Association of Liquid Biopsy Biomarkers with Survival Outcomes in Oropharyngeal Squamous Cell Carcinoma

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Abstract

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Background: Oropharyngeal squamous cell carcinoma (OPSCC) is increasingly associated with human papillomavirus (HPV) infection and often presents at advanced stages, contributing to substantial morbidity and mortality. Liquid biopsy offers a promising, non-invasive approach for cancer detection and monitoring; however, the prognostic significance of many reported biomarkers remains unclear.

Objective: To systematically assess the prognostic relevance of liquid biopsy biomarkers in OPSCC using multi-omic data from The Cancer Genome Atlas (TCGA), and to evaluate their potential to complement established molecular markers.

Methods: A systematic literature search identified diagnostic liquid-based biomarkers across molecular modalities, including DNA mutations, DNA methylation, gene expression, and miRNA expression. Biomarkers were mapped to TCGA OPSCC datasets, and their associations with overall survival were evaluated using univariate and multivariate Cox proportional hazards models, adjusting for HPV status, *CDKN2A* expression, and *TP53*/*NOTCH1* mutations. Pathway enrichment analysis was conducted to explore biological relevance.

Results: From 1,544 curated gene symbols corresponding to liquid-based biomarkers, 524 were significantly associated with worse overall survival in OPSCC ($p < 0.05$). DNA methylation markers near MIR21, VMP1, and KCNC1 exhibited the strongest prognostic performance (C-index > 0.69). Pathway enrichment analysis revealed that prognostic biomarkers were involved in key processes including extracellular matrix (ECM) organization, epithelial-mesenchymal transition (EMT), and oncogenic signaling via MAPK, Notch, and PI3K pathways. Importantly, 1,180 biomarkers provided independent prognostic value beyond established markers.

Conclusion: This study demonstrates that a subset of liquid-based biomarkers holds strong prognostic value in OPSCC, independent of established molecular indicators. Integrating multi-omic biomarkers into liquid biopsy

strategies may enhance risk stratification, support personalized treatment decisions, and improve monitoring of disease progression in OPSCC. Prospective validation is warranted to translate these findings into clinical practice

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Background

Oropharyngeal squamous cell carcinoma (OPSCC) incidence is rising globally with human papillomavirus (HPV), particularly HPV-16 being a major contributor in the United States (US) and Europe.[1] The incidence of head and neck cancers including OPSCC is projected to rise by 30% annually by 2030.[2] In North America, HPV-16 accounts for 63% of OPSCC cases.[2] OPSCC now surpasses the incidence of HPV-related cervical cancer in women[3], and predominantly affects white men in the US which is considered the "epicenter" of this "viral epidemic".[4] From 2001 to 2017, a total of 260,182 cases of OPC and 111,291 related deaths were reported nationwide. The increasing incidence of regional-stage OPSCC and associated mortality, particularly among older men, underscores a pressing public health concern.[5]

OPSCC affects the posterior one-third of the tongue, tonsils, soft palate, uvula and pharyngeal wall, leading to substantial morbidity, mortality, and financial burden.[6] In 2025, an estimated 59,660 new cases of oral and oropharyngeal cancer will account for 2.9% of all new cancer diagnoses, with approximately 12,770 deaths representing 2.1% of all cancer-related deaths.[7] Early detection is critical, localized OPSCC has a five-year relative survival rate of 88.4%, yet only 26% of cases are detected at this stage.[7] Most are diagnosed after regional spread, where the 5-year relative survival rate is 62%, or after distant metastasis, where it further drops to 29%.[7] The prevailing presentation is an advanced-stage tumor, presenting as a lateral neck mass, with many cases exhibiting regional metastasis at the time of diagnosis.[8, 9] This delay in diagnosis contributes significantly to treatment failure and poor prognosis.[10]

Although HPV vaccination offers a highly effective preventive strategy against OPSCC, its benefits are primarily limited to younger birth cohorts born after 1990. This leaves older individuals at risk and without viable screening or early detection methods.[4] Unlike cervical cancer, where the Pap smear has revolutionized early detection[11], there is no equivalent screening tool for OPSCC. This is largely due to the anatomical and biological characteristics of the oropharynx: the primary target sites of HPV infection are deep, often not visible or inaccessible to quick or easy direct visual inspection such as the tonsillar crypts and base of tongue. Moreover, OPSCC lacks a well-defined precancerous stage, with malignant transformation often occurring abruptly.[12] Cytology and HPV testing in the oropharynx are hindered by limited sensitivity and specificity, as exfoliated malignant cells are scarce and often mixed with normal epithelial cells, making abnormal cells difficult to detect.[13] Additionally, the feasibility of population-wide screening is limited by low yield and high cost. These challenges make OPSCC a high priority for research and prevention strategies.[3]

