Ultra-sensitive detection and quantification of HPV DNA in the plasma of patients with oropharyngeal squamous cell carcinoma (OPSCC) enrolled in the OPTIMA 2 treatment de-escalation trial

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Introduction

- Human papillomavirus (HPV) infection is a primary factor driving the increasing incidence of OPSCC.
- As patients with HPV+ OPSCC show significantly improved treatment response and prognosis, there is an urgent need to de-escalate treatment of HPV+ OPSCC that optimizes oncologic control while minimizing treatment-related toxicity.
- **Cell-free HPV DNA (cfHPV-DNA)** from plasma represents a promising noninvasive surrogate of disease burden in these patients.
- To enable cfHPV-DNA analysis as a strategy to monitor therapy response and guide treatment de-escalation, we developed a highly sensitive assay for HPV16/18 detection and quantification in plasma, based on SafeSEQ next-generation sequencing (NGS) technology.

Methods

- Longitudinal plasma samples were collected from patients with locoregional HPV+ OPSCC treated on OPTIMA 2 (NCT03107182), a de-escalation protocol of induction chemoimmunotherapy followed by risk/response stratified de-escalated locoregional therapy.
- Neck CT or MRI was obtained for all patients at baseline and following induction chemoimmunotherapy; radiographic response to induction therapy was assessed per RECIST 1.1 criteria.
- cfHPV-DNA was quantified in plasma samples (2mL plasma per time-point) collected at baseline and at 6-9 weeks after beginning induction therapy using the SafeSEQ cfHPV-DNA Test (Sysmex Inostics).

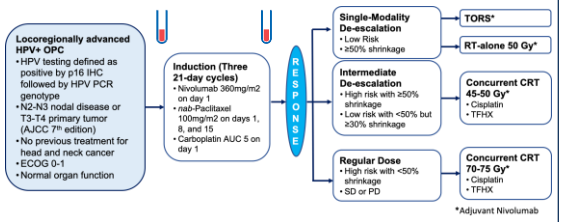


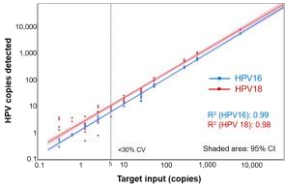
Figure 1. OPTIMA 2 study design. *TORS: Transoral Robotic Surgery; TFHX: paclitaxel, 5-fluorouracil, and hydroxyurea, with twice daily radiation in week-on week-off CRT platform*

Conclusions

- The SafeSEQ cfHPV-DNA Test exhibits robust quantitative detection of HPV across a broad range, enabling high-resolution molecular monitoring for HPV+ OPSCC patients.
- Prospective studies are underway to further evaluate the kinetics of cfHPV-DNA as a predictor of response to therapy in order to more precisely guide the management of patients with HPV+ OPSCC.

Results

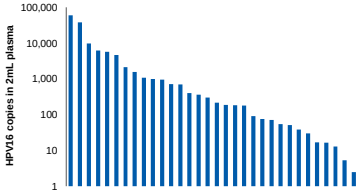
SafeSEQ cfHPV-DNA Test: Analytical performance



- Accurate quantification of plasma HPV 16 and 18 levels over 5 orders of magnitude.
- Low quantitative variability (<30% CV observed above 5 copies).
- Low level of background signal (<0.04 copies per sample across 20 healthy donor samples), indicating high specificity.

Figure 2. Quantification of HPV 16 and 18 in contrived samples. *Dilution series of contrived samples tested in replicate at 12 tiers ranging from 0.3 to 50,000 copies*

cfHPV-DNA detected in patient plasma samples collected at baseline



- In 32 (82%) of 39 patient plasma samples collected at baseline, cfHPV-DNA was detected at levels ranging from 2.5 to >59,000 copies.
- cfHPV-DNA was not detected above the assay cut-off (1.5 copies) in 7 of the 39 baseline samples tested.

Figure 3. cfHPV-DNA detected in 32 patient plasma samples collected at baseline.

