Detection of minimal residual disease in post-surgical drain fluid synergizes with pathology to predict recurrence in HPV-negative head and neck cancer patients

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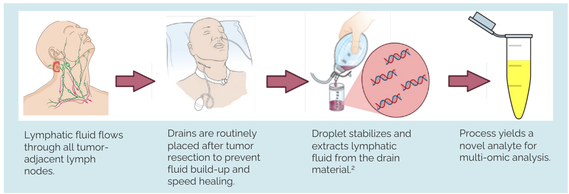
INTRODUCTION

Locoregional cancer relapse remains a major cause of failure in head and neck squamous cell carcinoma (HNSCC), particularly for HPV-negative patients whose 3-year locoregional failure rate is 32.5%.[1] There is major unmet need for an accurate diagnostic test that predicts risk of locoregional recurrence prior to adjuvant therapy selection.

AIM

We present a novel proximal assay ("Droplet") for minimal residual disease (MRD) profiled in lymphatic exudate collected via surgical drains ("lymph") and compare its performance to standard pathologic features for recurrence prediction.

METHOD



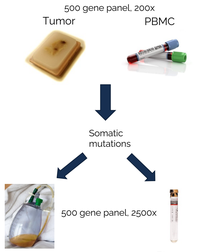
Lymphatic fluid flows through all tumor-adjacent lymph nodes.

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

Droplet stabilizes and extracts lymphatic fluid from the drain material.[2]

Process yields a novel analyte for multi-omic analysis.

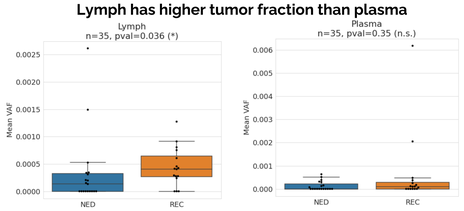
35 patients (HPV- H&N)



Lymph, plasma, and blood were collected from 46 HPV-negative HNSCC patients postoperatively at 24 hours along with resected tumor. Cell-free DNA was extracted from lymph and plasma and sequenced using the TruSeq Oncology 500 panel to a depth of >100 million reads at Droplet Biosciences. Somatic mutations were identified by exome sequencing (200x) tumor and blood. Nine patients had <2 somatic mutations in tumor and were excluded. Two patients were censored due to lack of clinical data, yielding 16 patients with disease recurrence (REC) and 19 with no evidence of disease (NED) with >1 year of follow-up. Two plasma samples were not available. Tumor-specific variants were force-called in lymph and plasma using a custom pipeline. Patients were considered MRD positive if the mean variant allele fraction (mVAF) was greater than 0.02% (the estimated limit of detection). Mann-Whitney U test was used for group comparisons. The Kaplan-Meier (KM) 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.

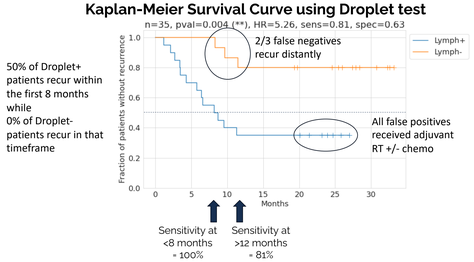
RESULTS

Lymph has higher tumor fraction than plasma



ctDNA levels were significantly higher in the lymph of REC patients compared to NED (median mVAF: REC = 0.041% ± 0.034%; NED = 0.013% ± 0.064%. p = 0.036), but not plasma (REC = 0.01% ± 0.15%; NED = 0% ± 0.02%. p = 0.53).

