Detection of Occult Recurrence Using Circulating Tumor Tissue Modified Viral HPV DNA among Patients Treated for HPV-Driven Oropharyngeal Carcinoma

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ABSTRACT

Purpose: Despite generally favorable outcomes, 15% to 25% of patients with human papillomavirus (HPV)-driven oropharyngeal squamous cell carcinoma (OPSCC) will have recurrence. Current posttreatment surveillance practices rely on physical examinations and imaging and are inconsistently applied. We assessed circulating tumor tissue modified viral (TTMV)-HPV DNA obtained during routine posttreatment surveillance among a large population of real-world patients.

Experimental Design: This retrospective clinical case series included 1,076 consecutive patients across 108 U.S. sites who were ≥ 3 months posttreatment for HPV-driven OPSCC and who had one or more TTMV-HPV DNA tests (NavDx, Naveris Laboratories) obtained during surveillance between February 6, 2020, and June 29, 2021. Test results were compared with subsequent clinical evaluations.

Results: Circulating TTMV-HPV DNA was positive in 80 of 1,076 (7.4%) patients, with follow-up available on all. At first positive surveillance testing, 21 of 80 (26%) patients had known recurrence while 59 of 80 (74%) patients were not known to have recurrent disease. Among these 59 patients, 55 (93%) subsequently had a confirmed recurrence, 2 patients had clinically suspicious lesions, and 2 had clinically "no evidence of disease" (NED) at last follow-up. To date, the overall positive predictive value of TTMV-HPV DNA testing for recurrent disease is 95% ($N = 76/80$). In addition, the point-in-time negative predictive value is 95% ($N = 1{,}198/1{,}256$).

Conclusions: These findings highlight the clinical potential for circulating TTMV-HPV DNA testing in routine practice. As a surveillance tool, TTMV-HPV DNA positivity was the first indication of recurrence in the majority of cases, pre-dating identification by routine clinical and imaging exams. These data may inform future clinical and guideline-endorsed strategies for HPV-driven malignancy surveillance.

See related commentary by Colevas, p. 4171

Introduction

The incidence of human papillomavirus (HPV)-driven oropharyngeal squamous cell carcinoma (OPSCC) in the United States over the last three decades has risen exponentially (1, 2). HPV-driven OPSCC is characterized by favorable survival characteristics generally, however 15% to 25% of patients relapse with locoregional or distant metastatic disease (3–10). Relapse can be associated with significant morbidity and mortality and can present with an atypical pattern of distant organ spread (8, 9, 11, 12). Relapses tend also to occur later in the disease's natural history, with some reports describing metastases occurring more than 10 years following initial treatment (8, 13). However, patients presenting with oligometastases (one to five lesions) could be treated with curative intent, underscoring the value of early recurrence detection (8, 11, 14).

Optimal intervals for systematic radiologic examination to monitor potential recurrence of HPV-driven OPSCC are not well defined. Current guidelines suggest clinical examinations and include PET/CT (PET-CT) imaging at 3 months postdefinitive treatment (15). There is wide variability in the use and scheduling of additional or subsequent imaging among patients without clinical evidence of disease (16), and patients with HPV-driven OPSCC exhibit decreased adherence to monitoring after year 2 of follow-up (17–21). As early recurrences are often asymptomatic, they may be missed in their earlier and potentially curable stages. Optimized surveillance strategies that rely on increased sensitivity for detecting clinically occult recurrent or persistent HPV-driven OPSCC are urgently needed.

Circulating tumor DNA (ctDNA) has emerged as a diagnostic tool to detect the presence of cancer or quantify tumor burden among patients with cancer (22, 23). Yet in the context of HPV-driven OPSCC, ctHPVDNA has produced promising but mixed results (24–36). Conventional ctHPVDNA testing does not distinguish HPV DNA attributed to active acute or chronic viral infection from tumor-associated HPV DNA. To distinguish between these two entities, an HPV-driven cancer-specific and ultrasensitive multi-analyte digital droplet PCR (ddPCR) assay that tests for circulating, cell free tumor tissue modified viral (TTMV)-HPV DNA was developed (37, 38). TTMV-HPV DNA is a unique biomarker produced during the fragmentation of integrated and/or episomal HPV DNA of malignant epithelial cells during the degradation of HPV-driven tumors. Circulating TTMV-HPV DNA can be detected and quantified using a combination of specific primer and probe sets with algorithmic HPV DNA fragment analysis, yielding high analytic sensitivity and specificity.

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Translational Relevance

Circulating, cell free tumor tissue modified viral (TTMV)–human papillomavirus (HPV) DNA is a unique biomarker of HPV-driven cancer, present in blood and saliva of patients with HPV-driven malignancy and distinct from HPV DNA from HPV infection. This is the first prospectively designed, retrospective consecutive clinical case series ($N = 1{,}076$) evaluating circulating TTMV-HPV DNA for surveillance of patients with HPV-driven oropharyngeal squamous cell carcinoma (OPSCC), postdefinitive treatment, in routine practice across 108 U.S. sites. TTMV-HPV DNA was positive in 7.4% of patients, demonstrated 95% positive predictive value for recurrent OPSCC, and was commonly the first indication of recurrence among asymptomatic patients. The negative predictive value of the assay was 95%. Our findings support the potential clinical utility of TTMV-HPV DNA testing. Given that HPV-driven OPSCC surveillance is limited to imaging and clinical examinations and is inconsistently applied, our data may inform and expand future clinical and guideline-endorsed strategies for HPV-driven malignant disease surveillance.

We present here the first prospectively designed study applied to a large-scale retrospective consecutive case series of patients with OPSCC undergoing TTMV-HPV DNA testing as part of routine clinical surveillance 3 or more months following the completion of definitive or curative-intent therapy. The primary aims of this analysis were to assess the real-world usage and positive predictive value (PPV) of circulating TTMV-HPV DNA for the identification of patients with recurrent HPV-driven OPSCC under routine surveillance.

