Abstract 6084

Evaluation of a tissue-free genome-wide methylome enrichment assay for detecting molecular residual disease (MRD) in patients with head and neck squamous cell carcinoma (HNSCC)

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BACKGROUND

- Squamous cell carcinomas comprise ~90% of head and neck cancers.[1]
- Although ~80% of patients with HNSCC are eligible for curative therapy, an estimated 20% to 35% will develop recurrence, typically within 2 years.[2–6]
- Recurrence is associated with poor survival, but early detection may enable salvage therapy.[3,6,7]
- We previously reported clinical validation of a tissue-free, genome-wide methylome enrichment MRD assay for recurrence detection in head and neck cancer.[8]
- Here we present performance of an updated classifier in a subset of patients with HNSCC and evaluate the effect of defining an indeterminate test category near the threshold.

STUDY DESIGN

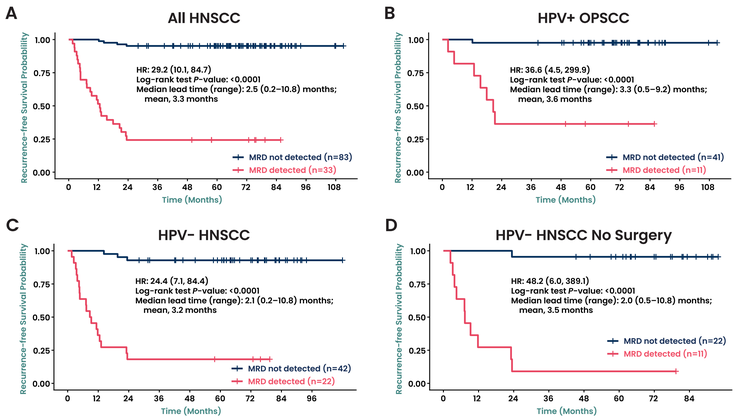
- Blood was collected ~3, 12, and 24 mo after curative-intent treatment from patients with stage I-IVb HNSCC. **(Table 1)**
- MRD testing was performed using an assay based on cell-free methylated DNA immunoprecipitation and high throughput sequencing (cfMeDIP-seq) as previously described.[8,9]
- First, an updated version of the previously validated classifier[8] was developed to enhance robustness for a commercial-ready test. The classifier was analyzed in a training set, followed by a pre-specified clinical validation analysis.
- Recurrence-free survival (RFS) was estimated using the Kaplan-Meier method, with differences assessed by the 2-sided log-rank test. Hazard ratios (HRs) were estimated using the Cox proportional hazards model.
- Next, to evaluate the effect of an indeterminate zone on classifier performance, a range encompassing 5% of training samples near the classification threshold was defined as indeterminate.
- Two sensitivity analyses with differing assumptions were used to estimate the potential range of effects of adding an indeterminate zone in the clinical validation set.
  - Case A: All indeterminate cases were assumed to be correctly resolved with subsequent testing.
  - Case B: Patients with any indeterminate samples were removed from all performance calculations.

RESULTS

The updated classifier demonstrated strong performance for recurrence detection in patients with HNSCC

- MRD detection using the updated classifier was highly prognostic for RFS in patients with HNSCC (HR, 29.2 [95% CI, 10.1–84.7]), independent of human papillomavirus (HPV) status. **(Figure 1)**
  - The classifier was also highly prognostic for RFS in patients with HPV-HNSCC without surgery (HR, 48.2 [6.0, 389.1]), a subgroup with high unmet need for tissue-free testing options.
- MRD detection was also associated with OS (HR, 60.2 [8.0-455.0]).
- Performance of the updated classifier **(Table 2)** did not significantly differ from that of the previously reported classifier.[7]

Figure 1. MRD positivity was predictive of RFS in patients with HNSCC (A), HPV+ oropharyngeal squamous cell carcinoma (OPSCC) (B), HPV- HNSCC (C), and HPV- HNSCC without surgery (D)



Among 117 patients in the full HNSCC validation set, median follow-up was 65.3 mo. The Kaplan-Meier (KM) method was used to compare RFS between groups using the 2-sided log-rank test. HRs were estimated using a Cox proportional hazards model. 1 patient with HPV- persistent disease was not included in HR calculations for panels A, C, and D.

