opportunity to improve overall assay performance.

### **Future Applications of ctDNA in Head and Neck Cancer**

Direct tumor sequencing and mutation detection in plasma cfDNA are unlikely to be a viable clinical approach due to the associated complexity and turn-around time.<sup>36</sup> However, as previously discussed based on the known mutational landscape of HNSCC, a ctDNA panel could be designed to detect and monitor cases in a rapid and cost-effective fashion.<sup>33</sup> Use of such a panel would enable researchers and clinicians to detect and monitor non-HPV-related malignancies, which still constitute the majority of HNSCC cases. This would allow trials of previously untreated locally advanced HNSCC to move beyond risk stratification by tumor-node-metastasis stage or HPV status and instead move to real-time response of tumors to therapy as judged by ctDNA dynamics. That could drive intensification of therapy in patients with lack of disease response via ctDNA or de-escalation of therapy based on a fall in ctDNA, thereby preventing treatment-related toxicity. Before either of these designs is pursued, we need not only a validated biomarker test but also an understanding of normal biomarker dynamics during treatment to identify responders/nonresponders.

Development of novel therapeutics in unresectable recurrent or metastatic HNSCC has been met with little success. Cytotoxic chemotherapy can cause severe adverse effects without significant meaningful response, limiting its use and the ability to receive further therapy. Although promising, immunotherapy has been found to have a low response rate of 13%, which is often not appreciated for several months.<sup>71–73</sup> Novel methods of detecting response are needed to better establish response to therapy and avoid exposure to toxic, ineffective agents. A ctDNA biomarker panel test could detect response prior to traditional radiographic imaging. Analogous to past breast cancer trials,<sup>74</sup> if a biomarker is able to be validated as predicting response

prior to conventional imaging (i.e., blood draw after 1 cycle vs. imaging after 3 cycles), a trial evaluating early switch of therapy in those nonresponsive to therapy is quite enticing. Such a trial has the potential to spare patients the toxicities of ineffective therapy and more rapidly administer beneficial therapies. Even more revolutionary would be evaluation of whether clonal resistance can be detected via a ctDNA panel and whether modification of therapy based on clonal resistance would alter patient outcomes. This scenario would take much more development, advancement of bioinformatics, and likely effective targeted therapies.

## CONCLUSION

Although several questions remain, including the cost-effectiveness of in-depth sequencing and improving the speed at which sequencing results are obtained, early studies published thus far have demonstrated promising results for implementing ctDNA monitoring into clinical care. If ctDNA monitoring proves truly impactful, it may not only make routine assessments of cancer progression and response to therapy cheaper and accessible but may also lead to significant improvements in early detection. In addition, we may begin to observe improvements in overall survival for patients with this difficult-to-monitor disease.

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