Materials and Methods

Patient population

After independent Institutional Review Board (IRB) approval, a chart review was conducted. A waiver of written informed consent was granted by a central, independent IRB (WCG IRB; sponsor protocol number: NAV11042020), in line with recognized ethical guidelines, in consideration of the limited risk associated with the retrospective nature of the data collection process. Eligible participants included patients diagnosed and treated for primary HPV-driven OPSCC without known distant metastatic disease who were at least 3 months postcompletion of standard definitive therapy (any modality). The 3-month follow-up window was selected based on prior observations of TTMV-HPV DNA clearance kinetics from the circulation posttreatment (37). Confirmatory tumor HPV status was provided by the treating clinical site using p16 IHC, direct HPV tissue detection by *in situ* hybridization (ISH) or PCR or with a detectable circulating TTMV-HPV DNA test result in lieu of HPV tissue detection methods. Participants must have had a minimum of one TTMV-HPV DNA test result obtained at least 3 months posttreatment during routine surveillance between February 6, 2020 and June 29, 2021 for study inclusion. Pretreatment or baseline, and on-treatment TTMV-HPV DNA testing results were not collected as part of this study, but were included for the case examples if available.

Clinical characteristics

Each participant's test requisition data included patient demographics, clinical disease status, and relevant International Classification of Diseases (ICD)-10 codes. All information is supplied by the ordering clinician at the time of test ordering, and prior to receipt of any test results. Data were extracted from a central laboratory information system to compile a working deidentified database that included the following provided by the ordering clinician: age, sex, ICD-10 disease diagnosis code, p16 IHC or HPV status, categorical interval following the completion of definitive treatment (3–6 months, 6–12 months, >12 months), clinical disease status as reported by the ordering clinician [active disease, indeterminate (IND) disease status (equivocal findings, on exam or imaging), no evidence of disease], test order and collection dates, and the TTMV-HPV DNA test results, including HPV high-risk strain and TTMV-HPV DNA score. Included participants were assigned a unique study number. Follow-up information regarding the presence or absence of recurrent disease during the study period was provided by ordering clinicians using appropriate imaging and/or biopsy as clinically indicated for TTMV-HPV DNA–positive cases.

Test characteristics

The TTMV-HPV DNA assay (NavDx, Naveris, Inc.) is a laboratory developed test (LDT) provided under the regulations of Clinical Laboratory Improvement Amendments (CLIA) 88 by a single national reference laboratory. The laboratory is CLIA certified for high complexity testing and accredited by the College of American Pathologists and the New York State Department of Health. TTMV-HPV DNA was quantified according to methods previously described (37, 38). Briefly, a 10-mL peripheral blood sample was drawn into a DNA stabilizing tube (Streck) and plasma was separated followed by DNA isolation. TTMV-HPV DNA is identified using ddPCR with droplet data reduced with a TTMV-HPV DNA score-specific algorithmic analysis. TTMV-HPV DNA is quantified and one of five high-risk HPV subtypes (16, 18, 31, 33, 35) is determined using multiple primers and probes in each reaction that span multiple amplicons for individual HPV strains. Analysis of droplets is performed using the K-Nearest Neighbors algorithm to identify clusters associated with specific amplicons and DNA fragment sizes. The counts for each of the clusters are summed in a weighted linear combination to create the TTMV-HPV DNA Score reflecting normalized TTMV fragments/mL plasma. TTMV-HPV DNA scores >7 (for HPV subtype 16) or >12 (for HPV subtypes 18, 31, 33, or 35) were considered positive. Tests with TTMV-HPV DNA scores between 5 to 7 (HPV 16) or 5 to 12 (HPV 18, 31, 33, or 35) were considered IND. Tests with TTMV-HPV DNA scores <5 were considered negative, regardless of HPV strain.

Data review

Aggregate laboratory data including clinical information provided on the test requisition were retrospectively reviewed as part of ongoing laboratory quality management processes and in compliance with the U.S. Health Insurance Portability and Accountability Act (HIPAA). All positive TTMV-HPV DNA test results obtained during the study period were subsequently compared with provider-reported clinical disease status to establish evidence of HPV-driven OPSCC recurrence, using standard clinical surveillance tools such as flexible endoscopy, radiologic (CT, MRI, or PET-CT) evaluations, and/or tissue biopsy when deemed clinically appropriate. Confirmation of recurrent HPV-driven OPSCC was ultimately provided by the ordering or treating physician through their review of their own patient's medical records. A deidentified curated database of test and clinical information was created and used for subsequent analysis.

Statistical analysis

TTMV-HPV DNA has been clinically available for use since February 2020. Patient characteristics and TTMV-HPV DNA results were analyzed descriptively while assay performance metrics were later calculated to determine PPV and accuracy of surveillance test predictions. PPV for recurrence was defined as the number of patients diagnosed with confirmed recurrent disease by the ordering or treating physician among all patients with a positive TTMV-HPV DNA test result divided by the total number of patients with a positive TTMV-HPV DNA test. An unpaired *t* test was used to compare median TTMV-HPV DNA values by disease status and time posttreatment intervals. All statistical tests were two-sided and a $P < 0.05$ was considered significant. Negative predictive value (NPV) was defined as the probability that a patient with a negative TTMV-HPV DNA result has no evidence of confirmed clinically identifiable HPV-driven recurrent OPSCC at the time of testing.

Data availability statement

The data generated in this study are not publicly available as information could compromise patient privacy but are available upon reasonable request from the corresponding author.

