Personalized circulating tumor DNA analysis in head and neck squamous cell carcinoma: preliminary results of the Liquid BIOpsy for MiNimal RESidual DiSease Detection in Head and Neck Squamous Cell Carcinoma (LIONESS) study

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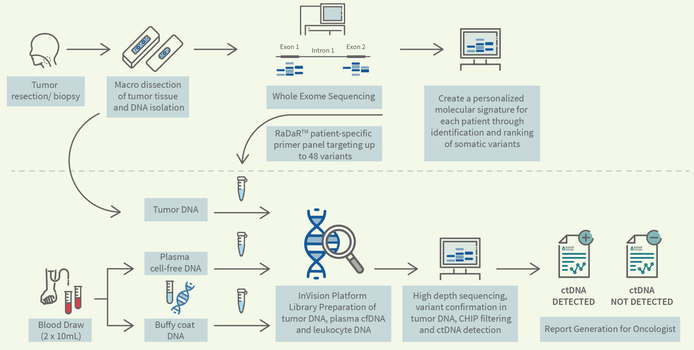
INTRODUCTION

- Head and neck squamous cell carcinoma (HNSCC) remains a substantial burden to global health. Despite evolving therapies, 5-year survival is <50% and unlike other cancers, reliable biomarkers to monitor treatment response do not exist.
- The detection of circulating tumor DNA (ctDNA) as a marker of minimal residual disease following curative-intent surgery holds promise for identifying patients at an increased risk of relapse, who may benefit from adjuvant radio(chemo)therapy or facilitate close monitoring with repeat resection if needed.
- Here, we use the RaDaR^{TM} assay to detect ctDNA in pre- and post-operative plasma samples (range 1-9, median 4) collected from the LIONESS study.

METHODS

- This is a single-center prospective experimental evidence-generating cohort study to assess ctDNA in patients with p16-negative HNSCC (stages I-IVB) who received primary surgical treatment with curative intent at the Hospital of the University of Munich, Germany.
- Plasma samples were collected 1-4 days pre-operatively (T0), 2-7 days post-operatively (T2), before start of adjuvant therapy (if any) and at follow-up visits (T3-T8). Whole exome sequencing was performed on formalin-fixed paraffin-embedded tumor tissue to a median depth of 250x.
- For each patient, up to 48 tumor-specific variants for RaDaR^{TM} assay design were selected to analyze serial plasma samples for evidence of minimal residual disease or recurrence. Variants were verified by deep sequencing of tumor tissue DNA and matched buffy coat DNA was sequenced to identify confounding CHIP mutations.

RaDaR^{TM} WORKFLOW



Acknowledgements

- We would like to thank the clinical patients and their families for participation in this study
- Inivata's Product Development, Computational Biology and UK Laboratory Clinical Operations teams

RESULTS

Patient demographics

Characteristics	Patients (n=11)
Stage	**Age, Median (Range)**
Stage III (n=5)	67 (59-78)
Stage IV (n=6)	60 (54-76)
Sex	
Male	9 (81.8%)
Female	2 (18.2%)

Tumor characteristics	
Location	
Oral cavity	4 (33.3%)
Oropharynx	1 (8.4%)
Larynx	4 (33.3%)
Hypopharynx	3 (25.0%)
Second primary tumor	1 (9.1%)

Table 1: Patient demographics of HNSCC patients enrolled in the LIONESS study to date.

Clinical sensitivity

- In a subset of 11 patients analyzed to date to evaluate the performance of RaDaR^{TM}, personalized panels were designed with between 40 and 52 somatic variants (median 48).

Figure 1: Box plots of estimated median variant allele frequency percentage (% VAF)

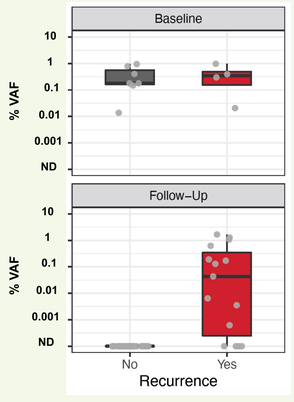


Figure 1: ctDNA levels in baseline samples taken prior to surgery ranged from 0.014% to 0.97% estimated variant allele frequency (% VAF) (top).
In post-surgery samples, ctDNA could be detected at levels as low as 0.0006% VAF, with levels below 0.01% VAF in 27% of ctDNA positive samples (bottom)

Figure 2: Longitudinal monitoring of serial plasma samples

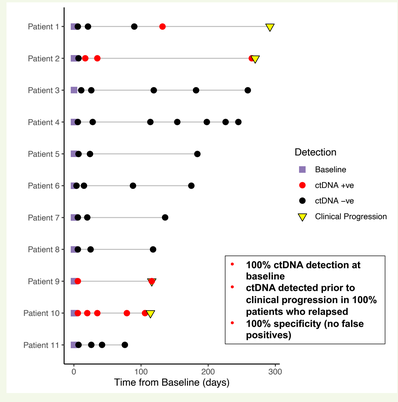


Figure 2: Longitudinal monitoring of serial plasma samples from 11 patients, indicating when ctDNA was detected and whether the patient subsequently relapsed.

