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WHITE PAPER

Molecular Residual Disease (MRD) Detection in HPV-Negative Head and Neck Cancer

Clinical Insights from the LIONESS Study

Head and neck squamous cell carcinoma (HNSCC) remains one of the most challenging malignancies in oncology, with early relapse and metastatic disease being primary drivers of poor outcomes. The LIONESS (Liquid bIOpsy for miNimal rESidual diSease detection in Head and Neck Squamous Cell Carcinoma) study represents a landmark prospective investigation into whether circulating tumor DNA (ctDNA) can identify patients at high risk of recurrence and detect relapse before clinical presentation.

Key clinical takeaway

In 76 surgically treated HNSCC patients, tumor-informed ctDNA testing detected recurrence in 91.3% of cases (21/23) with a median lead time of 119 days before clinical confirmation. In addition, postoperative ctDNA detection identified molecular residual disease that predicted treatment failure, potentially creating a window for early intervention.

Background and clinical context

The clinical challenge of HNSCC

Head and neck squamous cell carcinoma presents unique challenges that distinguish it from other solid tumors:

- **High recurrence rates:** Despite aggressive multimodal therapy, 30-50% of locally advanced HNSCC patients experience recurrence
- **Early relapse predominance:** Most recurrences occur within the first 2 years after treatment, when salvage options are most effective
- **Poor salvage outcomes:** Once recurrent disease is clinically apparent, treatment options are limited, and prognosis is poor

Limitations of current surveillance

Standard postoperative surveillance for HNSCC relies on:

- **Physical examination:** Limited by post-treatment tissue changes, fibrosis, and anatomic distortion
- **Cross-sectional imaging:** CT and MRI have difficulty distinguishing post-treatment changes from recurrent disease
- **PET/CT:** Useful but expensive, involves radiation exposure, and timing after treatment affects interpretation
- **Endoscopy:** Invasive, requires anesthesia, and primarily assesses mucosal surfaces while missing deeper recurrences

These modalities often detect recurrence only when tumors reach a size amenable to clinical or radiographic identification—potentially missing a critical window for intervention when disease burden is minimal.

The promise of molecular residual disease detection

Patient population

Molecular residual disease (MRD) represents microscopic cancer that persists after definitive treatment but remains undetectable by conventional methods. Its early detection could:

- Enable early intervention when disease burden is lowest
- Inform treatment response in the adjuvant setting
- Improve salvage treatment success rates through earlier detection

Study design and methods

Study overview

LIONESS was a single-center prospective cohort study conducted at the University Hospital, LMU Munich, designed to comprehensively evaluate the role of ctDNA in surgically treated patients with HNSCC. The study employed a tumor-informed approach using the RaDaR®* (Residual Disease and Recurrence) assay developed by NeoGenomics Laboratories.

Patient population and inclusion criteria

76 HNSCC patients receiving primary surgery with curative intent were enrolled. The cohort characteristics included:

The predominantly p16-negative (HPV-negative) cohort (93%) reflects traditional smoking- and alcohol-related HNSCC rather than HPV-associated oropharyngeal cancer, representing the population at highest risk for recurrence and poor outcomes.

Characteristic	Finding (N=76)
Stage I/II disease	37% (28/76)
Stage III/IV disease	63% (48/76)
p16-negative status	93% (71/76)
Total plasma samples collected	656 longitudinal samples
Median follow-up	29.5 months

The RaDaR ST assay: tumor-informed ctDNA detection

The study employed a sophisticated personalized approach to maximize sensitivity:

1. **Tumor tissue sequencing:** Whole-exome sequencing (WES) was performed on FFPE tumor tissue from surgical specimens.
2. **Personalized panel design:** Tumor-specific somatic variants were identified and used to create a customized assay for each patient.
3. **Serial sample collection:** Plasma and saliva samples were collected preoperatively, postoperatively, and during clinical follow-up.**
4. **High-sensitivity detection:** The RaDaR ST assay enabled ctDNA detection at extremely low variant allele fraction, as low as 3.3 ppm. This tumor-informed methodology provides superior sensitivity compared to tumor-naive approaches by tracking genomic variants known to be present in each patient's specific cancer.