Results

Clinical characteristics of the patient population

We conducted a retrospective consecutive case series analysis of patients with HPV-driven OPSCC who underwent circulating TTMV-HPV DNA testing as part of routine surveillance 3 months or more following the completion of therapy. From 108 clinical sites in the United States, there were 1,076 patients previously treated for HPV-driven OPSCC who received one or more TTMV-HPV DNA tests in the surveillance setting (**Table 1**). Each site contributed a median of three cases (range: 1–211), with 27 sites contributing 10 or more cases each, and the top five sites accounting for 59% of the total tests (**Fig. 1A**). The majority of patients were male (943, 88%), consistent with previously reported prevalence rates (39), with p16-positive tumors by IHC (1067, 99%). Mean age was 63 years (range: 33–97). Most patients had only a single test result in the surveillance setting (837, 78%), while 190 (18%), 43 (4%), and 6 (1%) had two, three, or four consecutive test results, respectively. In total, 1,370 tests were ordered among 1,076 patients. Of the 239 (22%) of patients having more than one test result, the median time after the initial test was 96 days for obtaining the second test (range: 9–367 days), 183 days for the third test (range: 64–402 days), and 259 days for the fourth test (range: 188–301 days), roughly correlating with standard appointment intervals although a large range was observed (**Fig. 1B**). As reported at each patient's first TTMV-HPV DNA surveillance test, 249 (23%), 238 (22%), and 589 (55%) patients were 3 to 6 months, 6 to 12 months, or more than 12 months into surveillance following completion of definitive or curative intent treatment, respectively. At the time of analysis, the median follow-up for the entire cohort was 9 months (range: 6–22 months).

Table 1. Summary of patient characteristics.

	***N* = 1,076 (%)**
Mean age, years (range)	63 (27–97)
Sex	
Female	133 (12)
Male	943 (88)
p16 status	
Positive	1,069 (99)
Negative[a]	1 (1)
Unknown[a]	6 (1)
# NavDx TTMV-HPV DNA test results	
1	837 (78)
2	190 (18)
3	43 (4)
4	6 (1)
Time posttreatment[b]	
3–6 months	249 (23)
6–12 months	238 (22)
>12 months	589 (55)

[a]p16 status was negative unknown at baseline as noted on the test requisition but reported as positive for HPV status on the test requisition or was positive for TTMV-HPV DNA.

[b]For patients with more than one TTMV-HPV DNA test, this was the interval posttreatment reported for the first test result obtained in surveillance.

Circulating TTMV-HPV DNA results in patients under surveillance for recurrence

Of the 1,076 patients tested during surveillance, 80 (7.4%) patients had at least one positive TTMV-HPV DNA result (**Table 2**; **Fig. 2A**). All 80 had confirmed HPV-driven OPSCC with 77 (96%) confirmed by both a p16 IHC result on tissue testing and a positive circulating TTMV-HPV DNA test result. 3 patients (4%) had a positive circulating TTMV-HPV DNA test result alone with unknown p16 IHC status. Of the TTMV-HPV DNA–positive patients, 21 of 80 (26%) were noted as having clinically active disease at the time of the first positive result (as indicated on the test requisition), whereas the remaining 59 patients (74%) had either IND clinical disease status or no evidence of disease (NED) indicated (**Fig. 2A**). The majority of the 80 patients were positive for TTMV-HPV subtype 16 (74, 93%), with 1 to 2 patients positive for each of TTMV-HPV subtypes 18, 31, 33, or 35. Notably, of the 80 patients who tested positive for TTMV-HPV DNA during surveillance, nearly half (38, 48%) were tested more than 12 months after completion of definitive therapy, while 27 (34%) were tested at 6 to 12 months, and 15 (19%) were tested at 3 to 6 months posttreatment completion. Similarly, among the 59 patients with either IND or NED status, 27 (46%) tested positive more than 12 months after completion of therapy.

Confirmation of clinically occult recurrence during surveillance in TTMV-HPV DNA–positive patients

Follow-up information was available for the entire subgroup of TTMV-HPV DNA–positive patients in the overall cohort ($N = 80$). All 21 patients with active disease status reported at the time of surveillance testing were confirmed to have recurrent disease. Of the 59 patients with IND or NED status for recurrent disease at the time of surveillance testing, 55 (93%) were later proven to have recurrent disease on subsequent imaging and/or biopsy, suggesting the presence of clinically occult recurrence at the time of the initial positive TTMV-HPV DNA test result. To date, the overall PPV of TTMV-HPV DNA testing for identification of recurrent disease on a single test performed 3 months or after treatment completion is 95% ($N = 76/80$), where PPV is defined as the probability that a patient with a positive TTMV-HPV DNA result has clinically identifiable HPV-driven recurrent OPSCC at or subsequent to the time of testing (**Fig. 2B**). Amongst patients with reported clinically active disease at the time of testing, the PPV of recurrent disease was 100% ($N = 21/21$). The PPV of detecting occult recurrence in patients with clinically IND disease status or with NED at the time of testing was 93% ($N = 55/59$). Importantly, longer follow-up may identify recurrent cancer in the 4 IND and NED patients under surveillance.