In all cases with clinical recurrence to date (4/4), ctDNA was detected prior to clinical progression, with lead times ranging from 108 to 248 days (median 136 days).

Longitudinal monitoring for residual disease and recurrence

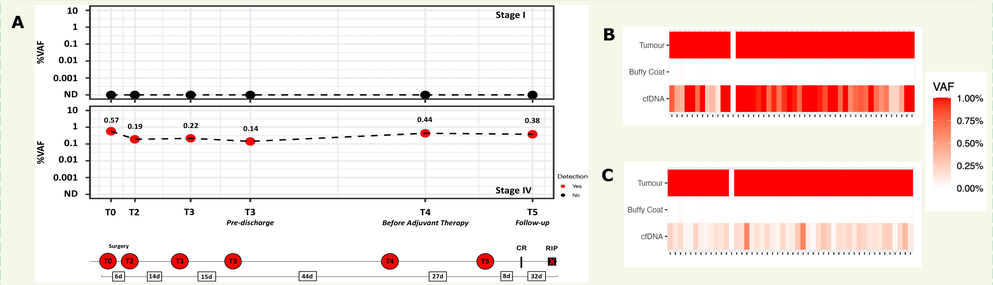


Figure 3: (**A**) Longitudinal monitoring of ctDNA taken from plasma pre-operatively (T0) and post-operatively (T2-T5) from a patient with two primary tumors (Stage I and stage IV SCCs located in the floor of the mouth and larynx respectively). Post-operative detection of ctDNA was observed 108 days ahead of clinical recurrence (CR). **(B, C)** heat maps, each column representing a different variant, each row a different sample type. **(B)** baseline/pre-op showing ctDNA detection at 0.57% VAF; **(C)** post-op ctDNA analysis showing residual disease, with detection of variants in this personalized panel (0.19% VAF).

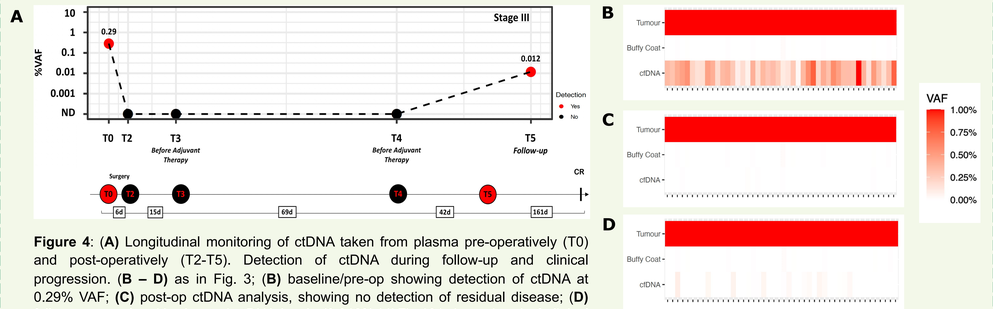


Figure 4: (**A**) Longitudinal monitoring of ctDNA taken from plasma pre-operatively (T0) and post-operatively (T2-T5). Detection of ctDNA during follow-up and clinical progression. (**B – D**) as in Fig. 3; (**B**) baseline/pre-op showing detection of ctDNA at 0.29% VAF; **(C)** post-op ctDNA analysis, showing no detection of residual disease; (**D**) follow-up sample with elevated ctDNA levels (0.012% VAF), 161 days ahead of clinical recurrence (CR).

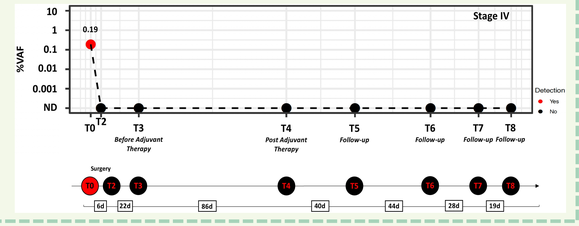


Figure 5: Longitudinal monitoring of ctDNA from a patient where no clinical progression was observed. Plasma was taken at various time points, including pre-operatively (T0) and post-operatively (T2-T8).

CONCLUSION

This study illustrates the potential of ctDNA as a biomarker of recurrence in HNSCC, demonstrating the feasibility of personalized ctDNA assays for the detection of minimal residual disease post-treatment. In this cohort, ctDNA was detected with 100% specificity and 100% sensitivity for patients who subsequently relapsed, with lead times ahead of clinical recurrence ranging from 108-248 days. Early detection of relapse using ctDNA could indicate patient populations where earlier therapeutic intervention may be beneficial.

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