Time Dependency for Human Papillomavirus Circulating Tumor DNA Detection after Chemoradiation as a Prognostic Biomarker for Localized Anal Cancer

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ABSTRACT

Purpose: Although detection of ctDNA weeks after surgery is linked to recurrence for other solid tumors, the optimal time point for ctDNA assessment as a prognostic biomarker following chemoradiation for anal cancer is undefined.

Experimental Design: Patients with stages I to III anal cancer treated with chemoradiation between December 2020 and March 2024 were evaluated for human papillomavirus (HPV) ctDNA status at baseline, at the end of chemoradiation, and during surveillance using a droplet digital HPV ctDNA PCR assay, targeting HPV E6 and E7 oncogenes for 13 oncogenic HPV types. Median recurrence-free survival (RFS) according to HPV ctDNA status was estimated via Kaplan–Meier and compared using a log-rank test.

Results: Detection of HPV ctDNA at ≥3 months after chemoradiation was associated with recurrence (80% vs. 2%; OR, 168; 95% confidence interval (CI), 13.6–2,080; $P < 0.0001$) and inferior RFS [4.9 months vs. not reached; HR, 39.2; 95% CI, 4.6–330; $P < 0.0001$] relative to HPV ctDNA–negative status. Sensitivity and specificity for recurrence according to HPV ctDNA detection were 89% and 95%, respectively, with positive and negative predictive values of 80% and 98%, respectively. Differences in RFS according to HPV ctDNA status were not observed at the end of treatment (median RFS, not reached for both; HR, 1.6; 95% CI, 0.35–7.4; $P = 0.48$).

Conclusions: With a novel, highly sensitive assay, detection of HPV ctDNA at least 3 months after chemoradiation was associated with unfavorable survival. Future clinical trials should incorporate this 3-month post-treatment time point to identify patients with HPV-positive anal cancer at elevated recurrence risk according to HPV ctDNA status.

See related commentary by Bercz et al., p. 2261

Introduction

Squamous cell carcinoma of the anal canal is a rare malignancy with an increasing incidence in the United States (1). More than 90% of cases are linked to prior infection with human papillomavirus (HPV), for which HPV types 16 and 18 are the most prevalent (2). Over the past two decades, the proportion of patients presenting with advanced stage at the time of initial diagnosis has increased (3). Nonetheless, the majority of patients still initially have locoregional, nonmetastatic anal cancer, for which standard treatment is concurrent chemoradiation. Depending on the initial stage, 20% to 40% of patients with localized anal cancer do not achieve cure (4). In this setting, salvage surgery with abdominoperineal resection is used for nonmetastatic persistent or recurrent anal cancer after chemoradiation (5), and palliative systemic therapies are reserved for those who develop distant, incurable metastatic disease (6). New therapeutic options are needed to cure more patients with anal cancer at elevated risk for treatment failure after upfront chemoradiation.

Identification of ctDNA as a surrogate for the presence of minimal residual disease—biochemical detection using a blood-based assay in the absence of concomitant endoscopically and/or radiographically evident cancer—is highly prognostic for recurrence after curative-intent therapies in patients with solid tumors (7). Most commonly, ctDNA assays utilized a tumor-informed methodology, in which cancer-specific somatic mutations are identified as DNA fragments in the plasma upon release into the circulation by remnant micrometastatic deposits of cancer (8). One potential limitation using this approach to prognosticate recurrence risk after chemoradiation in patients with anal cancer is that HPV-associated cancers are characterized by low tumor mutation burden (9), a feature that may confound the high sensitivity required for detection of low levels of ctDNA using a mutation-informed approach.