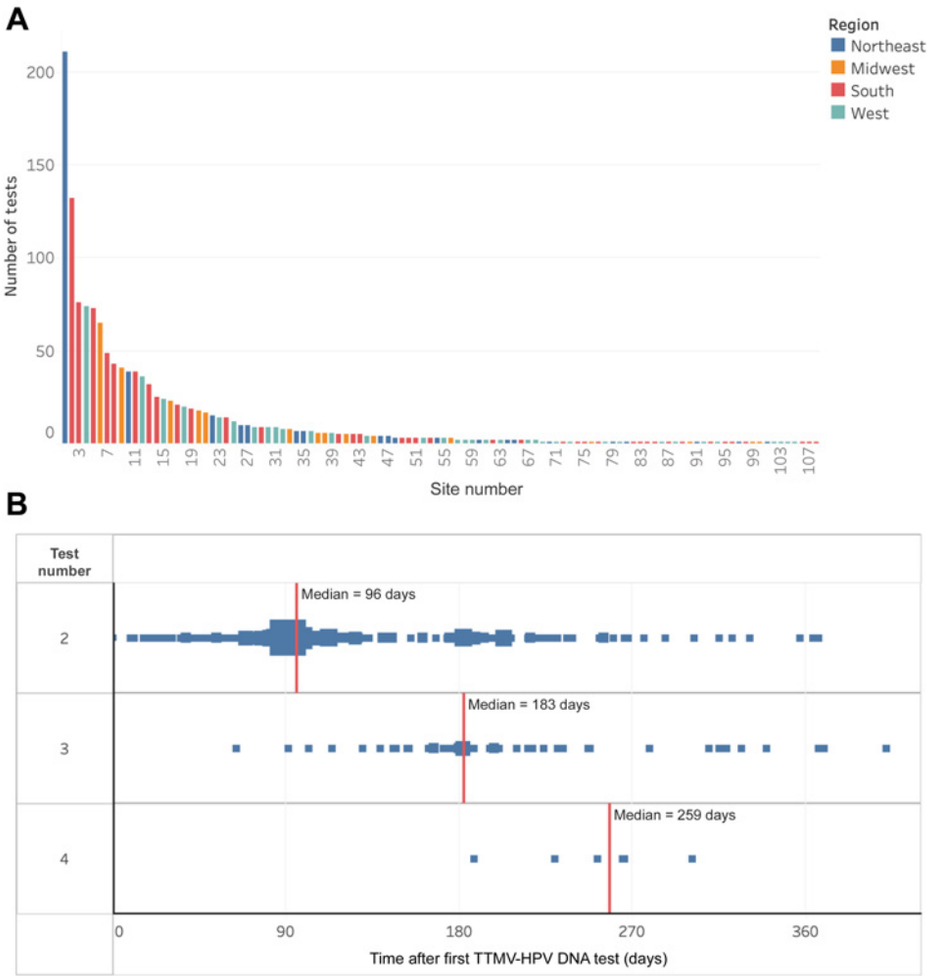

Figure 1.
Real-world ordering of TTMV-HPV DNA testing. **A,** Number of tests ordered by each of the 108 sites across the entire cohort of 1,076 patients ($N = 1{,}370$ total tests) where each bar represents an individual site. The median number of tests per site was 3 (range: 1–211). Colors represent the site region, with Northeast (blue), Midwest (orange), South (red), and West (green) regions represented. **B,** Timing of test ordering for patients with two, three, or four TTMV-HPV DNA tests. Each box represents a single test, plotted at the number of days the test was ordered following the first TTMV-HPV DNA test (time = 0). The red line corresponding to the median number of days the second, third, and fourth tests occurred. Of the 1,076 patients within the cohort, 239 patients had a second test at a median time of 96 days following the first test, 43 patients had a third test at a median of 183 days after the first test, and 6 patients had a fourth test at a median of 259 days after the first test, roughly following standard appointment intervals.

Of the 4 patients with a positive TTMV-HPV DNA result that are currently maintaining IND or NED status, two have clinically suspicious lesions. One has a biopsy-negative, nonhealing base of tongue ulcer at the primary site (TTMV-HPV DNA score: 9), accompanied by slowly resolving imaging findings. The TTMV-HPV DNA signal has been attributed to resolving necrotic tumor tissue. The latest TTMV-HPV DNA result, obtained 3 months after the first positive test, remains in the IND range (TTMV-HPV DNA score: 7). This patient continues active surveillance. The second patient had a suspicious sub-centimeter pulmonary nodule, negative for squamous cell carcinoma (SCC) on image-guided biopsy. This patient's TTMV-HPV DNA tests remain persistently positive, with subsequent tests at 1, 3, and 7 months following the first positive test showing TTMV-HPV DNA scores ranging from 32 to 44. The other 2 patients are currently clinically NED (TTMV-HPV DNA scores ranging from 16–79), with 1 to 3 additional TTMV-HPV DNA tests showing persistently positive or increasing TTMV-HPV DNA over time. Careful examination and radiologic surveillance of these two patients is ongoing.

Distribution of TTMV-HPV DNA levels among positive test results

In all 80 patients with a positive TTMV-HPV DNA test result, the median TTMV-HPV DNA score was 85 (range: 7–123,148; **Fig. 2C**). When stratifying by reported clinical disease status, patients with known clinically active disease exhibited a median TTMV-HPV DNA score of 135 (range: 7–123,148) while those with IND or NED status exhibited a median TTMV-HPV DNA score of 58 (range: 8–23,296), and there was no statistically significant difference between the groups (**Fig. 2D**; $P = 0.27$; unpaired t test). When stratifying by discrete elapsed time intervals since treatment, the median TTMV-HPV DNA score were 48.5 (range: 7–16,957) and 124 (range: 8–123,148) in patients who were less than 12 months and more than 12 months posttreatment, respectively (**Fig. 2E**), and again this difference was not statistically significant ($P = 0.22$; unpaired t test). For the four patients with IND or NED status at the time of testing that remain clinically negative for recurrent disease, TTMV-HPV DNA scores were less than 100 for all positive tests (median: 30, range: 9–79; **Fig. 2C–E**). In addition, all 4 patients in this negative subgroup were less than 12 months out from completion of treatment at the time of their positive test result. The small sample of clinically negative patients in this subgroup precluded statistical comparison.

TTMV-HPV DNA testing as the first indication of recurrence during routine surveillance

Two case examples are provided (**Fig. 3**) illustrating how TTMV-HPV DNA provided the initial indication of recurrence during routine surveillance of otherwise asymptomatic patients. For these case

Table 2. Patients with TTMV-HPV DNA positive results.

	Active disease on first test *N* = 21 (%)	IND/NED on first test *N* = 59 (%)	Total *N* = 80 (%)
Mean age, years (range)	63 (46–76)	64 (44–97)	63 (44–97)
Sex			
Male	19 (90)	56 (95)	75 (94)
Female	2 (10)	3 (5)	5 (6)
p16 status			
Positive	19 (90)	58 (98)	77 (96)
Negative	0	0	0
Unknown[a]	2 (10)	1 (2)	3 (4)
HPV strain/subtype			
HPV 16	18 (86)	56 (95)	74 (93)
HPV 18	1 (5)	0	1 (1)
HPV 31	0	1 (2)	1 (1)
HPV 33	2 (10)	0	2 (3)
HPV 35	0	2 (3)	2 (3)
Time posttreatment[b]			
3–6 months	2 (10)	13 (22)	15 (19)
6–12 months	8 (38)	19 (32)	27 (34)
>12 months	11 (48)	27 (46)	38 (48)

[a]p16 status was unknown at baseline as noted on the test requisition.
[b]From the time of a first positive TTMV-HPV DNA test result.

examples, all TTMV-HPV DNA test results to date, including pretreatment and additional posttreatment follow-up tests are reported in order to provide a full patient history and timeline. In the first case, TTMV-HPV DNA testing was positive 7 months prior to imaging confirmation in an otherwise asymptomatic patient (**Fig. 3A**). In the second case, the first TTMV-HPV DNA test yielded a positive result more than 24 months after completion of primary therapy, also in an asymptomatic patient (**Fig. 3B**). In both cases, upon identification and confirmation of recurrent disease through appropriate imaging and/or biopsy, the patients underwent appropriate treatment and posttreatment TTMV-HPV DNA tests have resolved to undetectable levels.

