ctDNA analysis of lymph detects minimal residual disease and correlates with recurrence in HPV-negative head and neck cancer patients

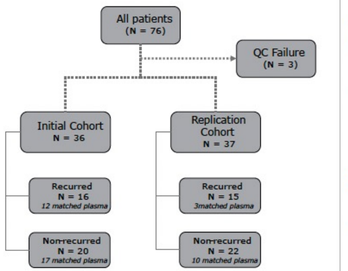
Wendy Winckler[1], Zhuosheng Gu[1], Seka Lazare[1], Adam Benson[1], Noah Earland[2], Marra S. Francis[1], Adam Harmon[1], Megan Long[1], Maciej Pacula[1], Ashley Tellis[1], Aadel A. Chaudhuri[3], Jose P. Zevallos[4]

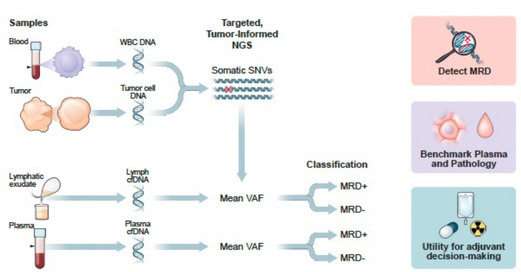
[1]Droplet Biosciences, Cambridge, MA [2]Washington University, St. Louis, MO
[3]Mayo Clinic, Rochester, MN [4]University of Pittsburgh Medical Center, Pittsburgh, PA

Introduction

Relapse is a major cause of failure after surgery in human papillomavirus (HPV) negative head and neck squamous cell carcinoma (HNSCC), with up to 50% of patients recurring within 2 years. Clinicopathologic criteria for adjuvant treatment are imprecise and have not changed for decades. Using an ultra-sensitive targeted sequencing approach, we demonstrate that circulating tumor DNA (ctDNA) in lymphatic exudate collected via surgical drains ("lymph") 24 hours after surgery identifies MRD and outperforms plasma in an initial cohort of 36 HNSCC patients. We then replicated the lymph ctDNA test in an independent, multi-site cohort of 37 HNSCC patients. Lymph performance was particularly enhanced in locoregional relapse in both cohorts and generalized to early-stage patients. Analysis of matched plasma collected at this early timepoint was not predictive of recurrence. We demonstrate a liquid biopsy approach using a historically overlooked biofluid to potentially enable precision adjuvant therapy and achieve superior oncologic outcomes. *(Abstract updated with additional data and analyses.)*

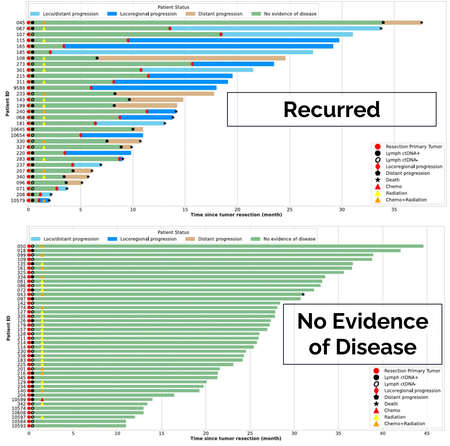
Methods and Materials





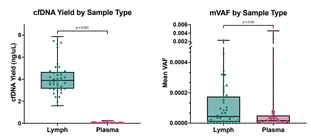
Lymph and blood were collected from 76 HPV-negative HNSCC patients postoperatively at 24 hours along with resected tumor and sequenced using a custom HNSCC-focused 6.3 Mb panel. Somatic mutations were identified by sequencing tumor and blood (>250x). Lymph and plasma were sequenced to >6,000x coverage. Patient-specific somatic variants detected in tumor were directly genotyped to examine those positions in lymph and plasma using a custom pipeline. One patient was excluded due to a blood sample mismatch and two patients were excluded because tumor samples failed sequencing, resulting in a total study population of 73 patients. Of the 73 patients, 36 patients were studied in an initial cohort and 37 were reserved for the replication cohort. Forty-two patients had matched plasma samples available (29 initial cohort,13 replication cohort). A sample was classified as positive if the mean ctDNA variant allele fraction (VAF) exceeded a calibrated cut-off accounting for tumor mutation burden and coverage. The Kaplan-Meier estimator with log-rank test and Cox proportional-hazards model were used for survival analyses. Logistic regression models were performed with 5-fold cross-validation.

Results



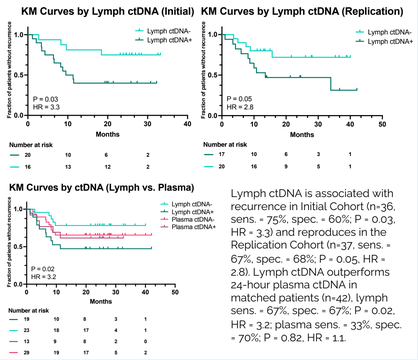
Swimmer plot of recurred (n=31) and non-recurred (n=42) patients describing lymph ctDNA status, treatment course, recurrence type and outcome.

