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Implementation of Human Papillomavirus Circulating Tumor DNA to Identify Recurrence during Treatment De-Escalation

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Following the recent advancements in the ability to quantify Human Papillomavirus circulating tumor DNA (HPV ctDNA) from the plasma of patients with HPV+ oropharyngeal squamous cell carcinoma (OPSCC), several academic and commercial teams are now evaluating the potential of quantitative HPV ctDNA analysis in various clinical settings, including for disease control following de-escalation and detection of early recurrence following definitive therapy. A recent important review published in *Oral Oncology* last month by Chatfield-Reef et. al.¹ highlighted the emerging techniques and some of the current challenges in this field.

For HPV+ OPSCC, a large number of clinical trials are attempting to advance new treatment de-escalation strategies that maintain excellent survival outcomes while minimizing long-term toxicities.²⁻⁶ However, these trials carry the risk of de-escalating patients that would have otherwise benefited from standard treatment; and, the identification of such patients, with ctDNA or other biomarkers, remains of paramount importance. Retrospective data with small patient cohorts suggest that HPV ctDNA is difficult to detect in patients with early stage disease (26% of patients with T1, 48% of patients with T2 disease)^{7,8}, levels correlate with disease burden,^{9,10} rapid clearance after chemoradiation (CRT) is associated with improved locoregional control¹¹, and levels increase at the time of recurrence^{9,12}. Importantly, very limited data has been published from low risk OPSCC patients and the published studies have few failures, preventing the rational design of HPV ctDNA trials in this setting.

We recently completed a phase II clinical trial of low-risk HPV+ OPSCC to determine whether treatment stratification by neck dissection, to more accurately pathologically stage patients, could minimize treatment modalities and thereby improve quality of life. Our trial cohort therefore provided the unique opportunity to prospectively evaluate HPV ctDNA clearance following various modes of therapy in low-risk disease and to test the utility of HPV ctDNA for early detection of recurrence during surveillance.

Twenty-seven T1 and T2 patients with limited neck disease had plasma drawn before treatment and, 35.7% (5/14) of T1, and 69% (9/13) of T2 patients had detectable HPV ctDNA at baseline. Baseline HPV ctDNA levels were significantly higher in patients with T2 disease compared with T1 disease (averages of 2,467 vs 130.7 copies per milliliter plasma, respectively, $P<0.05$). There were no differences in baseline plasma HPV ctDNA counts based on N classification or presence of extracapsular extension. All patients had complete clearance of plasma HPV ctDNA at 4 weeks post treatment regardless of modality (Figure 1).

In total, we evaluated 81 post-treatment plasma specimens from patients in this cohort with a median follow up duration of 33.3 months (range: 0.56–39.6 months). One patient had detectable ctDNA at baseline that became undetectable 4 weeks post-surgery. Then, HPV ctDNA was detected six months post-treatment and persisted through the next 5 time points (Figure 2). Importantly, 24 months after treatment (18 months after initial biochemical recurrence) a local recurrence was clinically identified. All post-treatment samples from other patients had no detectable biochemical recurrence and no other patient recurred clinically/radiologically.

Ongoing de-escalation strategies use various modalities to allow for de-intensified adjuvant treatment^{3–6}. While our trial utilized the pathologic findings from neck dissection as a clinical biomarker to guide primary treatment modality, we were able to leverage the cohort to gain insight into the clearance of HPV ctDNA in early stage disease. Our data demonstrate that all patients had undetectable plasma HPV ctDNA within one month of completion of treatment regardless of treatment modality and future recurrence. However, one of these patients developed recurrent disease, suggesting that plasma HPV ctDNA clearance after completion of definitive therapy may not be predictive of future recurrence in low risk HPV+ OPSCC. These data are consistent with current working mathematical models of ctDNA shedding,¹³ which suggest that tumor size, death and other shedding characteristics may limit the amount of ctDNA in circulation to below detectable levels with current technology. In the future, this problem may be addressed by development of more sensitive assays, thereby enhancing the ability to detect ctDNA from otherwise-undetectable residual/recurrent disease.

Despite this limitation, recent published data in a variety of settings suggest that plasma HPV ctDNA may be a complementary biomarker for identification of residual or recurrent disease.^{7–9,11,12} We show data that HPV ctDNA was detectable 18 months prior to overt clinical or radiographic recurrence. With only one recurrent patient, the interpretation of clinical utility should be tempered, but our finding supports the need for future studies that formally validate the utility of longitudinal HPV ctDNA kinetics in low risk patients. It remains to be seen how early detection of recurrence will translate into new clinical trials and/or if treating recurrent HPV+ OPSCC patients early will positively impact patient outcomes. These are all extremely exciting future areas of study that need to be rigorously studied before HPV ctDNA is used for clinical decision making. Nonetheless, our data adds to the growing body of evidence supporting the potential multi-setting clinical utility of HPV ctDNA, and continues to suggest that HPV ctDNA could have a transformative impact for the outcomes of HPV+ OPSCC patients worldwide.

Methods

Thirty-four patients with low-risk HPV+ OPSCC (T1–3, N0–N1) were enrolled (NCT02784288). Patients underwent upfront neck dissection with stratification into one of three treatment groups based on post-operative pathology. Patients with a single lymph node < 6 cm with no extracapsular extension (ECE), perineural (PNI), or perivascular invasion (PVI) underwent transoral surgery. Patients with two or more positive nodes without adverse features (ECE or positive margins) or a single node in which PVI or PNI was noted were treated with radiotherapy (RT) alone. Patients with ECE underwent CRT. Plasma was isolated from blood collected in Streck tubes from 27 patients prior to the neck dissection, one-month post-treatment (surgery, RT, or CRT), and every 3 months thereafter.

CfDNA was isolated following the QIAamp MinElute ccfDNA Mini Kit protocol. Analytical and methodologic details of our ctDNA assay are described.¹⁴ Briefly, the assay amplifies a 77bp region of HPV16_E6 with forward 5'-CGAAACCGGTTAGTATAAAAGCAG-3', reverse 5'GTCGCTCCTGTGGGTCCT-3' and

probe 5'-FAM-CATTTTATGCACCAAAAGAGAACTGCAATGTTTC-MGBNFQ-3', and uses an *RPP30*-HEX reference to assess quality (Assay-ID: dHsaCP2500350, Bio-Rad).

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Figure 1: HPV16 ctDNA is undetectable in low stage disease as soon as 4 weeks after treatment. Plasma samples from patients were analyzed before and after treatment. Heatmap shows the absolute quantification of HPV16 ctDNA copies detected in each sample. Clinical variables associated with each patient, including T and N stage (AJCC 8), extracapsular extension (ECE) and treatment strategy are shown. Note that one patient with detectable baseline ctDNA missed the 1 month draw.

Figure 2: Longitudinal ctDNA quantification in recurrent patient.

Ten plasma samples drawn from pre-treatment to 2.5 years post-treatment were analyzed for HPV16 ctDNA, and all samples except the 4 week and 3 month time points had detectable HPV16 ctDNA copies.