Negative predictive value of circulating TTMV-HPV DNA in patients under surveillance for recurrence

The NPV of TTMV-HPV DNA testing was calculated, where NPV is defined as the probability that a patient with a negative TTMV-HPV DNA result has no evidence of confirmed clinically identifiable HPV-driven recurrent OPSCC at the time of testing. In total, 1,256 TTMV-HPV DNA tests were negative (**Table 3**). The ordering physician indicated that patient had active disease in 58 of the 1,256 requisition forms for these tests (4.6%), while in the remaining 1,198 (95.4%) tests the patient was indicated as having no evidence of active disease (defined as clinically NED or IND disease status). Among these 1,198 negative tests, the majority (683, 57%) were obtained more than 12 months after completion of therapy, with 282 (24%) being 6 to 12 months posttreatment and 233 (19%) being 3 to 6 months posttreatment. Of the 58 tests where active disease was indicated at the time of the negative TTMV-HPV DNA result, the inverse was true with nearly half of tests (26, 45%) obtained 3 to 6 months after completion of definitive therapy, and 13 (22%) and 19 (33%) at 6 to 12 months and more than 12 months posttreatment, respectively. The overall NPV of TTMV-HPV DNA testing for identification of recurrent disease on a single test performed ≥3 months after treatment completion is 95% (1,198/1,256).

Discussion

This study is the first to demonstrate the broader clinical application of a unique circulating tumor biomarker, TTMV-HPV DNA, in HPV-driven oropharyngeal carcinoma during routine posttreatment surveillance. We observed a PPV of 95% (76/80) to date and an NPV of 95% (1,198/1,256), providing clinical evidence for TTMV-HPV DNA as a promising circulating biomarker that can facilitate earlier detection of recurrence in asymptomatic patients who have completed curative-intent therapy. The ability for a novel circulating tumor biomarker to detect minimal residual disease (MRD) is not a new concept, but one gaining momentum among head and neck cancers as the sensitivity, specificity, and availability of HPV DNA-directed assays evolves. TTMV-HPV DNA testing appears to be an effective aid in the surveillance of HPV-driven OPSCC, with a positive test being an indication for further clinical investigation with imaging-based modalities.

Detection of TTMV-HPV DNA was the first indication of recurrence among 72% (55/76) of patients with confirmed recurrent disease upon follow-up, predating clinical symptoms or imaging detection; and 49% (27/55) of those identified were more than 12 months posttreatment, a time of less intense surveillance. Although recommendations for the optimal frequency and duration of follow-up for patients with head and neck cancer are outlined in National Comprehensive Cancer Network guidelines (15), after a first re-staging CT, MRI, and/or PET-CT at 12 weeks posttreatment, patterns of subsequent imaging are often variable. Although further data will refine surveillance intervals, our cohort study data suggests TTMV-HPV DNA testing every 3 to 4 months during years 1 to 2 posttreatment and every 6 months in years 3 to 5 as a reasonable first approximation.

The interval between completion of therapy and testing may inform whether or not there is a need to confirm a positive test before proceeding with radiologic studies. Using a less sensitive TTMV-HPV DNA assay in the clinical research setting, with the first test very proximate to completion of curative intent therapy, Chera BS, and colleagues proposed confirmatory testing of a positive result within 4 to 6 weeks and careful clinical examination before proceeding with a restaging PET-CT to rule out clinical evidence of recurrence (38). Using the current assay, we found that an initial positive test supported proceeding with confirmatory investigation in the majority of cases, especially when testing was weeks to months after completion of therapy, a time when patients have likely cleared any residual TTMV-HPV DNA. Furthermore, in patients with a positive test result, PET imaging may be preferred to evaluate for rare metastatic sites that CT neck and chest imaging may miss. Ultimately, future studies will be needed to select optimal testing intervals and clarify the need for confirmatory testing during surveillance.

Important to any surveillance strategy that aims to offer early detection is the notion of earlier intervention to improve overall disease outcomes, which remains an open question in the head and neck cancer community (40–44). In some cases, patients with oligometastatic HPV-driven disease recurrence may be aggressively treated with local therapies to promote long-term remission or cure (8, 11, 14). The ease of access and implementation is also an important element of circulating biomarker testing. Having a widely available blood-based metric to monitor patients may improve surveillance utilization and improve healthcare access and efficiency. Discussions of relative cost of surveillance by blood testing versus imaging are evolving (45, 46). However, as the cost and charges and schedules of PET/CT use and clinical examinations are