Assessment of recurrence risk according to ctDNA status can be performed as soon as 2 to 4 weeks after surgical resection for the majority of other (i.e., non-anal) gastrointestinal malignancies curable by surgery (10). In contrast, the antitumor cytotoxicity of

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Translational Relevance

In this translational study of 65 patients with stages I to III anal cancer, detection of HPV ctDNA using a tumor-agnostic assay at least 3 months after chemoradiation was strongly associated with recurrence (median RFS not reached vs. 4.9 months; HR, 39.2; $P < 0.0001$) and was both sensitive and specific for the detection of metastatic recurrence. This prognostic effect was not observed at the end of treatment. These results highlight that solid tumors like anal cancer treated with radiotherapy warrant consideration for treatment-specific time assessments using ctDNA methodologies that differ in relation to other solid tumors treated with curative-intent surgery. Detection of HPV ctDNA 3 months after chemoradiation identifies patients with localized anal cancer unlikely to be cured and provides guidance toward design of future interventional trials, seeking to cure more patients with anal cancer.

radiation endures as long as 6 months after treatment completion of localized anal cancer (11). To date, the optimal timing for assessing recurrence risk using a ctDNA-informed approach after chemoradiation in patients with anal cancer remains undefined. In order to address this unknown, we evaluated survival outcomes in patients with localized anal cancer treatment with curative-intent chemoradiation in association with HPV ctDNA detection using an extremely sensitive HPV ctDNA assay broadly covering 13 oncogenic HPV types.

Materials and Methods

Patient identification

Blood samples were collected in Streck tubes, and plasma was isolated from patients with squamous cell carcinoma of the anus or rectum who underwent definitive chemoradiation therapy at our institution. Informed written consent was obtained from each participant prior to any research conduct. Radiation consisted of 50 to 58 Gy to the primary tumor and 43 to 47 Gy to elective nodes more than 25 to 29 daily fractions in combination with weekly chemotherapy (cisplatin 20 mg/m^2 i.v. weekly and 5-fluorouracil as a continuous i.v. infusion at 300 mg/m^2/day on days of radiation; ref. 12). HPV status ["HPV-positive (HPV+)," "HPV-negative," or "tumor not available"] was determined using detection of p16 expression by IHC (expression of p16 protein—E6H4 clone; Ventana Medical Systems) and/or DNA *in situ* hybridization (Patho-Gene HPV types 16/18/31/33/51 probe), as previously described (13) in patients with archival formalin-fixed, paraffin-embedded anal cancer available for testing. Tumors with expression of p16 by IHC or with detection of HPV DNA by *in situ* hybridization were considered "HPV positive." Tumors with no expression of p16 and also with no detected HPV ctDNA by *in situ* hybridization were classified as "HPV negative." Patients with anal margin cancers that were surgically excised were not included.

HPV ctDNA assessment

Blood was collected prior to treatment initiation (baseline), at the end of chemoradiation (week 5), and during surveillance (at 3, 6, 12, 18, 24, 30, and 36 months after completion of chemoradiation, when feasible). Patients were monitored for recurrence with radiographic assessment by CT or MRI at months 3, 6, 12, 18, 24, 30, and 36 after the time of completion of chemoradiation (hereafter referred to as month 3/6/12/etc of follow up) and with endoscopic evaluation at follow-up months 3, 6, 12, 18, and 24 after treatment completion. All participants provided informed consent for sample collection and analysis of their clinical and pathology data prior to any research conduct under an Institution Review Board–approved laboratory protocol at MD Anderson Cancer Center. All research was conducted in accordance with the Declaration of Helsinki.

From 4 mL plasma at each time point, a digital droplet PCR (ddPCR)–based assay (Bio-Rad QX200 ddPCR system) that targets HPV E6 and E7 oncogenes was utilized for HPV ctDNA detection. This multiplex assay detects 13 high-risk oncogenic HPV types (HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, and -68). The human endogenous retrovirus group 3 (*ERV3*) gene was used as a single-copy human gene for DNA quality control. This assay was previously shown to have high analytical sensitivity (limit of quantification [LoQ] = 16 copies/mL), accuracy, precision, linearity, HPV-type specificity, and robustness using oligonucleotides and plasmids encoding HPV type-specific E6/E7 coding regions (14). The assay also has a sensitivity of 91.7% and specificity of 97.7% for a diagnosis of HPV+ oropharyngeal cancer (14). Based on the assay's LoQ of 16 copies/mL for all 13 high-risk HPV types, a plasma sample with ≥16 HPV copies/mL was classified as "HPV ctDNA detected," whereas a sample with <16 copies/mL was considered "HPV ctDNA not detected." HPV quantification for samples positive for detection was reported in copies/mL.