Longitudinal cfHPV-DNA analysis

- Plasma samples collected at 2 serial time-points (baseline and 6-9 weeks after beginning induction therapy) were available for analysis in 31 patients. cfHPV-DNA was detected at baseline in 25 patients.
- All 25 patients with baseline cfHPV-DNA showed a decrease in cfHPV-DNA level at follow-up, with complete clearance observed in 20/25 (80%) of patients; this is consistent with tumor response (shrinkage) to induction therapy observed in 24/25 (96%) patients.
- Of 5 patients with persistent cfHPV-DNA detected post-induction therapy (*indicated by yellow highlighted rows in Table 1*): 1 patient progressed on induction therapy, 1 patient demonstrated subsequent recurrence and death, and 1 patient demonstrated concern for distant metastasis followed by death.

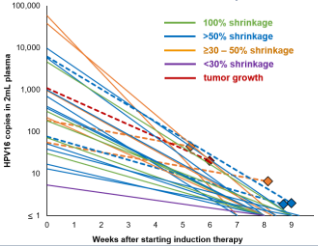


Figure 4. Longitudinal cfHPV-DNA analysis. *Trend lines are colored according to radiographic response to induction therapy. Dashed lines indicate patients with persistent cfHPV-DNA at follow-up; residual cfHPV-DNA data points are indicated with a ♦*

Patient ID	Clinical stage	Clinical risk	Baseline cfHPV16	Follow-up sample		Post-induction response (% tumor shrinkage)	Clinical follow-up / outcome
				cfHPV16	Weeks from baseline		
UC005	T3N0	LR	**4,622.75**	*0.00*	9	100	NER
UC017	T2N2b	LR	**302.63**	*0.42*	8	100	NER
UC023	T4N2c	HR	**180.45**	*0.00*	9	100	NER
UC064	T2N2b	HR	**71.73**	*0.00*	8	100	NER
UC030	T2N2b	LR	**30.09**	*0.00*	8	100	NER
UC019	T3-4N2b	HR	*0.05*	*0.00*	8	100	NER
UC011	T3N1	HR	**76.57**	**1.83**	9	87.7	NER
UC014	T4N2c	HR	**6,173.74**	**1.97**	9	81.6	Concern for lung met (deceased)
UC016	T4N3	HR	**9,740.48**	*0.00*	9	74.6	NER
UC027	T2N2c	HR	**401.31**	*0.68*	8	73.3	NER
UC008	T1N2	HR	**694.18**	*0.00*	7	68.8	NER
UC024	T1N2a	LR	**51.71**	*0.33*	8	65.7	NER
UC013	T2N2b	LR	**5,705.97**	*0.00*	8	64.5	NER
UC067	T1N2b	LR	**365.26**	*0.00*	8	63.9	NER
UC068	T2N1	HR	*0.82*	*0.00*	9	63.0	NER
UC028	T1N2b	HR	**12.86**	*0.00*	9	60.7	NER
UC062	T2N2b	LR	**16.72**	*0.00*	8	55.3	NER
UC018	T2N1	LR	**38.44**	*0.00*	9	55.1	NER
UC066	T1N2b	LR	*0.38*	*0.00*	8	54.6	NER
UC015	T2N2b	LR	*1.09*	*0.00*	8	53.3	NER
UC009	T1N2a	LR	**985.17**	*0.00*	8	51.9	NER
UC022	T2N2b	HR	*0.10*	*0.00*	8	50.7	NER
UC070	T1N2b	HR	**946.06**	*0.00*	8	48.0	NER
UC020	T2N2b	LR	**54.77**	**6.50**	8	46.9	Recurrence (deceased)
UC003	T1N2	LR	**37,855.87**	*0.00*	8	45.5	NER
UC063	T1N2b	LR	**215.82**	*0.00*	8	45.5	NER
UC001	T2N2a	LR	*0.16*	*0.00*	8	38.5	NER (deceased)
UC039	T2N2b	HR	**59,050.56**	*0.75*	7	37.5	NER
UC040	T2N2	LR	**184.92**	**44.03**	5	30.0	NER
UC007	T2N2b	HR	**5.32**	*0.00*	8	28.6	NER
UC073	T4N2b	HR	**1,084.91**	**19.94**	6	2% GROWTH	NER

Table 1. Summary of patients included in longitudinal cfHPV-DNA analysis. *HPV values in grey italics are below assay cut-off; LR: Low risk; HR: High risk; NER: No evidence of recurrence.*