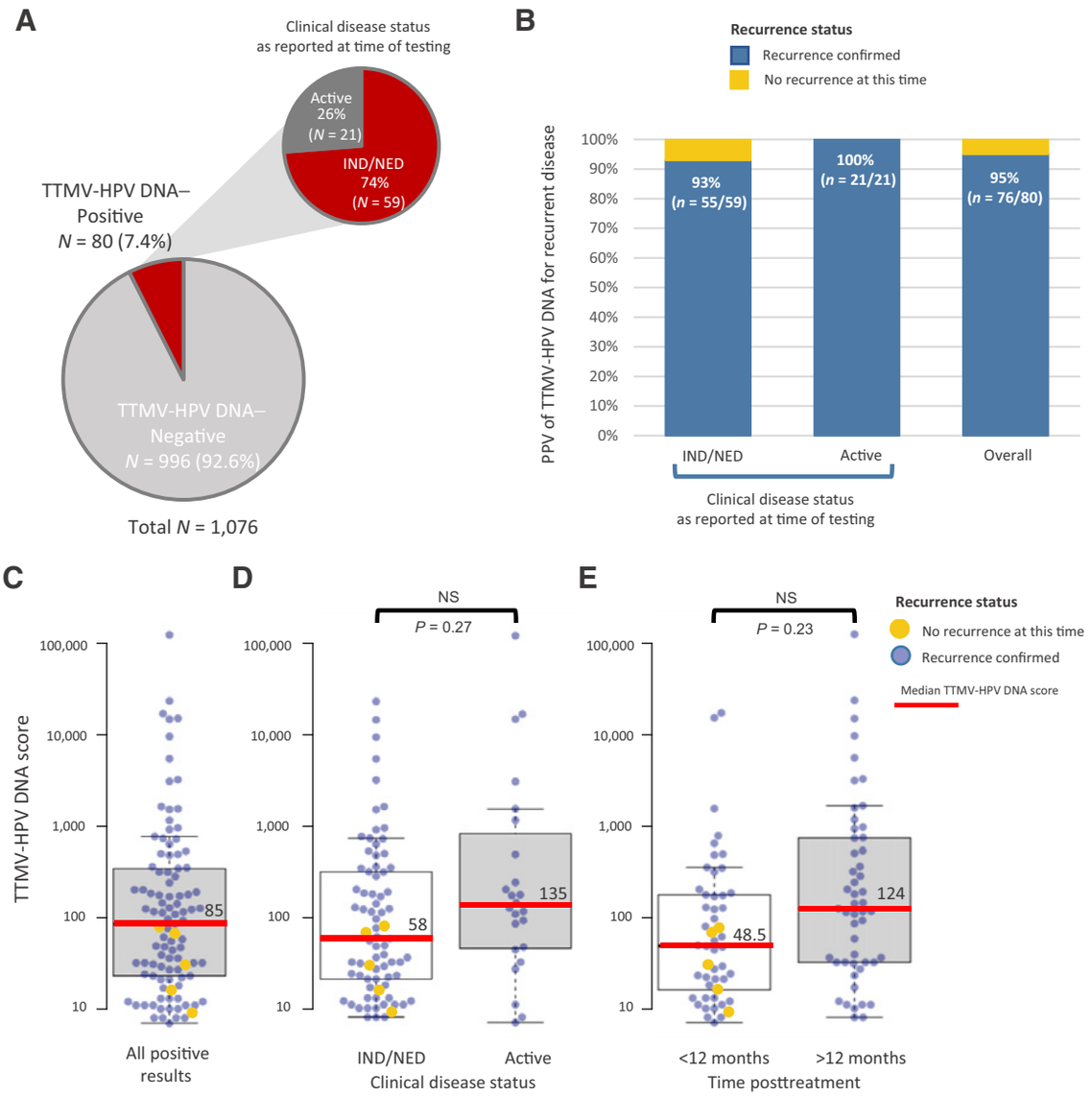

Figure 2.

Percentage of positive TTMV-HPV DNA test results, PPV of TTMV-HPV DNA for recurrent disease, and distribution of TTMV-HPV DNA levels among positive test results. **A,** Overall percentage of positive TTMV-HPV DNA test results (7.4%, $N = 80/1,076$) and, of those, the fraction with IND clinical disease status or NED (74%, $N = 59$) at the time of the first positive result (as indicated on the test requisition) or with clinically active disease at the time of the first positive result (26%, $N = 21$). **B,** PPV of TTMV-HPV DNA as representing the probability that a patient with a positive TTMV-HPV DNA result has clinically identifiable HPV-driven recurrent OPSCC at or subsequent to the time of testing. In patients reported as clinically IND or NED at the time of testing, the PPV of TTMV-HPV DNA for recurrent disease was 93% ($N = 55/59$ patients). In patients reported as having active disease at the time of testing, the PPV was 100% ($N = 21/21$ patients). The overall PPV of TTMV-HPV DNA for recurrent disease across all patients was 95% ($N = 76/80$). PPV was calculated by dividing the number of true positives by the total patients (true positives plus false positives). Median value and distribution of TTMV-HPV DNA scores: (**C**) across all patients with a positive test result ($N = 80$ patients, 101 tests), (**D**) in patients with IND or NED clinical disease status at the time of testing ($N = 59$ patients, 77 tests) versus those with active disease status at the time of testing ($N = 21$ patients, 24 tests), and (**E**) in patients with a test less than 12 months ($N = 42$ patients, 50 tests) versus less than 12 months ($N = 42$ patients, 51 tests) postcompletion of treatment. Colored dots represent the current recurrence status for each patient, showing tests corresponding to patients remaining negative for recurrent disease at this time ($N = 4$ patients, 5 tests; yellow dots) versus those with confirmed recurrences ($N = 76$ patients, 96 tests; blue dots). Data are presented within Beeswarm box plots, where the boundary of the box closest to zero indicates the 25th percentile; the boundary denoted by the red line marks the median, with the red number indicating the median value; and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 10th and 90th percentiles. Points above and below the whiskers indicate outliers outside the 10th and 90th percentiles. Statistical comparison was performed by an unpaired *t* test, and *P* values are presented above the box plots.