Statistical analysis

Associations between HPV ctDNA status at baseline and clinicopathologic factors were evaluated using a χ^2 test. Associations between age at diagnosis and baseline HPV ctDNA status were evaluated using a Wilcoxon rank-sum test. Recurrence-free survival (RFS) was calculated as the date from treatment start until the date of clinical recurrence or failure to clear all of the initial cancer by 6 months, whichever came first. Recurrence was defined not as a biochemical event based upon HPV ctDNA detection but instead as a clinical event based upon radiographic detection, endoscopic assessment, and/or clinical assessment/examination at the discretion of the evaluating physician. Patients without recurrence were censored at the last follow-up date. RFS for the entire cohort and by select subgroups was estimated by the Kaplan–Meier method. Comparisons between baseline HPV ctDNA levels and selected clinical features were performed with a Wilcoxon test. Time-dependent outcomes were compared using a log-rank test. A two-sided P value ≤0.05 was considered significant.

Data availability

Beyond identification of HPV type and quantification of HPV ctDNA (all described later), there was no additional genomic sequencing data obtained from these analyses. The data generated in this study are available upon request to the corresponding author.

Results

Demographics

From December 2020 to March 2024, 65 patients with localized anal cancer treated with chemoradiation were enrolled in this translational study (Supplementary Table S1). Median age was 62.8 years (range, 43–84), and 50 (77%) were female. There were 29 (45%) patients with stage III disease at diagnosis. Among the

52 patients with available archival formalin-fixed, paraffin-embedded tumors, 51 (98%) were HPV+ anal cancers.

HPV ctDNA was detected in 44 (68%) patients at baseline prior to chemoradiation. HPV16 was detected in 43 (98%) of 44 positive cases, and one case was positive for HPV33. Among the 52 HPV+ cases confirmed by matched tumor testing, the sensitivity and specificity for HPV ctDNA detection of untreated, localized anal cancer in this cohort were 71% and 100%, respectively. Median copy number at baseline for those with detected HPV ctDNA was 139.4 copies/mL (range, 18.9–26,770). As shown in **Table 1**, there was no difference in age or gender between the groups with or without detectable HPV ctDNA prior to chemoradiation. However, detectable HPV ctDNA at baseline was associated with T3 to T4 stage [OR, 5.1; 95% confidence interval (CI), 1.5–18; $P = 0.01$], node-positive status (OR, 4.3; 95% CI, 1.4–13.0; $P = 0.01$), and stage III clinical stage (OR; 5.6; 95% CI, 1.6–19.3; $P = 0.007$). Higher median absolute numbers of HPV ctDNA copies/mL blood were observed for a greater T stage (364 vs. 26 for T3/T4 vs. T1/T2, respectively; $P = 0.04$) and for a node-positive status (387 vs. 13 for node-positive vs. node-negative, respectively; $P = 0.006$) at the time of initial presentation (Supplementary Fig. S1A and S1B). Among patients with no detectable HPV ctDNA at baseline, 100% of subsequent samples obtained after treatment initiation remained under the LoQ of 16 copies HPV ctDNA per mL plasma, and none of these patients experienced disease recurrence after completion of chemoradiation.

HPV ctDNA as a prognostic biomarker for recurrence

At the time of data cut-off (May 2024), 61 (94%) of 65 patients had completed one or more surveillance visits (i.e., ≥3 months after completion of chemoradiation; Supplementary Table S2). After a median follow of 16.3 months (IQR, 7.1–26.8 months), 10 patients had cancer recurrence (Supplementary Fig. S2). Patients with recurrence had a higher median HPV ctDNA count at baseline than those who did not develop recurrence (1,168 vs. 53; $P = 0.004$), as seen in Supplementary Fig. S1C. The presence or absence of HPV ctDNA detection during follow-up for patients with versus without HPV ctDNA ≥16 copies per mL plasma at baseline is shown in **Fig. 1A** and **B**, respectively. Of note, one patient developed symptomatic metastatic disease after the week 5 draw, at which time HPV ctDNA was 1,289 copies/mL. Among the 10 patients who had recurrence, two did not have HPV ctDNA detected in surveillance. Both had HPV+ disease as confirmed by expression of p16 on baseline tumor tissue, and both had detectable levels of HPV ctDNA (247 and 275 copies/mL). One patient experienced a localized recurrence and proceeded to salvage surgery, and the other had a lymph-node only extraregional recurrence.

