Personalised circulating cell-free tumour DNA analysis for detection of minimal residual disease and recurrence in patients with head and neck squamous cell carcinoma

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INTRODUCTION

- Head and neck squamous cell carcinoma (HNSCC) remains a substantial burden to global health with 5-year survival of <50%. Despite improvements in treatments for HNSCC, many patients develop recurrences.
- Circulating cell-free tumour DNA (ctDNA) is a recently identified biomarker available from blood samples which remains largely uncharacterised in the context of surgical treatment of patients with HNSCC. The detection of 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™ assay (Figure 1) 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-centre 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
- A total of 131 plasma samples from 21 patients 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-T12). Whole exome sequencing (WES) was performed on formalin-fixed paraffin-embedded tissue obtained from 25 unique tumours to a median depth of 250x.
- For each patient tumour, up to 48 tumor-specific variants for RaDaR™ assay design were selected to analyse 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.

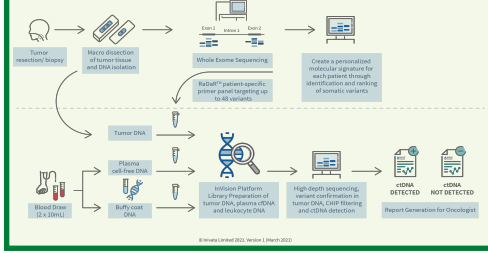

Figure 1. RaDaR Workflow

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Conflict of Interest
S.F., P.B., C.R., M.C., O.G. and C.W. declare no conflict of interest. K.H., S.H., C.P., P.E., K.M., and G.M. are employees and shareholders of Inivata Ltd (Cambridge, UK).

RESULTS

Patient demographics

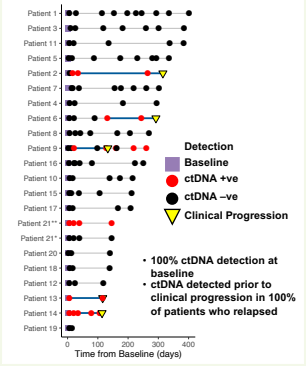
Table 1. Patient demographics of HNSCC patients enrolled in the LIONESS study to date.

Characteristics	Patients (n=21)
Stage	**Age, Median (Range)**
Stage III (n=9)	67 (51-78)
Stage IV (n=12)	62 (48-76)
Sex	
Male	17 (81%)
Female	4 (19%)

Tumour Characteristics	
Location	
Oral cavity	5 (22%)
Oropharynx	3 (13%)
Larynx	9 (39%)
Hypopharynx	6 (26%)
Second primary tumour	3 (14%)*

*One patient had a second primary tumour in the kidney.

Longitudinal monitoring



- **100% ctDNA detection at baseline**
- **ctDNA detected prior to clinical progression in 100% of patients who relapsed**

Figure 2. Longitudinal monitoring of 131 serial plasma samples from 21 patients, indicating when ctDNA was detected and whether the patient subsequently relapsed.

Patient 21 had two stage IVb synchronous tumours detected, one in the hypopharynx (*) and a second in the oropharynx (**). WES revealed that these tumours were molecularly distinct since only 7.8% of the detected variants were shared (hypopharynx, 299 somatic variants; oropharynx, 193 somatic variants). There was no overlap in the variants used in the RaDaR assays designed for these two tumours.

In all cases with clinical recurrence to date (5/5), ctDNA was detected prior to clinical progression, with lead times ranging from 108 to 298 days (shown in horizontal blue lines).

Personalised assays for detecting residual disease and recurrence

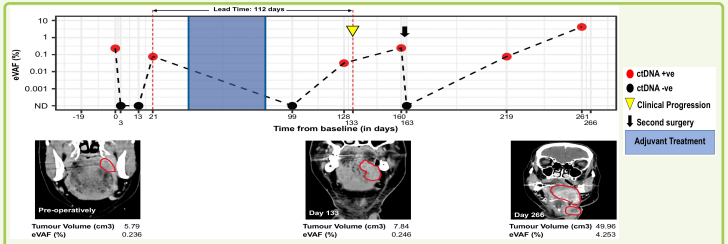


Figure 3. ctDNA detected before surgical resection of a stage III cancer of the lateral tongue, but not 3 and 13 days post-op. ctDNA detected 21 days post-op, which decreased after completion of adjuvant treatment only to rise again by day 128, prior to clinical progression. ctDNA levels were undetectable following a second surgical intervention at 160 days after the first surgery. They started to rise again 219 days after the first surgery prior to detection of a second recurrence, which was unresectable. Coronal CT images demonstrate a tumour mass in the lateral tongue pre-operatively, at the time of the first recurrence and at the time of the second recurrence. Tumour volume and estimated variant allele frequency (eVAF) are shown.

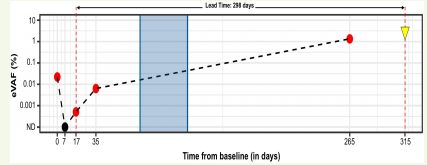


Figure 4. Detection of molecular recurrence in a stage IVb hypopharyngeal cancer patient with a lead time of 298 days ahead of confirmation of clinical recurrence reflected in the detection of metastatic disease being disseminated to the lung and bones. WES of the lung lesion indicated that 31% (53/172) of the detected variants were also present in the primary hypopharyngeal tumour. RaDaR panels designed for the two tumours shared 48% (20/42) of the priority variants in common and gave almost identical results when assessing ctDNA levels.

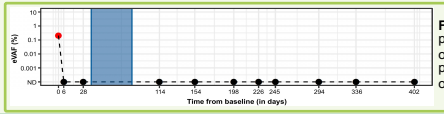


Figure 5. Longitudinal monitoring of ctDNA from a patient where no clinical progression was observed. Plasma was taken at various time points, including pre-operatively (day 0) and post-operatively (day 6 to day 402).

CONCLUSION

This study illustrates the potential of ctDNA as a biomarker for monitoring of minimal residual disease as well as recurrence in patients with HNSCC and demonstrates the feasibility of personalised ctDNA assays for detection of disease post-treatment and with consequences for further therapy planning. Early detection of relapse using ctDNA could indicate patient populations where earlier therapeutic intervention may be beneficial.

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