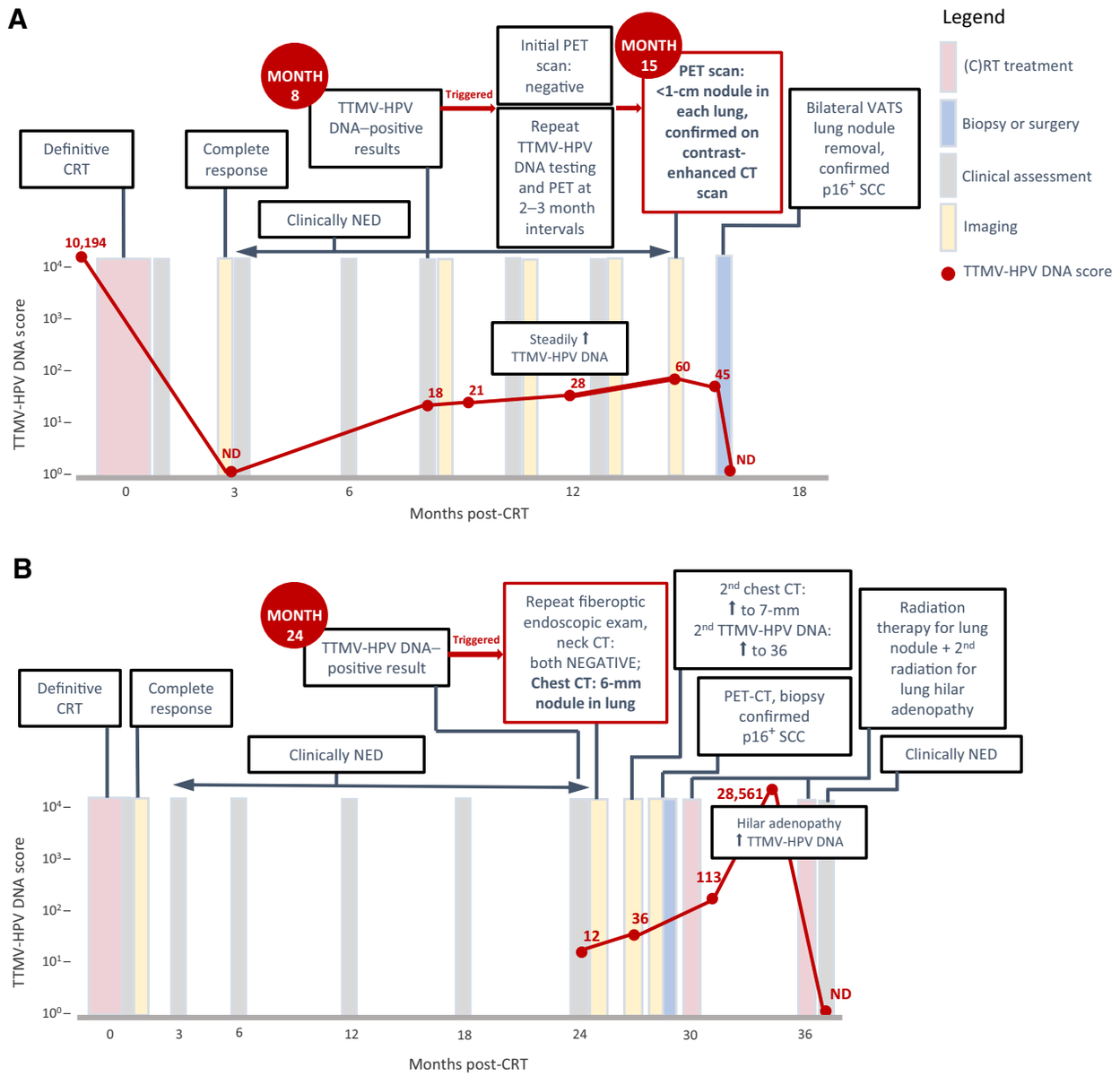

Figure 3.

TTMV-HPV DNA testing as the first indication of recurrence during routine surveillance. **A,** TTMV-HPV DNA testing detected occult recurrence 7 months prior to imaging in an asymptomatic patient. A 62-year-old male presented with T3N2cM0 [American Joint Committee on Cancer (AJCC) 7th edition] p16-positive squamous cell carcinoma of the right tongue base and had a positive prebiopsy TTMV-HPV DNA test (score: 10,194). This patient received definitive chemoradiation therapy with concurrent cisplatin, with resolution of the TTMV-HPV DNA score to negative/not detected at 3 months posttreatment. During surveillance, TTMV-HPV DNA became positive 8 months posttreatment (score 18; imaging and clinical examination negative), with continued small increases over the next 7 months (TTMV-HPV DNA score range: 21–28) and with continued negative imaging and clinical examination. At 15 months posttreatment, a <1-cm, increasingly PET-avid nodule was identified in each lung (TTMV-HPV DNA score: 60) and confirmed on contrast-enhanced CT scan. Therapeutic wedge resections revealed both to be HPV-16, p16-positive, OPSCC. Postoperatively, the TTMV-HPV DNA test result resolved again to negative or not detectable. **B,** TTMV-HPV DNA testing detected occult recurrence 24 months posttreatment in an asymptomatic patient. A 66-year–old male never-smoker presented with p16-positive SCC of right tonsil extending to tongue base, with a single ipsilateral 1.5-cm level IIA lymph node (AJCC 2017 8th edition, stage 1 disease). Definitive chemoradiation therapy with concurrent cisplatin and posttreatment fiberoptic endoscopic examination were given. PET-CT imaging showed a complete response. Five consecutive surveillance examinations over 24 months were negative, and the patient was clinically NED. At 24 months, a TTMV-HPV DNA test was obtained and was positive (TTMV-HPV DNA score: 12, HPV 16). While concurrent 24 month endoscopy and neck CT were negative, chest CT revealed a 6-mm right upper lobe nodule. Follow-up testing at 27 months showed increasing TTMV-HPV DNA (score: 36) and increased nodule size to 7 mm with hypermetabolic findings on PET/CT (SUV 3.1). Biopsy confirmed metastatic SCC and stereotactic body radiation therapy (SBRT) was provided, but the TTMV-HPV DNA score remained elevated at 113. Hilar adenopathy evolved, and the TTMV-HPV DNA score increased to 28,561. After repeat pulmonary radiation, the TTMV-HPV DNA score resolved to negative/not detectable and the patient is currently asymptomatic. ND, not detected; VATS, video-assisted thoracoscopic surgery. CRT, chemoradiation therapy.

Table 3. TTMV-HPV DNA negative test results.