We evaluated associations between calendar time, HPV ctDNA detection, and clinical recurrence. As shown in **Fig. 2A**, HPV ctDNA detection at completion of chemoradiation was not associated with recurrence [median RFS not reached (NR) for both; HR, 1.6; 95% CI, 0.35–7.4; $P = 0.48$]. In contrast, RFS was significantly worse for those with versus without HPV ctDNA (median RFS detection at the planned 3 months follow-up clinical assessment after completion of chemoradiation (4.9 months vs. NR; HR, 35.4; 95% CI, 1.8–700; $P < 0.0001$; **Fig. 2B**). Among 41 patients evaluated at the 6-month follow-up clinical assessment following chemoradiation completion (**Fig. 2C**), we observed a confirmatory association between HPV ctDNA detection and inferior median RFS (5.6 months vs. NR; HR, 32.0; 95% CI, 1.8–560; $P < 0.0001$).

Some patients who developed clinical recurrence had undetectable HPV ctDNA at their 3-month surveillance follow-up, yet converted to an "HPV ctDNA detected status" at a later subsequent time point, either prior to or at the time of clinical recurrence. In order to broaden the surveillance window for ctDNA detection, we evaluated 3 months as the earliest cut-point for evaluating HPV ctDNA ≥16 copies per mL plasma as a biomarker for anal cancer recurrence (**Fig. 3**). In this study, detection of HPV ctDNA at ≥ 3-month after completion of chemoradiation was highly prognostic for recurrence (median RFS 4.9 months vs. NR; HR, 39.2; 95% CI, 4.6–330; $P < 0.0001$). At this landmark time point, the sensitivity and specificity of HPV ctDNA detection for recurrence were 89% and 95%, respectively. Positive and negative predictive values for an HPV ctDNA level ≥16 copies per mL plasma in relation to recurrence during the surveillance period were 80% and 98%, respectively.

We also assessed for univariate associations between baseline features and RFS (Supplementary Fig. S3). Higher T stage (60% vs. 37%; OR, 2.5; 95% CI, 0.63–10.1; $P = 0.19$), node-positive status (80% vs. 51%; OR, 3.8; 95% CI, 0.74–20.0; $P = 0.11$), and stage III disease (70% vs. 37%; OR, 3.9; 95% CI, 0.91–17.0; $P = 0.07$) were not linked to subsequent recurrence. However, male gender was

Table 1. Clinical characteristics according to pretreatment HPV ctDNA detection status.

	HPV ctDNA < 16 copies/mL	HPV ctDNA ≥ 16 copies/mL	
	***N* = 21**	***N* = 44**	***P* value**
Age (years; SD)	63.0 (11.0)	61.0 (9.1)	0.45
Gender (%)			0.71
Female	17 (81)	33 (75)	
Male	4 (19)	11 (25)	
T stage			0.01
1–2	17 (81)	20 (45)	
3–4	4 (19)	24 (55)	
N stage			0.01
0	14 (67)	14 (32)	
1	7 (33)	30 (68)	
Clinical stage			0.007
1–2	17 (81)	19 (43)	
3	4 (19)	25 (57)	

