Liquid biopsy for detection of molecular residual disease and recurrence in head and neck squamous cell carcinoma

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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 locoregional recurrences.
- Detection of cell-free circulating tumour 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 personalised treatment strategies.
- Here, we use the RaDaR® assay to detect ctDNA in pre- and post-operative plasma (range 2-14, median 7) and saliva samples from 52 patients in the LIONESS study (**Table 1**).

METHODS

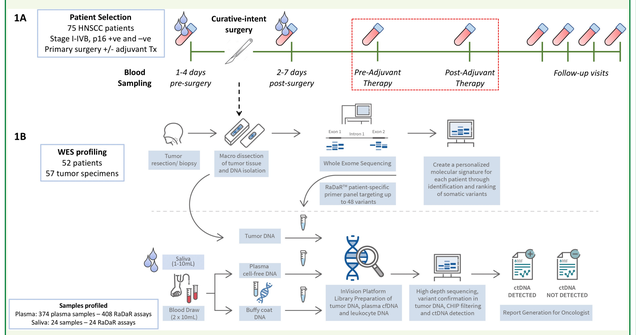


Figure 1. LIONESS study design and RaDaR workflow. (A) Blood samples were collected before and after curative-intent surgery and at different timepoints post-operatively. The red box indicates that not all patients received adjuvant therapy. Pre- and post-surgery saliva samples were also collected from 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 personalised RaDaR assays and profiling plasma and saliva samples for evidence of molecular residual disease and recurrence. WES variants were verified by deep sequencing of tumour tissue DNA and matched buffy coat DNA to identify confounding CHIP mutations.

Table 1. Patient Demographics (*N*=52)	
Males **Females**	43 (83%) 9 (17%)
Median age (years) at diagnosis (range)	64 (35-83)
Stage	
I II III IV	6 (12%) 8 (15%) 20 (38%) 18 (35%)
pT status	
T1 T2 T3 T4	8 (15%) 12 (23%) 24 (47%) 8 (15%)
Tumour Characteristics – Location (*N*=52)*	
Oral Cavity	15 (29%)
Oropharynx p-16 positive p-16 negative	6 (12%) 1 5
Larynx	21 (40%)
Hypopharynx	10 (19%)

* Three patients had two primary tumours (oral cavity, oropharynx and kidney). One patient had a lung metastasis biopsy and a second one had a diagnostic biopsy specimen profiled in addition to the primary resection specimens. These are not included in the above table.

RESULTS

Table 2. Assay Characteristics			
Panel Design	57 panels targeting 17-60 variants (median: 48)		
Samples Profiled Plasma Saliva	374 unique samples from 52 patients 24 unique samples from 8 patients		
Detection		Baseline (Pre-op)	Post-op*
	Plasma	45/52 (87%)	11/52 (21%)
	Saliva	7/8 (87.5%)	3/6 (50%)
Plasma Detection Levels	Median eVAF: 0.047% (range: 0.0005% - 18.4%) eVAF <0.01% in 31% 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.
eVAF, estimated variant allele frequency.

