Postoperative lymphatic exudate in HPV-negative head and neck cancer detects recurrence prior to adjuvant treatment selection

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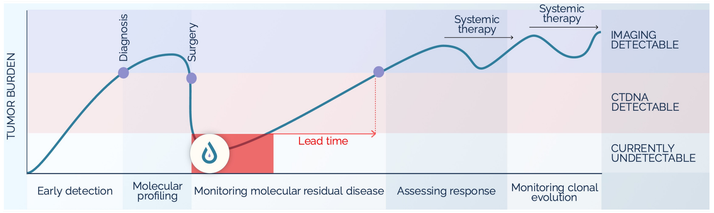
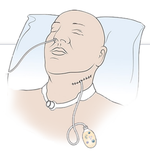
Introduction

Recurrence after surgery remains a major cause of failure in head and neck squamous cell carcinoma (HNSCC), particularly for HPV-negative patients whose 2-year failure rate is > 50%. There is unmet need for an accurate diagnostic test that predicts risk of recurrence prior to adjuvant therapy selection. We evaluated ctDNA in lymphatic exudate ("lymph") collected via surgical drains at 24 hours after surgery to detect molecular residual disease (MRD) and compared its performance to plasma and adverse pathology features. Using a tumor-informed, ultra-deep sequencing approach, we demonstrate that lymph ctDNA is more prognostic of recurrence than clinicopathologic features alone and superior to plasma ctDNA from the same timepoint. Our approach overcomes the major limitations of plasma-based MRD assays by measuring proximally to the site of tumor early enough to make adjuvant therapy decisions.

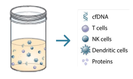


Methods and Materials



Drains are routinely placed after tumor resection to prevent fluid build-up and speed healing[1].

Droplet provides an ambient-stable collection kit. Drains are emptied into tubes and shipped overnight.



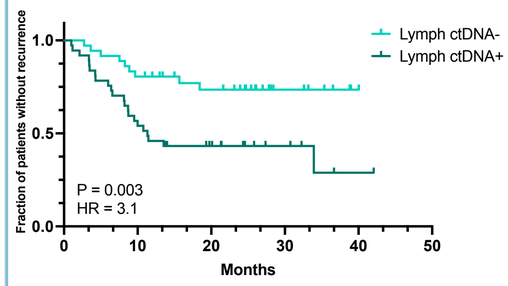
Droplet processes using proprietary extraction to yield DNA, proteins, and viable cells.

DNA is sequenced using a tumor-informed, custom 699 gene panel and results are reported.

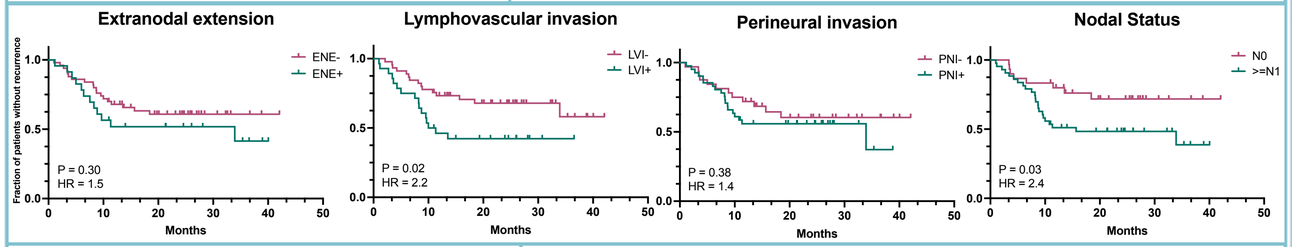
Lymph, plasma and blood were collected from 76 HPV- HNSCC patients 24 hours after surgery. Tumor tissue was collected at surgery. 73 patients passed QC. Samples were sequenced using a 699-gene custom panel to a median of 6322x (lymph and plasma cfDNA) or 533x (tumor and blood genomic DNA). Patient-specific somatic mutations detected in tumor were directly genotyped in lymph and plasma. A base-specific error model (BEM) was estimated at each somatic mutation position to quantify the background noise for single nucleotide variants (SNVs). MRD status was determined using a calibrated cutoff based on mean variant allele fraction after applying a non-linear correction for coverage and mutation load variation. Wilcoxon signed-rank test was used for group comparisons. The Kaplan-Meier estimator with log-rank test and Cox proportional hazards model were used for survival analysis.