Study objectives

The LIONESS study had three primary objectives:

- **Surgical clearance assessment:** Determine whether postoperative ctDNA detection can serve as a biomarker for incomplete surgical tumor clearance.
- **MRD detection:** Evaluate the ability of RaDaR ST to detect molecular residual disease after surgery.
- **Early recurrence detection:** Assess whether the presence of ctDNA can predict relapse prior to clinical confirmation.

* Data was generated by RaDaR assay. RaDaR ST has been compared to RaDaR across stages and tumor types, demonstrating 97% concordance and rendering the clinical performance of the assays equivalent.
** RaDaR ST is validated for use in plasma.

Key findings

Ultrasensitive ctDNA detection

The study demonstrated remarkable sensitivity in ctDNA detection:

- 91.3% clinical sensitivity in relapsed patients and detection as low as 3.3 ppm
- 35% of positive samples had ctDNA levels in the critical ultrasensitive region (eVAF: 100 ppm).

This extraordinary sensitivity is critical for HNSCC, where early detection of small-volume recurrent disease could make the difference between successful salvage therapy and incurable disease.

Exceptional performance in ctDNA monitoring

The primary clinical finding: ctDNA detection predicts clinical relapse with remarkable accuracy.

Metric	Value	Range	Clinical impact
Recurrences detected	**91.3%**	21 of 23 cases	Near-perfect sensitivity
Median lead time	**119 days**	14-500 days	4+ month intervention window

The 119-day (approximately 4 months) median lead time (up to 500 days) is clinically significant, providing a substantial window for potential intervention before tumors become clinically apparent.

Postoperative MRD detection and clinical outcomes

Among the 53 patients without clinically confirmed relapse during follow-up:

- 6 patients (11.7%) had detectable ctDNA immediately postoperatively, indicating MRD
- 4 of these 6 patients received adjuvant therapy, which resulted in persistent ctDNA clearance
- Of the remaining 2, one patient did not have a post-adjuvant sample. The other patient exhibited ctDNA clearance post CRT, followed by re-emergence >16 months later, yet remained relapse-free throughout a total follow-up of 45 months until study closure

For patients with clinically confirmed relapse

- In 11 of 23 patients (48%) with recurrence, ctDNA was detected within four weeks from surgery, when adjuvant therapy is still feasible.

Correlation with tumor characteristics

The study identified several important correlations between ctDNA levels and disease features:

Tumor volume correlation
Strong linear correlation between larger tumor volumes on staging CT and higher estimated variant allele frequency (eVAF). This validates ctDNA as a quantitative biomarker that reflects tumor burden.

Critical insight

Postoperative ctDNA detection identified patients at high risk even when imaging suggested complete resection. Adjuvant therapy appeared to successfully clear MRD in 4 patients who might otherwise have recurred, suggesting that ctDNA in the adjuvant setting can assess therapy response.

Pathologic stage and nodal involvement
Both factors showed a strong correlation with preoperative ctDNA shedding:

- **Pathologic tumor stage (pT):** Higher T stage associated with higher preoperative ctDNA levels
- **Lymph node involvement (pN):** Presence and extent of nodal disease correlated with ctDNA detection

Pattern of recurrence
A trend toward higher eVAF was observed in cases with:

- Regional recurrence (nodal relapse) versus local recurrence
- Distant metastases versus local relapse

Clinical implications for head and neck oncologists

Transforming postoperative risk stratification

The LIONESS study suggests ctDNA could revolutionize how we stratify surgical patients.