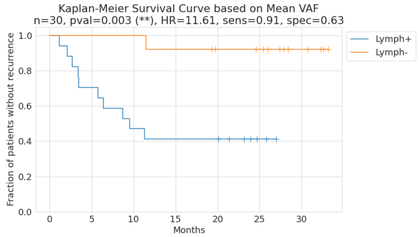
Kaplan-Meier Survival Curve using Droplet test



50% of Droplet+ patients recur within the first 8 months while 0% of Droplet- patients recur in that timeframe

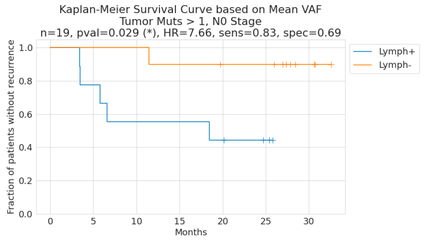
KM survival analyses showed lymph accurately predicts recurrence (sensitivity = 81%, specificity = 63%; p = 0.004, Hazard ratio (HR) = 5.6). Patients who have more ctDNA in lymph recur significantly more often and earlier than patients who have low or undetectable levels of lymph ctDNA.

Locoregional recurrences only



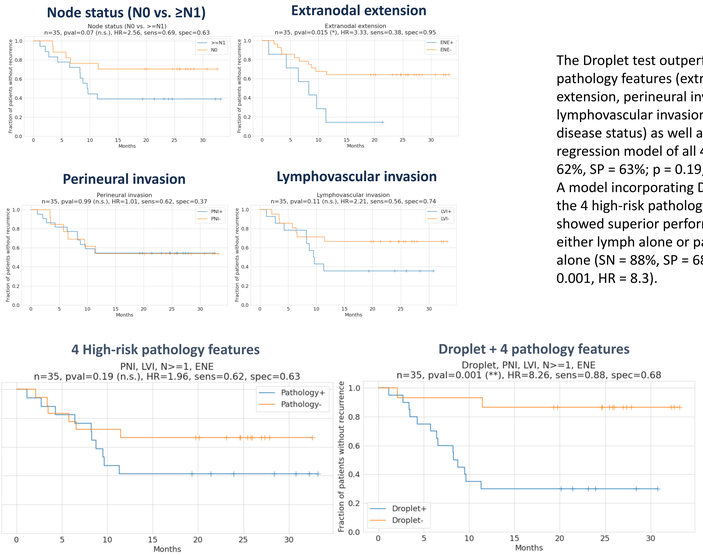
Performance was enhanced when the cohort was limited to locoregional relapse (Sensitivity = 91%, specificity = 63%; p = 0.003, HR = 11.6. N=30).

Node-negative



Performance is consistent when the cohort is limited to N0 patients (Sensitivity = 83%, specificity = 69%; p = 0.029, HR = 7.7. N=19).

Pathology feature performance in this cohort



Droplet synergizes with pathology, increasing sensitivity by 26% points when combined.

The Droplet test outperformed pathology features (extranodal extension, perineural invasion, lymphovascular invasion, and nodal disease status) as well as a logistic regression model of all 4 (SN = 62%, SP = 63%; p = 0.19, HR = 2.0). A model incorporating Droplet plus the 4 high-risk pathology features showed superior performance over either lymph alone or pathology alone (SN = 88%, SP = 68%; p = 0.001, HR = 8.3).