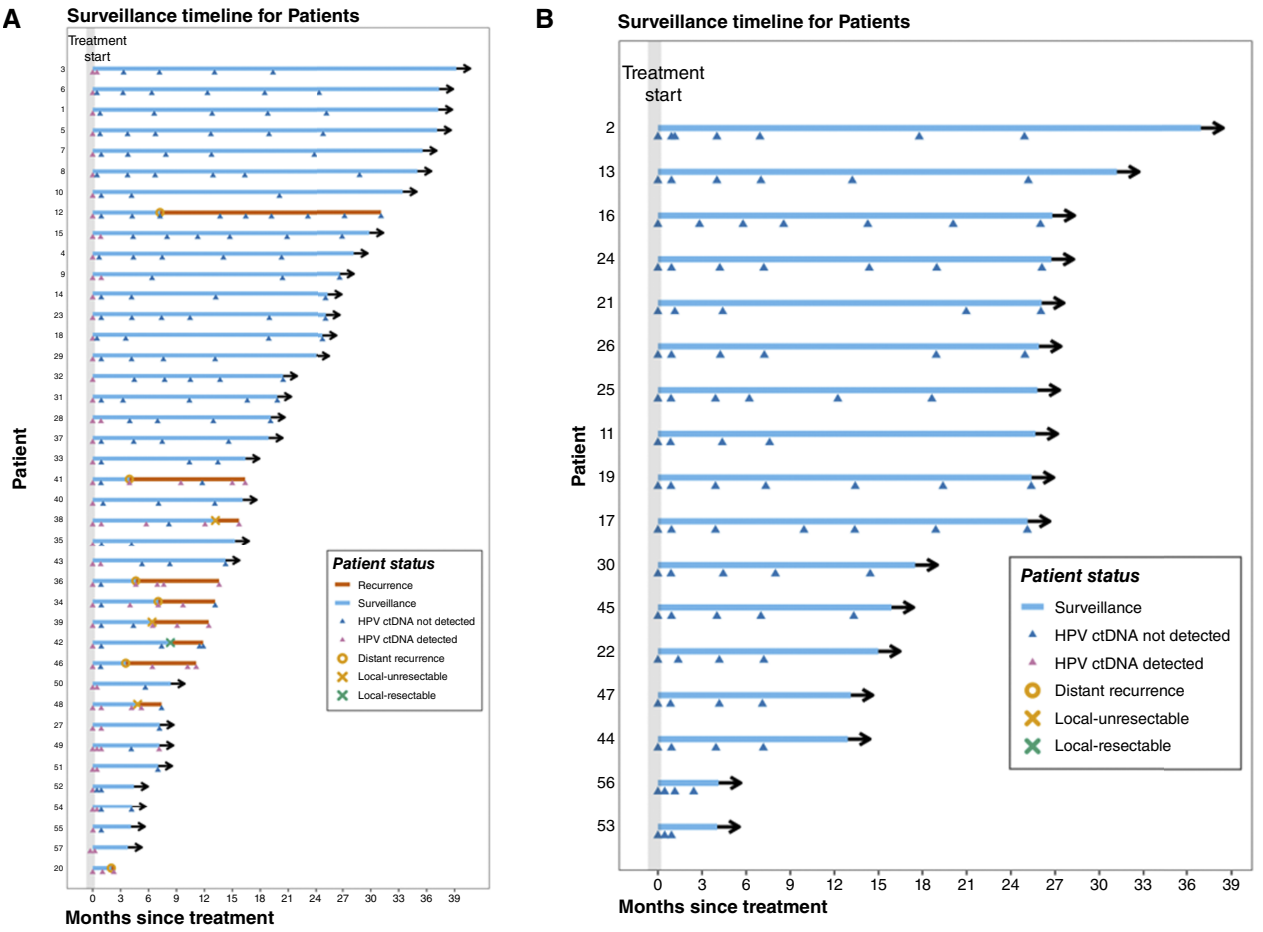

Figure 1.
Swimmer plots for patients with (**A**) and without (**B**) detectable HPV ctDNA at baseline.

associated with recurrence (36% vs. 11%; OR, 4.7; 95% CI, 1.1–19.6; $P = 0.04$). Patients with HPV ctDNA at baseline had a trend toward increased risk for recurrence relative to those without detectable baseline ctDNA (100% vs. 63%; OR, 12.6; 95% CI, 0.70–227; $P = 0.09$). HPV ctDNA detection at ≥3-month following chemoradiation was most prognostic for recurrence in this cohort of patients with anal cancer (80% vs. 2%; OR, 168; 95% CI, 13.6–2080; $P < 0.0001$).