Current paradigm
Adjuvant therapy decisions are based on:

- Pathologic stage and surgical margins
- Extranodal extension (ENE)
- Lymphovascular invasion (LVI)
- Perineural invasion (PNI)

Potential future paradigm
ctDNA-guided approach could enable:

- **MRD-positive patients:** Consider treatment intensification even with favorable pathology
- **MRD-negative patients:** Potential de-escalation in borderline cases where toxicity is a concern, pending data showing de-escalation is safe
- **During adjuvant therapy:** Serial monitoring to assess treatment response, and potentially inform next steps

Surveillance strategy implications

The 119-day median lead time (14-500 days) creates opportunities to reimagine surveillance:

For ctDNA-negative patients

- Potentially reduce the frequency of invasive endoscopic examinations, once data supports that surveillance de-escalation is safe
- Provide a level of reassurance in addition to a patient's standard of care surveillance imaging
- Reduce healthcare costs and patient burden

For ctDNA-positive patients

- Intensify surveillance with more frequent clinical and radiographic assessment
- Optimize timing of salvage interventions by prompting earlier detection while disease burden is lower

Conclusions

The LIONESS study represents a significant advance in our understanding of molecular residual disease in head and neck squamous cell carcinoma. By demonstrating 91.3% sensitivity for recurrence detection with a median 119 days/ ~4 months lead time, this work establishes that tumor-informed ctDNA analysis can identify disease progression before conventional surveillance methods.

Additionally, the detection of postoperative ctDNA, indicative of molecular residual disease, can identify patients at high risk of relapse, providing treatment response information to inform the adjuvant and surveillance conversations.

Glossary of terms

ctDNA – Circulating tumor DNA – fragments of DNA released by cancer cells into the bloodstream or other body fluids

MRD – Molecular Residual Disease – microscopic cancer that persists after treatment but is undetectable by conventional clinical or imaging methods

RaDaR ST assay – Residual Disease and Recurrence assay – a personalized tumor-informed liquid biopsy test that tracks patient-specific somatic mutations identified through tumor sequencing

Tumor-informed assay – A ctDNA test that uses information from sequencing of the patient's tumor to identify specific mutations to track in blood or other biofluids

eVAF – Estimated Variant Allele Frequency – the proportion of circulating DNA molecules containing a specific tumor mutation; a quantitative measure of ctDNA burden

WES – Whole-Exome Sequencing – sequencing of all protein-coding regions of the genome to comprehensively identify somatic mutations in tumor tissue

Lead time – The interval between detection of ctDNA and clinical confirmation of recurrence through imaging or biopsy

ENE – Extranodal Extension – spread of tumor through the capsule of involved lymph nodes into surrounding soft tissue; a high-risk feature requiring adjuvant therapy

HNSCC – Head and Neck Squamous Cell Carcinoma – cancers arising from the mucosal surfaces of the head and neck region, including the oral cavity, oropharynx, larynx, and hypopharynx

p16 – A protein marker used as a surrogate for HPV-associated oropharyngeal cancer; p16-negative tumors are typically smoking/alcohol-related with worse prognosis

Patient communication strategies

When discussing the potential role of ctDNA monitoring:

- **Set appropriate expectations:** "Blood tests that look for cancer DNA are being studied but are not yet proven to help patients live longer or better."
- **Explain research context:** "Studies like LIONESS show these tests can detect cancer earlier, but we don't yet know if treating earlier actually helps."
- **Discuss clinical trials:** "We can explore whether you might be eligible for trials studying these new tests."
- **Emphasize standard surveillance:** "For now, the most important things are regular check-ups, examinations, and imaging as recommended."
- **Address anxiety:** "We are making progress in detecting cancer earlier, and this research is very promising for the future."

Patients should be made aware that one negative MRD test does NOT indicate their cancer will not recur. Additional monitoring is needed.

Take-home message

The LIONESS study proves that ctDNA can detect HNSCC recurrence months before clinical manifestation. The next critical step is determining whether this early detection window can be exploited to improve patient outcomes through timely intervention, but the future is promising, and physicians should prepare for a potential paradigm shift in postoperative management and surveillance.

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