ID	Stage	TNM	Tumor Site	ENE	Other high risk path features	Adjuvant Tx	Recurrence
DF142	I	T1N0	Tongue	Neg	yes	none	No
DF165	II	T2N0	Buccal mucosa	Neg	no	none	Local
DF097	II	T2N0	Tongue	Neg	yes	none	No
DF115	IVa	T2N2c	Tongue	Neg	yes	RT	Local
DF157	III	T3N0	Tongue	Neg	yes	RT	No
DF086	III	T3N0	Buccal mucosa	Neg	yes	RT	No
DF081	IVa	T3N2b	Buccal mucosa	Neg	no	RT	No
DF114	IVa	T4aN0	Larynx	Neg	no	RT	No
DF064	IVa	T4aN0	Alveolar Ridge	Neg	yes	RT	Local
DF179	IVa	T4aN0	Tongue/FOM	Neg	yes	RT	No
DF220	IVa	T4aN0	Larynx	Neg	no	none	Local
DF072	IVa	T4aN1	Maxillary Alveolus	Neg	no	RT	No
DF071	IVa	T4aN2c	mandibular alveolar ridge/buccal mucosa & tongue	Neg	yes	none	Local/Distant
DF068	IVa	T4aN2c	Larynx	Neg	yes	RT	Local
DF181	IVb	T4aN3b	Mandibular alveolar ridge	Pos	yes	RT	Distant
DF208	IVb	T4aN3b	Mandibular alveolus	Pos	yes	none	Local/Distant
DF185	IVa	T4N2b	Tongue	Neg	yes	none	Local/Distant
DF108	IVa	T4aN0	Tongue	No	no	no	Distant
DF126	I	T1N0	Mandibular alveolus	No	yes	RT	No
DF127	III	T3N0	Tongue	No	yes	RT	No
DF129	IVa	T4aN0	Maxillary sinus	No	no	RT	No
DF140	IVa	T4aN2b	Larynx	No	yes	Chemo +RT	No
DF143	IVb	T3N3b	Tongue	Yes	yes	RT	Distant
DF183	IVa	T3N2b	Floor of Mouth	No	yes	RT	No
DF199	IVc	T4aN2c	Tongue	No	yes	RT	Distant
DF207	IVb	T4aN3b	Buccal mucosa	Yes	yes	Chemo +RT	Distant
DF211	II	T2N0	Tongue	No	yes	RT	No
DF214	IVa	T4aN0	Midline Maxillary alveolus	No	yes	RT	No
DF215	IVa	T4aN0	Mandibular Alveolar Ridge	No	no	RT	Local
DF216	IVb	T1N3b	mandibular alveolus/buccal mucosa	Yes	yes	Chemo +RT	No
DF225	IVa	T4aN1	Floor of Mouth	No	yes	RT	No
DF230	IVa	T4aN2b	Maxillary	No	yes	RT	No
DF233	IVb	T4aN3b	Tongue	Yes	yes	Chemo +RT	Distant
DF234	IVa	T4aN0	Tongue/Floor of mouth	No	yes	RT	No
DF240	IVb	T3N3b	Tongue	Yes	yes	Chemo +RT	Local

CONCLUSIONS

- Postoperative ctDNA analysis from surgical lymphatic fluid represents a novel MRD approach in HPV-negative HNSCC
- Lymph significantly outperforms plasma for prediction of recurrence, including patients with locoregional relapse and patients with N0 disease
- The Droplet assay gives superior prediction of recurrence than a multi-feature pathology model
- The observed synergy between lymph MRD testing and traditional pathology suggests that incorporating postoperative lymph analysis has the potential to:
  1. Augment traditional pathology
  2. Provide more personalized adjuvant treatment
- Validation in a large, prospective multi-institutional cohort of patients is ongoing

REFERENCES

[1]Ang KK, Zhang Q, Rosenthal DI, Nguyen-Tan PF, Sherman EJ, Weber RS, et al.. Randomized phase III trial of concurrent accelerated radiation plus cisplatin with or without cetuximab for stage III to IV head and neck carcinoma: RTOG 0522. *J Clin Oncol Off J Am Soc Clin Oncol* (2014) 32(27):2940–50. doi: 10.1200/JCO.2013.53.5633

[2]Earland N, Semenkovich NP, Ramirez RJ, Gerndt SP, Harris PK, Gu Z, Hearn AI, Inkman M, Szymanski JJ, Whitfield D, Wahle BM, Xu Z, Chen K, Alahi I, Ni G, Chen A, Winckler W, Zhang J, Chaudhuri AA, Zevallos JP. Sensitive MRD detection from lymphatic fluid after surgery in HPV-associated oropharyngeal cancer. Clin Cancer Res. 2023 Nov 8. doi: 10.1158/1078-0432.CCR-23-1789.

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