Longitudinal monitoring for residual disease and recurrence

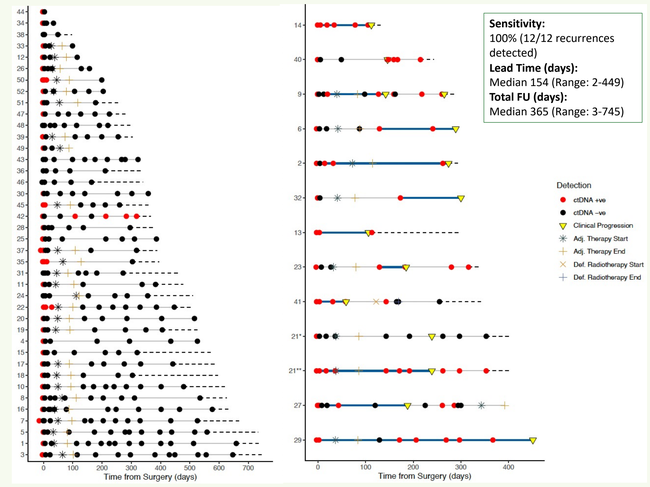


Figure 2. Longitudinal monitoring of post-operative serial plasma samples from 52 patients, indicating when ctDNA was detected and whether the patient subsequently relapsed. 40 patients did not show any evidence of clinical progression (**left**) while 12 patients had confirmed clinical progression (**right**). ctDNA was undetectable pre-operatively in 7 patients. 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 tumours detected, one in the hypopharynx (*) and a second in the oropharynx (**).

Correlation of tumour volume with pre-op ctDNA eVAF

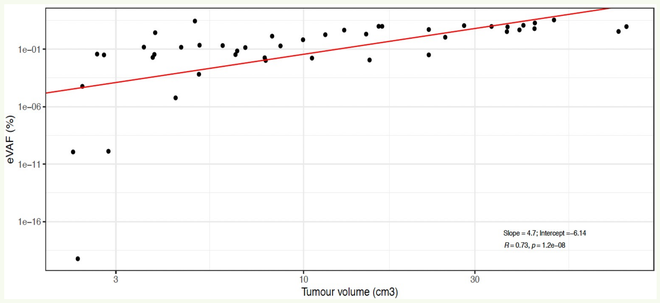


Figure 3. Tumour volumes were calculated from staging CT images and plotted against eVAF values from pre-operative ctDNA. A moderate to strong linear correlation was demonstrated between larger tumour volumes and higher eVAFs (Spearman correlation). Values are shown in a log10 scale.

Personalised ctDNA assays for detecting MRD and recurrence

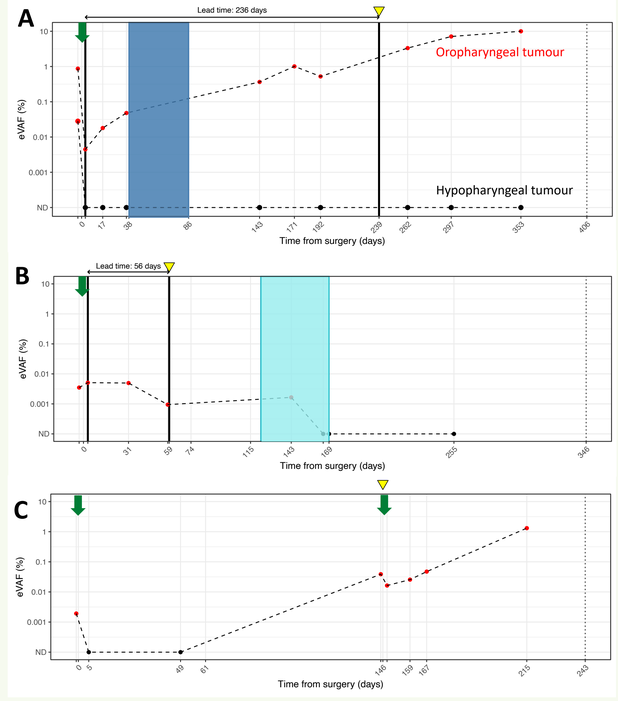


Figure 4. Representative examples of longitudinal ctDNA monitoring. A) ctDNA detected before surgical resection of two pT3 synchronous tumours of the hypopharynx and oropharynx, respectively. ctDNA levels remained undetectable for the hypopharyngeal tumour. ctDNA was detected at all timepoints for the oropharyngeal tumour, 236 days ahead of histological confirmation of liver metastases. **B)** Pre-op ctDNA detection in a patient with a pT2 pN0 hypopharyngeal tumour, 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 remained undetectable. **C)** Patient with a pT1 pN0 laryngeal cancer. ctDNA was detected prior to surgery and again shortly before clinical confirmation of a local recurrence (pT4 pN1). ctDNA remained detectable following a second surgical intervention, later confirmed as distant metastasis.