Discussion

In this study, we report that HPV ctDNA detection after completion of chemoradiation is highly prognostic for recurrence in patients with localized anal cancer. The performance of HPV ctDNA as a prognostic biomarker was time-dependent after completion of chemoradiation using surveillance follow-up. Inferior RFS was observed when HPV ctDNA was detected 3 months after chemoradiation, but this effect was not observed when HPV ctDNA was assessed at the end of treatment. These results may inform clinicians on the timing of testing HPV ctDNA after chemoradiation as definitive treatment for nonmetastatic anal cancer.

HPV ctDNA detection at this 3-month time point was significantly prognostic for recurrence when other clinical and pathologic risk factors like clinical stage at presentation and gender were not. In addition, previous retrospective series have linked a T3 or T4 primary tumor status, lymph node involvement, and male gender with poorer survival for anal cancer after chemoradiation (15). These findings are consistent with previous smaller series, evaluating ctDNA detection as a prognostic biomarker for recurrence of anal cancer after completion of chemoradiation (16–18), although the optimal timing for testing has not previously been specified. These studies assessed for HPV-16 and HPV-18 in the ctDNA, and our assay here broadens detection of less common oncogenic HPV types that can cause anal cancer. Although HPV ctDNA was detected in 68% of participants with localized disease prior to treatment, it is important to distinguish this clinical context as different from detection of HPV ctDNA *after* chemoradiation, which was associated with development of subsequent incurable anal cancer in 9/10 recurrent cases—six with distant metastases and three with locally advanced unresectable disease. This assay was reliably able to forecast clinical development of residual, metastatic cancer representative of treatment failure, a feature that has been validated using the same assay for cervical cancers (14, 19).

The timing of when to assess patients for recurrence risk after chemoradiation had heretofore remains unreported. In the Anal Cancer Trial II (ACT II), a fraction of patients with localized anal

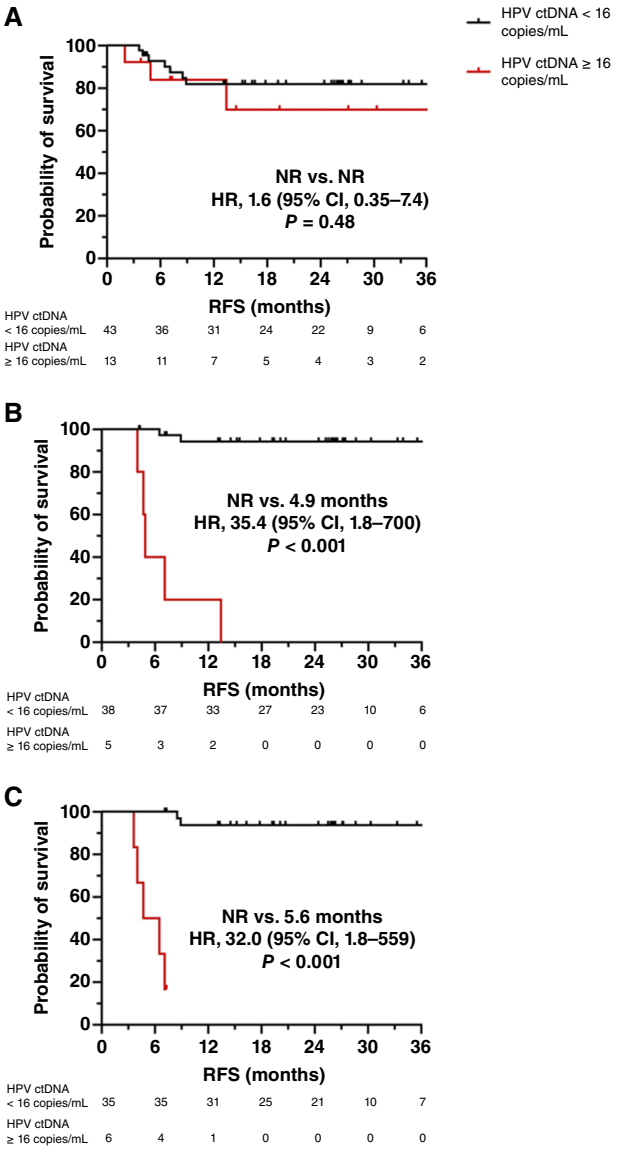

Figure 2.
RFS according to HPV ctDNA detection at the end of treatment (**A**) and 3 months (**B**) and 6 months (**C**) after chemoradiation.