Results

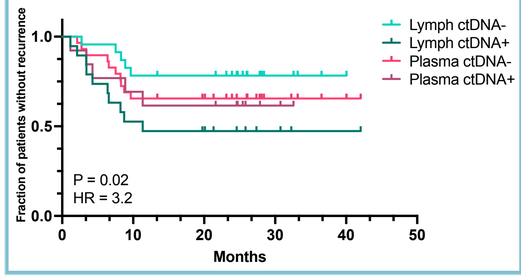
Lymph ctDNA



We previously demonstrated that lymph ctDNA detects recurrence in HPV(-) HNSCC across two independent multi-site cohorts. We pooled the cohorts into a single 73 patient group to better power subgroup analyses. In aggregate, lymph ctDNA in the pooled cohort demonstrated 71% sensitivity and 64% specificity to detect HNSCC recurrence (p = 0.003, HR = 3.1). When compared to pathology at this early time point, lymph ctDNA outperformed each for recurrence detection (extranodal extension (p = 0.30), lymphovascular invasion (p = 0.02), perineural invasion (p = 0.38), and positive lymph nodes (p = 0.03)).



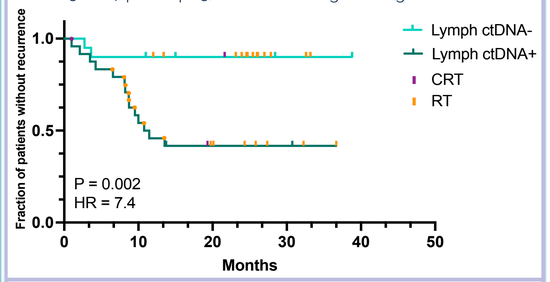
Lymph vs. Plasma ctDNA



Forty-two of the patients across the aggregate cohort had matched plasma drawn 24 hours after surgery. Applying the same ultra-sensitive NGS approach used for lymph, plasma was not predictive of MRD at this timepoint (sensitivity = 33%, specificity = 70%; p = 0.8, HR = 1.1; positive predictive value (PPV) = 38%, negative predictive value (NPV) = 66%). Lymph correctly identified MRD twice as often in the matched cohort (sensitivity = 67%, specificity = 67%; p = 0.02, HR = 3.2; PPV = 53%, NPV = 78%). Plasma ctDNA performed worse in locoregional disease (n = 34, sensitivity = 29%, specificity = 70%; p = 0.95, HR = 0.95), as has been previously reported.

RT + "consider systemic" cohort (n = 44)

*T3 or T4, pN2 or pN3, LVI+ or PNI+. Neg for margins and ENE.



We next evaluated lymph ctDNA in a critical subpopulation: patients considered intermediate risk by pathology (negative for ENE or margins but with one or more other adverse pathologic feature). Current NCCN guidelines recommend radiotherapy and to "consider systemic therapy" for these patients. Lymph ctDNA accurately stratified recurrence in this group with high sensitivity (SN = 88%, SP = 64%; P = 0.002, HR = 7.4, n = 44). Within this cohort, 100% of patients who received RT alone and later recurred were positive for lymph ctDNA at 24 hours, suggesting an opportunity to use molecular data in concert with pathology to identify patients who may benefit from adjuvant treatment intensification.

Conclusions

Postoperative lymph represents a novel proximal analyte for MRD detection in HPV- HNSCC designed specifically for use in the immediate post-surgical window when adjuvant therapy decisions must be made. Lymph ctDNA is significantly associated with recurrence and outperforms both time-matched plasma and pathology. Accurate MRD identification at this early timepoint has the potential to augment traditional pathology and personalize adjuvant treatment paradigms in HPV-negative HNSCC.

References

- [1]Earland N, Semenkovich NP, Ramirez RJ, et al. Sensitive MRD Detection from Lymphatic Fluid after Surgery in HPV-Associated Oropharyngeal Cancer. Clin Cancer Res. 2024;30(7):1409-1421.

Contact Information

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