Liquid blOpsy for miNimal rESidual diSease detection in Head and Neck Squamous Cell Carcinoma (LIONESS) – A personalized cell-free tumor DNA analysis for patients with HNSCC

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INTRODUCTION

- Despite improvements in multimodal treatment options for patients with head and neck squamous cell carcinoma (**HNSCC**), survival has only improved modestly over the past decades as patients frequently develop recurrences.
- Detection of cell-free circulating tumor DNA (**ctDNA**) post-operatively and during clinical follow-up has the potential to identify patients with molecular residual disease (**MRD**) or those at an increased risk of relapse who may benefit from personalized treatment strategies.
- Here, we use the RaDaR® assay to detect ctDNA in pre- and post-operative plasma samples (range 1-14, median 6) from 46 patients in the LIONESS study (**Table 1**).

METHODS

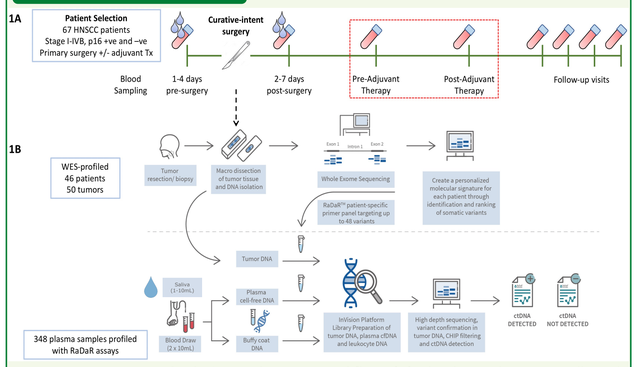


Figure 1. LIONESS study design and RaDaR workflow. (A) Blood samples were collected before and after curative-intent surgery and at different timepoints post-operatively. Red box indicates that not all patients received adjuvant therapy. Pre- and post-surgery saliva samples were also collected from all patients. **(B)** Formalin-fixed paraffin-embedded tissue from the surgical specimen was whole exome sequenced to a median depth of 250x to identify patient-specific somatic variants for designing personalized RaDaR assays and profiling plasma and saliva samples for evidence of molecular residual disease and recurrence. WES variants were verified by deep sequencing of tumor tissue DNA and matched buffy coat DNA to identify confounding CHIP mutations.

Table 1. Patient Demographics (*N*=46)

Males **Females**	38 (82.6%) 8 (17.4%)
Median age (years) at diagnosis (range)	64.5 (35-83)
Stage	
I	6 (13%)
II	5 (11%)
III	19 (41%)
IV	16 (35%)
Tumor Characteristics – Location (*N*=46)*	
Oral Cavity	12 (26%)
Oropharynx p-16 positive p-16 negative	7 (15%) 1 6
Larynx	20 (44%)
Hypopharynx	7 (15%)

* Three patients had a second primary tumor (oral cavity, oropharynx and kidney) and one patient a lung metastasis. These are not included in the above table.

RESULTS

Table 2. Assay Characteristics

Panel Design	50 panels targeting 17-60 variants (median: 48)		
Samples Profiled Plasma Saliva	 348 (from 46 patients) 24 (from 8 patients)		
Detection		Baseline (Pre-op)	Post-op*
	Plasma	39/45 (87%)	11/46 (24%)
	Saliva	7/8 (87.5%)	3/6 (50%)
Plasma Detection Levels	Median eVAF: 0.051% (range: 0.0005% - 18.4%) eVAF <0.01% in 30% of ctDNA positive samples		
Saliva Detection Levels	Median eVAF: 0.1038% (range: 0.001% - 11.0263%)		

* Post-op is defined as 2 days to 12 weeks from surgery and prior to any adjuvant treatment

Longitudinal monitoring for residual disease and recurrence

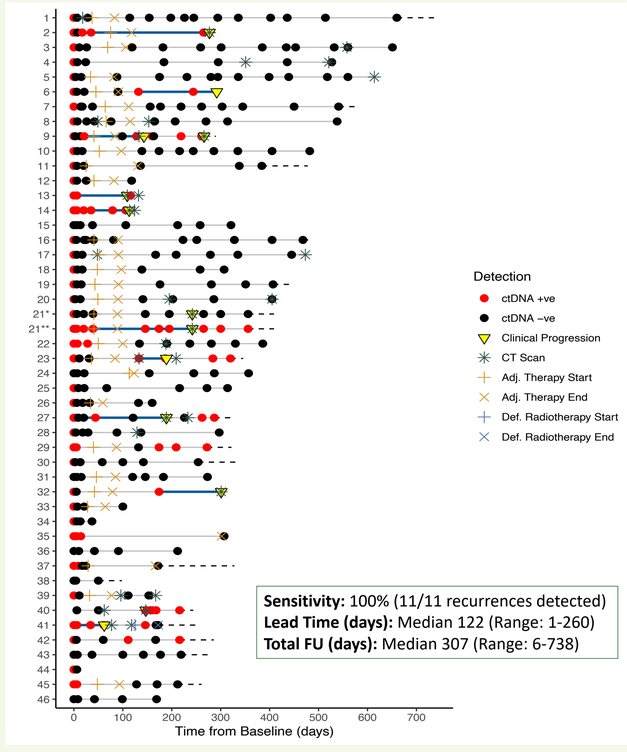
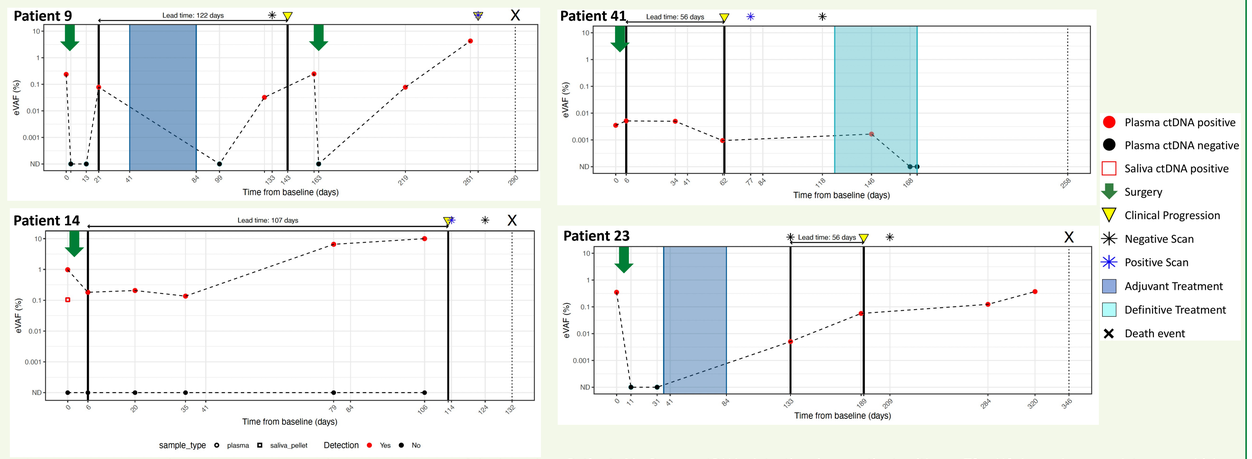