Design of personalised panels: Resection vs. diagnostic biopsy

- A pre-operative diagnostic biopsy and a primary resected tumour specimen from three patients (patient 37, **tumour 1;** patient 19, tumour 2; patient 28, tumour 3 **in Table 3**) were whole exome sequenced and two separate personalised RaDaR assays were designed in each case.
- Plasma ctDNA detection profiles and %eVAF detection levels were identical for both panels for patient 37. In patients 19 and 28, plasma samples were profiled using only the panels designed for the resected primary tumour – Plasma profiling on the diagnostic biopsy panel is pending
- Equally robust personalised RaDaR panels could be designed in all cases using a diagnostic biopsy, which could lead to faster clinical decisions.

Table 3. WES and panel variant overlap between diagnostic biopsy and primary resected tumour

Tumour	Tissue Specimen	WES Variants	WES variant overlap	Total panel variants	Panel variant overlap
1	Resection	25,333	31%	17	53%
	Diagnostic Biopsy	26,132		56	
2	Resection	25,316	88%	49	61%
	Diagnostic Biopsy	25,489		48	
3	Resection	25,781	95%	26	54%
	Diagnostic Biopsy	26,084		43	

Conflict of interest: Nothing to declare.

ctDNA detection in saliva

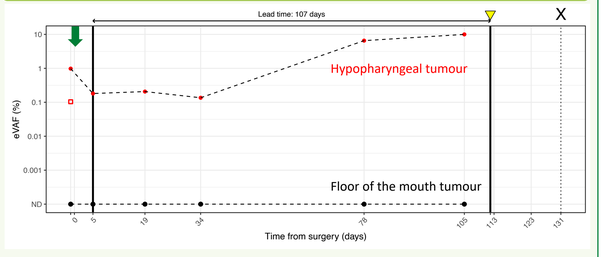
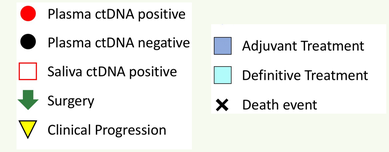


Figure 5. Patient with a local recurrence on a background of a previous pT2 floor of the mouth tumour that had been treated surgically and with adjuvant radiotherapy, and a synchronous hypopharyngeal tumour (pT4a pN0). Plasma ctDNA from the hypopharyngeal tumour was detected at all timepoints with a lead time of 107 days ahead of clinical progression. Pre-op ctDNA from the tumour in the floor of the mouth (pT1) was detected only in the saliva sample while plasma was negative for all timepoints.



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

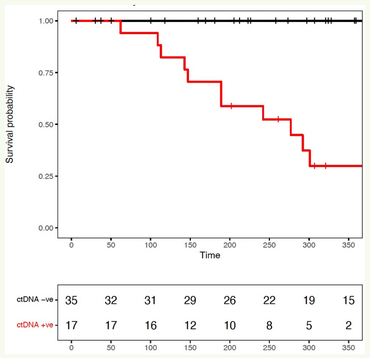


Figure 6. Survival analysis in 52 patients. Patients were stratified according to ctDNA detection at any time point post-surgery (red curve) or no ctDNA detection (black curve). There was a significant difference (p-value = 3e-7 (Mantel-Haenszel)) in recurrence rates between patients who tested ctDNA positive post-surgery and those that did not.

CONCLUSION

- This study illustrates the potential of ctDNA as a biomarker for monitoring for molecular residual disease following treatment with curative intent.
- Detection of ctDNA post-surgery was found to be associated with disease relapse with a median lead time of 154 days ahead of clinical confirmation.
- Earlier detection of disease relapse using ctDNA could indicate patient populations where earlier therapeutic intervention may be beneficial.
- In future, ctDNA analysis with subsequent ctDNA-guided treatment may improve disease outcomes and reduce morbidity for patients with HNSCC.

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