Application of an indeterminate zone may enhance performance of the updated classifier, particularly in patients with HPV+ disease

- Addition of the indeterminate zone to the updated classifier improved association of MRD detection with RFS in patients with HNSCC (Case A: HR, 54.5 [95% CI, 18.4-161.2]; Case B: HR, 47.5 [15.2-148.6]), particularly among those with HPV+ OPSCC (Case A: HR, 100.8 [11.7-868.5]; Case B: HR, 73.4 [7.8-688.7]).
- Use of the indeterminate zone improved the association of MRD detection with OS (Case A: HR, 89.3 [11.8-676.4]; Case B: HR, 92.7 [11.9-725.0]).
- The indeterminate zone improved specificity and positive predictive value of the classifier in patients with HNSCC. **(Table 2)**

Table 1. Demographics and clinical characteristics

		Patients (N=117)
Age at diagnosis, median y (range)		62 (27, 88)
Sex, n (%)	Female	24 (21%)
	Male	93 (79%)
Race, n (%)	White	87 (74%)
	Non-white	15 (13%)
	Missing	15 (13%)
Smoking status, n (%)	Current	20 (17%)
	Former	50 (43%)
	Never	32 (27%)
	Missing	15 (13%)
Stage (AJCC, 8th Edition), n (%)	I	45 (38%)
	II	27 (23%)
	III	18 (15%)
	IV	27 (23%)
Treatment, n (%)	CRT	42 (36%)
	RT	41 (35%)
	Surgery	14 (12%)
	Surgery + CRT	8 (7%)
	Surgery + RT	12 (10%)
Cancer site/ HPV status, n (%)	Hypopharynx	3 (3%)
	Larynx	16 (14%)
	Oral cavity	27 (23%)
	Oropharynx	63 (54%)
	HPV+	52 (83%)
	HPV-	11 (17%)
	Unknown primary	4 (3%)
	Other	4 (3%)

AJCC, American Joint Committee on Cancer; CRT, chemoradiation therapy; HPV, human papilloma virus; RT, radiation therapy.

Table 2. Performance of genome-wide methylome enrichment assay with and without an indeterminate zone (IZ) for recurrence detection in patients with HNSCC (N=117)

Population	IZ*	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
All HNSCC (N=117; n=101 for Case B)	No	87% (70%, 95%)	91% (83%, 95%)	76% (60%, 88%)	95% (88%, 98%)
	A / B	87% / 80%	98% / 98%	93% / 89%	96% / 95%
HPV+ OPSCC (n=52; n=45 for Case B)	No	88% (53%, 98%)	91% (79%, 96%)	64% (35%, 85%)	98% (87%, 100%)
	A / B	88% / 80 %	100% / 100%	100% / 100%	98% / 98%
HPV- HNSCC (n=65; n=56 for Case B	No	86% (67%, 95%)	91% (78%, 96%)	83% (63%, 93%)	93% (81%, 98%)
	A / B	86% / 80%	95% / 95%	90% / 86%	93% / 93%
HPV- HNSCC no surgery (n=34; n=31 for Case B)	No	92% (65%, 99%)	95% (78%, 99%)	92% (65%, 99%)	95% (78%, 99%)
	A / B	92% / 89%	95% / 95%	92% / 89%	95% / 95%

**The performance of the classifier with the indeterminate zone was estimated using 2 sensitivity analyses with differing assumptions. In case A, all indeterminate cases were assumed to be correctly resolved with subsequent testing. In case B, all indeterminate cases were excluded.*

CONCLUSIONS

- Tissue-free, blood-based MRD testing using a genome-wide methylome enrichment platform is prognostic for recurrence in patients with HNSCC treated with curative intent, regardless of HPV status.
- This tissue-free approach offers expanded access for MRD testing in a broad population of patients with HNSCC, including those who did not undergo surgery and may not have tissue samples available for tumor-informed testing.
- Introduction of an indeterminate threshold may improve test performance, particularly in the group of patients with HPV+ OPSCC; further exploration in additional cohorts is warranted.
- This methylome-based testing platform offers a robust, sensitive, and minimally invasive approach for early detection of recurrence to assist with clinical decision-making for patients with HNSCC.

Limitations and Future Directions

- Results were based on a single-center study using retrospective analysis of prospectively collected samples.
- Future studies are needed to generate real-world data on indeterminate resolution and to understand the utility of longitudinal methylome profiling for clinical decision-making.

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