Figure 2. Longitudinal monitoring of serial plasma samples from 46 patients, indicating when ctDNA was detected and whether the patient subsequently relapsed. A dashed line indicates length of follow-up to date. A blue line indicates lead time from ctDNA detection to confirmed clinical recurrence. Patient 21 had two pT3 synchronous tumors detected, one in the hypopharynx (*) and a second in the oropharynx (**).

Personalized ctDNA assays for detecting MRD and recurrence

Figure 3. Representative examples of longitudinal ctDNA monitoring



Patient 9: Pre-op ctDNA detection in a patient with a pT3 pN0 tumor of the lateral tongue. ctDNA was detected at day 21 (17 days from surgery), 122 days ahead of histological confirmation of disease relapse. The patient eventually developed a second recurrence.

Patient 14: Patient with a local recurrence on a background of a previous pT2 floor of the mouth tumor that had been treated surgically and with adjuvant radiotherapy, and a synchronous hypopharyngeal tumor (pT4a pN0). Plasma ctDNA from the hypopharyngeal tumor was detected at all timepoints with a lead time of 107 days ahead of clinical progression. Pre-op ctDNA from the tumor in the floor of the mouth (pT1) was detected only in the saliva sample while plasma was negative for all timepoints.

Patient 41: Pre-op ctDNA detection in a patient with a pT2 pN0 hypopharyngeal tumor, which remained detectable post-operatively until histological confirmation of a local recurrence (cT3 cN0 cM0), with a lead time of 56 days ahead of clinical progression. At the end of definitive radiotherapy treatment, ctDNA levels were undetectable.

Patient 23: Patient with a pT4a pN2b oropharyngeal tumor. ctDNA was detected before surgery and again following completion of adjuvant treatment. Lead time for detection of molecular relapse was 56 days ahead of clinical confirmation of recurrence. CT scans performed at time points close to the plasma collections positive for ctDNA were negative.

Plasma vs. Saliva ctDNA profiling

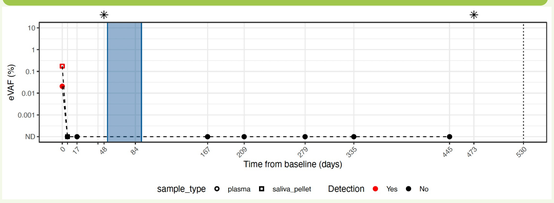


Figure 4. Patient with a pT1 pN2b oropharyngeal tumor showing positive ctDNA detection prior to surgery in both plasma (0.0208% eVAF) and saliva (0.175% eVAF). Following surgery, ctDNA clearance was observed in both plasma and saliva and remained undetected for all subsequent plasma timepoints analyzed.

CONCLUSION

The use of ctDNA as a biomarker in this HNSCC patient cohort has significant potential to guide treatment decisions and improve disease outcome. In future, ctDNA analysis with subsequent ctDNA-guided treatment may reduce morbidity for HNSCC patients.

Post-surgery ctDNA detection is associated with shorter relapse-free survival

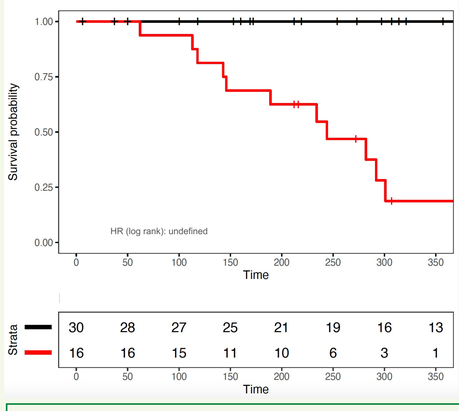


Figure 5. Survival analysis in 46 patients. Patients were stratified according to ctDNA detection at any time point post-surgery (red curve) or no ctDNA detection (black curve). There was a significant difference (p-value = 2e-7 (Mantel-Haenszel)) in recurrence rates between patients who tested ctDNA positive post-surgery and those that did not.

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