Lymph is enriched in ctDNA



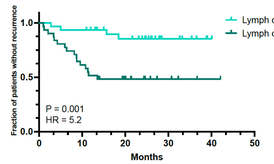
We observe significantly higher cfDNA content (>65x) in lymph compared to plasma. Mean VAF is increased >2x in lymph vs. plasma (n=42).

Lymph but not plasma ctDNA correlates with recurrence



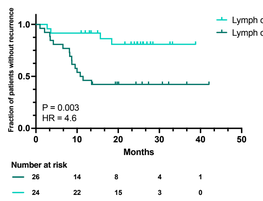
Lymph ctDNA is associated with recurrence in Initial Cohort (n=36, sens. = 75%, spec. = 60%; P = 0.03, HR = 3.3) and reproduces in the Replication Cohort (n=37, sens. = 67%, spec. = 68%; P = 0.05, HR = 2.8). Lymph ctDNA outperforms 24-hour plasma ctDNA in matched patients (n=42), lymph sens. = 67%, spec. = 67%; P = 0.02, HR = 3.2; plasma sens. = 33%, spec. = 70%; P = 0.82, HR = 1.1.

Locoregional recurrences only



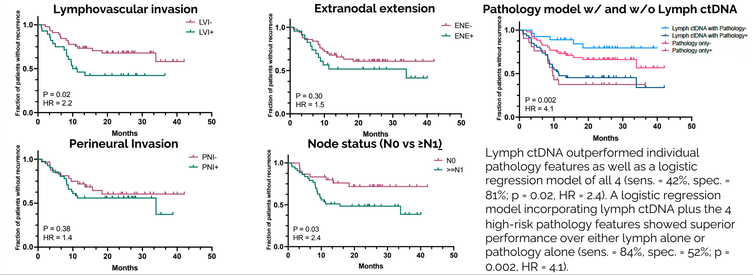
Performance was enhanced when the cohort was limited to locoregional relapse (n=62, sens. = 80%, spec. = 64%; P = 0.001, HR = 5.2).

ENE-negative patients

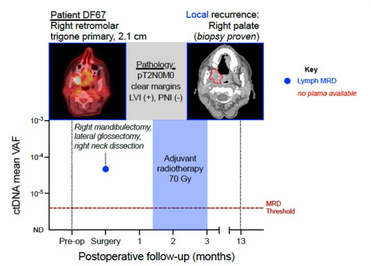


We observe strong performance in patients without extranodal extention (ENE), indicating low or intermediate pathologic risk (n=50, sens. = 79%, spec. = 65%, P = 0.003, HR = 4.6).

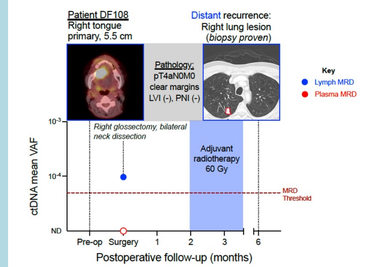
Pathology feature performance in this cohort



Lymph ctDNA outperformed individual pathology features as well as a logistic regression model of all 4 (sens. = 42%, spec. = 81%; p = 0.02, HR = 2.4). A logistic regression model incorporating lymph ctDNA plus the 4 high-risk pathology features showed superior performance over either lymph alone or pathology alone (sens. = 84%, spec. = 52%; p = 0.002, HR = 4.1).



Patient DF067 was T2N0M0 with LVI and received 70 Gy of adjuvant radiotherapy but no chemotherapy. Postoperative lymph was positive for ctDNA at 24 hours. Thirteen months later, DF067 suffered a locoregional recurrence in the right palate.



Patient DF108 was T4aN0M0 with no evidence of other high risk pathological features and received 60 Gy adjuvant RT but no chemotherapy. Lymph was positive for ctDNA while the 24-hour plasma sample was negative. Six months after surgery, the patient recurred distantly in the right lung.

Conclusions

- Post-surgical lymphatic fluid is a practical, differentiated proximal biofluid for ctDNA MRD detection 24 hours after head and neck cancer surgery
- Lymph ctDNA stratified survival outcomes at the 24h post-surgical timepoint while plasma ctDNA did not, and demonstrated enhanced performance in locoregional recurrence
- Lymph ctDNA significantly stratifies patients who lack high risk pathology features such as extranodal extension, outperforming individual pathology features and synergizing in a combination model
- Detection of lymph ctDNA 24 hours after head and neck cancer surgery could represent a precision biomarker for adjuvant decision-making
- Future studies will validate these findings in larger prospective multi-institutional settings and extend this approach to additional cancer types

Contact Information

- For more information, please contact: Wendy Winckler at wwinckler@dropletbiosci.com