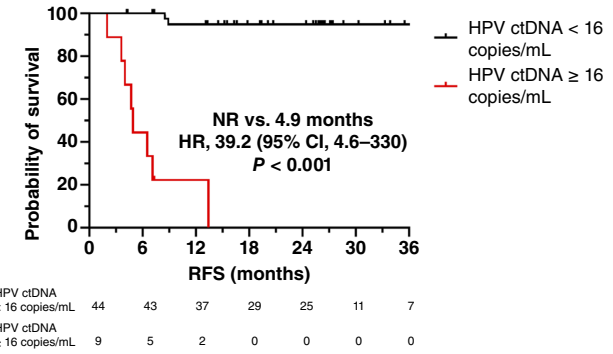

Figure 3.
RFS ≥3 months after chemoradiation according to HPV ctDNA detection.

cancer who remained with clinical and/or radiographic evidence of disease at 3 months after chemoradiation achieved a complete clinical response by the 6-month time point, without recurrence and with a subsequent favorable long- term survival outlook (11). These findings had affirmed the continual antitumor activity that remains ongoing for as long as 6 months after chemoradiation has been completed for anal cancer. One prior series evaluated clinical outcomes according to changes in HPV ctDNA patterns during chemoradiation in patients with anal cancer, for which HPV ctDNA was quantified not as an absolute number but rather reported as a percentage fraction relative to *EMC7* gene quantification. In that study, only pretreatment HPV ctDNA percentage (above or below median levels) was assessed in response to survival outcomes, and persistent HPV ctDNA was linked to distant metastatic disease in only 31% cases (18). Our data complement these preceding studies by clarifying the time dependency of HPV ctDNA clearance in assessing recurrence risk after chemoradiation for localized anal cancer. In our series, there was no difference in RFS between those with or without detected HPV ctDNA at the time of treatment completion, and an HPV ctDNA–positive status at treatment completion was not prognostic for recurrence. In addition, by including a 13-type HPV panel, we extend coverage for detection of more oncogenic HPV types that allow for greater detection of HPV ctDNA beyond HPV-16/-18 cancers like anal cancer. Most of the patients who experience treatment failure in our cohort had detectable ctDNA at their initial follow-up during surveillance. However, one patient did not have detectable ctDNA until their planned 6-month surveillance evaluation. In other cancers like colorectal cancer (20), sensitivity for ctDNA detection in identifying clinical recurrence improves over time with serial assessments during surveillance after completion of curative-intent therapies like surgery and adjuvant chemotherapy. Based on our findings, we would recommend initiation of ctDNA assessment 3 months after completion of chemoradiation for localized anal cancer and continuing throughout surveillance.

The majority (68%) of patients in our cohort had detectable ctDNA at baseline prior to initiation of chemoradiation. It is true; however, that not all patients had HPV ctDNA levels above the LoQ despite having known, clinically evident disease. Patients with baseline HPV ctDNA excessing 16 copies/mL were more likely to have clinically higher T stage and nodal involvement. Sensitivity for detection, therefore, appeared limited for lower stages of initial disease presentation. The ability to detect ctDNA in pretreatment specimens of other gastrointestinal cancers treated with chemoradiation like rectal adenocarcinoma has been previously reported. Using tumor-informed methodologies, sensitivity for detection of ctDNA for treatment-naïve rectal cancer approximated 75% (21, 22), similar to our findings here for anal cancer. Although this limitation for ctDNA assessment remains critical in the clinical management of localized gastrointestinal cancers, our primary focus in this work was correlating HPV ctDNA status with survival outcomes after chemoradiation, and this HPV ctDNA assay performed well in risk stratifying patients with localized anal cancer temporally after chemoradiation.