	Active disease at time of test $N = 58$ (%)	IND/NED at time of time $N = 1{,}198$ (%)	Total $N = 1{,}256$ (%)
Time posttreatment			
3–6 months	26 (45)	233 (19)	258 (21)
6–12 months	13 (22)	282 (24)	296 (24)
>12 months	19 (33)	683 (57)	702 (56)

variable across the United States, such studies are most useful in the local context where specific contracts drive the actual costing and out of pocket expense for patients.

TTMV-HPV DNA testing offers the ability to provide more accessible and frequent surveillance with earlier and more directed confirmatory follow-up testing. As seen in the case examples, patients were identified who were candidates for earlier initiation of therapy, and therapy was modified based on subsequent, post-intervention, testing. The current practice of using TTMV-HPV DNA testing identifies unrecognized patients who may be candidates for curative salvage treatment. However, earlier identification of disease could lead to earlier therapy-related toxicity exposure or financial burden and does not always improve survival outcomes. Therefore, long-term follow-up in randomized control trials will be necessary to quantify the timing and benefit of surveillance driven interventions.

Understanding the quantitative range and temporal trends of repeat TTMV-HPV DNA results and how they relate to disease recurrence and clinical parameters is of significant interest. Patients with known clinically detected disease at the time of assay testing had a median TTMV-HPV DNA score of 135, while those patients with no known clinical recurrence or indeterminate findings on prior imaging had a median TTMV-HPV DNA score of 58 ($P = 0.27$). Given similar ranges and medians between these groups, establishing a value cut-off for determining likelihood of clinically detectable disease is not plausible. That said, we did observe that all 4 patients being monitored for clinical recurrence with indeterminate disease status and detectable TTMV-HPV DNA had scores <100 with a median score of 30. The remaining 55 patients who later had clinically confirmed recurrence had a median TTMV-HPV DNA score of 102 but with values ranging from as low as 7. Furthermore, a positive test does not indicate the location of recurrence, although more data collected over time may provide insight regarding this question–as HPV DNA values have been linked with disease subsites in the metastatic setting (35). We speculate that the rate of increase over time may be more predictive of disease activity with exponential increases suggesting rapid disease growth while linear increases with shallower slopes may indicate more indolent or limited disease progression. These trends could further drive decision-making regarding when to reimage and when to start or change salvage systemic therapy versus using more locally ablative techniques.

Application of circulating TTMV-HPV DNA is also being explored in the definitive or neoadjuvant treatment setting. Chera BS, and colleagues described a favorable risk HPV DNA profile using TTMV-HPV DNA based on pretreatment values and assay clearance during treatment which could be used to risk-stratify patients to deintensified or even intensified therapy (37). A currently accruing study (ReACT) is stratifying patients with intermediate risk clinical features (smokers and those with T4 tumors) to deintensified therapy if they show favorable TTMV-HPV DNA metrics during treatment (NCT 04900623). One could certainly envision using TTMV-HPV DNA in the neoadjuvant setting or after transoral robotic surgery to risk stratify patients to modified or deintensified therapy based on assay clearance metrics (47, 48). Establishing whether baseline TTMV-HPV DNA values may be prognostic is of importance. Similarly, using TTMV-HPV DNA in the advanced or metastatic setting to gauge therapeutic response to chemotherapy, immunotherapies, and targeted agents may more rapidly identify disease progression or response to improve therapeutic selection and outcomes.

Our results are generalizable to the broader head and neck cancer community and reflect a real-world sample and application, however, the authors acknowledge some important limitations. First, the majority of patients (78%) had a single surveillance test result obtained during the study period with more than half (55%) greater than 12 months from completion of therapy. Sequential values obtained in this cohort over time would be of interest, as would baseline or pretreatment and on-treatment test results. The vast majority of positive or detectable cases reflected HPV subtype 16 disease (93%) and thus more data is needed to validate these observations in non-HPV16 high-risk subtypes, although we expect similar findings. In addition as this was a cross-sectional analysis of the NPV of the test, whether a negative TTMV-HPV DNA result remains predictive of the absence of recurrence 3, 6, or 12 months after a negative blood test is currently not known. Further follow-up will be needed to clarify this point and help further refine the role of TTMV-HPV DNA testing in the surveillance setting. The length of time a negative test remains predictive is the subject of continued study as the cohort ages.

These findings collectively demonstrate the clinical potential of circulating TTMV-HPV DNA testing to detect occult recurrence among patients with HPV-driven OPSCC in routine practice. In the majority of cases, the presence of detectable TTMV-HPV DNA was the first indication of recurrence. These data may help inform clinical guidelines and surveillance practice patterns in the future.

Authors' Disclosures

B.M. Berger reports personal fees from Naveris, Inc. during the conduct of the study as well as personal fees from Naveris, Inc. outside the submitted work. G.J. Hanna reports personal fees from Naveris, Inc. during the conduct of the study as well as grants from Repertoire, KSQ Therapeutics, and American Society of Clinical Oncology (ASCO) Conquer Cancer Foundation outside the submitted work. M.R. Posner reports personal fees from Merck, Calliditas, Hookipa, Cel Sci, Coherus, Kura, Icon, BioNnet, and Naveris outside the submitted work. S.P. Naber reports personal fees from Naveris, Inc. during the conduct of the study as well as personal fees from Tufts Medical Center outside the submitted work. C. Del Vecchio Fitz reports personal fees from Naveris, Inc. during the conduct of the study. C. Kuperwasser reports personal fees and other support from Naveris, Inc. during the conduct of the study. No disclosures were reported by the other authors.

Authors' Contributions

B.M. Berger: Conceptualization, data curation, formal analysis, supervision, validation, investigation, methodology, writing–original draft, writing–review and editing. **G.J. Hanna:** Investigation, methodology, writing–original draft, writing–review and editing. **M.R. Posner:** Investigation, writing–original draft, writing–review and editing. **E.M. Genden:** Writing–review and editing. **J. Lautersztain:** Data curation, investigation, writing–review and editing. **S.P. Naber:** Data curation, writing–review and editing. **C. Del Vecchio Fitz:** Conceptualization, data curation, formal analysis, supervision, validation, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing.

C. Kuperwasser: Conceptualization, supervision, methodology, writing–original draft, writing–review and editing.

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