Though our work was completed as a single-institution study, we nevertheless present a large cohort size for an orphan malignancy at a high-volume, tertiary comprehensive cancer center. Our cohort number may have been limited to complete a multivariate analysis

for assessment of clinical and biochemical risk factors associated with clinical recurrence, but our findings do remain consistent with those of other solid tumors for ctDNA detection reliably forecasting treatment failure prior to the development of eventual incurable disease. Although patients were not treated with a fluoropyrimidine and mitomycin C combination as a radiation sensitizer, use of a platinum and fluoropyrimidine doublet has been demonstrated to be effective as an alternative for chemoradiation for the treatment of localized anal cancer, and this regimen has been the primary systemic therapy used as concurrent chemotherapy with radiation at our institution for 2 decades (12, 23). In addition, the half-life of these cytotoxic agents is short and should not affect the time dependency for the 3-month landmark in prognosticating the recurrence risk after treatment completion with radiotherapy for these patients. Therefore, we believe that these findings are applicable, regardless of the selection of cytotoxic regimen used with radiotherapy.

In summary, detection of HPV ctDNA 3 months after chemoradiation, but not at the end of treatment, is significantly prognostic for recurrence using a highly sensitive ddPCR HPV ctDNA assay in a tumor-agnostic methodology. Patients with no detectable HPV ctDNA were unlikely to recur, perhaps a reflection of low HPV copy number inherent to the respective anal cancer. Validation of a negative HPV ctDNA detection status as a biomarker predictive for curative outcomes with chemoradiation may be considered in future, larger prospective clinical trials. Identification of patients likely to recur by this 3-month landmark according to HPV ctDNA status proffers a potentially earlier interventional window in identifying patients with localized anal cancer destined for recurrent or persistent disease otherwise before the historic precedent with 6 months of surveillance using radiographic imaging and endoscopy. Although no adjuvant therapies have yet proven effective in prospective trials in improving survival after completion of chemoradiation for anal cancer, the design of future trials could be informed by our data showing prognostic utility of an HPV-detected ctDNA status at this 3-month landmark time point in identifying patients at high risk for recurrence. In support of this, a first-in-kind clinical trial to incorporate detection of HPV ctDNA as an integral biomarker for study entry with combination immunotherapy of the anti–PD-L1 antibody atezolizumab and the anti–T-cell immunoreceptor with Ig and ITIM domains antibody tiragolumab for patients with localized anal cancer with detectable HPV ctDNA after chemoradiation is forthcoming at our institution.

Authors' Disclosures

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Authors' Contributions

V.K. Morris: Conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing. **W. Xiao:** Data curation, software, formal analysis, validation, investigation, methodology, writing–review and editing. **K. Lin:** Data curation, software, formal analysis, investigation, writing–review and editing. **C.W. Wong:** Data curation, software, formal analysis, investigation, methodology, writing–review and editing. **M.T. Wotman:** Investigation, methodology, writing–original draft, writing–review and editing. **E.B. Holliday:** Data curation, investigation, writing–review and editing. **R.W. Huey:** Resources, data curation, investigation, writing–review and editing. **S.S. Noticewala:** Data curation, investigation, writing–review and editing. **E.B. Ludmir:** Data curation, investigation, writing–review and editing. **A.H. Bent:** Investigation, writing–review and editing. **K. Ludford:** Investigation, writing–review and editing. **C. Messick:** Investigation, writing–review and editing. **E.J. Koay:** Investigation, writing–review and editing. **G. Smith:** Investigation, writing–review and editing. **T. Konishi:** Investigation, writing–review and editing. **B. Bednarski:** Investigation, writing–review and editing. **G.J. Chang:** Investigation, writing–review and editing. **A.C. Koong:** Investigation, writing–review and editing. **Y.N. You:** Investigation, writing–review and editing. **P. Das:** Investigation, writing–review and editing. **M.L. Gillison:** Conceptualization, resources, data curation, software, formal analysis, supervision, funding acquisition, validation, investigation, visualization, methodology, project administration, writing–review and editing.

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Note

Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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