Therefore, minimally invasive, sensitive screening methods are needed, particularly for high-risk individuals, including those with immune deficiencies, on immunosuppressive therapy, or with a history of OPSCC prone to recurrence or secondary development. The gold standard for OPSCC diagnosis is a biopsy followed by pathology review, which can be stressful, invasive, painful, costly, or not readily available in low-resourced areas. Less-invasive alternatives, particularly circulating biomarkers, may be one solution for developing screening tools.[10,14]

Biomarkers are measurable indicators of biological processes and offer immense potential to improve cancer screening, diagnosis, prognosis, and treatment monitoring.[15] An ideal biomarker

should be cost-effective, minimally invasive, highly sensitive and specific, and offer prognostic information. In head and neck cancers including OPSCCs, researchers are investigating biomarkers in accessible fluids such as saliva and blood–known as liquid-based biomarkers–to develop less invasive and more accurate diagnostic and prognostic tools.[10,16]

Existing tissue-based biomarkers provide important prognostic information in OPSCC. The most validated is p16 immunohistochemistry, a surrogate marker for HPV-associated tumors, which are strongly linked to improved survival outcomes.[17] Molecular confirmation using HPV DNA or RNA testing further refines prognostic stratification.[18] In contrast, TP53 mutations, commonly observed in HPV-negative OPSCC, are associated with worse survival.[19] EGFR overexpression has also been correlated with poor prognosis and resistance to radiation therapy, particularly in HPV-negative disease.[20] More recently, PD-L1 expression has emerged as a potential marker with prognostic and predictive relevance, though its role is still evolving.[21]

Although these tissue-based biomarkers from excised tumors are being used to assess prognosis in OPSCC, there are several critical limitations and clinical gaps that highlight the necessity of liquid-based approaches. First, tissue sampling is inherently invasive and represents a single snapshot of the tumor at the time of surgery, failing to capture dynamic tumor evolution and treatment-induced changes.[15] Liquid-based biomarkers such as circulating tumor DNA (ctDNA), methylation markers, and circulating miRNAs, offer a non-invasive, repeatable, and real-time window into tumor biology throughout the disease course.[15,22] This capacity enables dynamic risk stratification and early detection of relapse, which is particularly important in HPV-positive OPSCC, where most individuals have good initial responses but still experience variable long-term outcomes.[23] Moreover, studies have demonstrated that tumor heterogeneity—both spatial (within different regions of the tumor) and temporal (evolving during treatment and progression)—can limit the accuracy of tissue biomarkers.[24,25] Liquid-based biomarkers capture a more comprehensive, systemic profile of the tumor, including contributions from metastatic and subclonal populations, which might not be represented in a single tumor section.[26]

Liquid-based biomarkers identified for diagnostic purposes may result from background noise or non-tumor-related processes. Prioritizing the prognostic significance of these biomarkers could yield a more robust and clinically relevant strategy. Biomarkers that consistently correlate with disease progression, treatment response, or recurrence are more likely to be directly involved in tumorigenesis and, therefore, more reliable for clinical applications. By mapping these prognostic biomarkers to known tumorigenesis pathways, we can better understand their biological relevance and potential utility in clinical settings. This approach not only has the potential to enhance the specificity and sensitivity of liquid-based biomarkers for diagnosis but also paves the way for personalized treatment strategies based on real-time tumor dynamics.

Numerous studies have identified a range of liquid-based biomarkers often referred to as “liquid biopsy” for the detection of OPSCC. While certain molecular biomarkers such as HPV status, *TP53* and *NOTCH1*, or p16 *(CDKN2A)* mutations have demonstrated prognostic value[27,28,29] , the clinical significance of many other identified biomarkers has yet to be systematically evaluated. Comprehensive and independent analyses of these liquid-based biomarkers in OPSCC are still lacking, and it remains unclear whether these biomarkers can enhance or complement the prognostic accuracy of established molecular markers across different modalities.

Existing multi-omics datasets of The Cancer Genome Atlas (TCGA)[30] allow for assessing the prognostic value of known biomarkers in cancer. The TCGA project provides public access to clinical information, gene expression, protein expression, DNA mutation, and DNA methylation levels of different cancer types, and overall survival.

A systematic assessment of the prognostic value of OPSCC biomarkers could help us understand the limitations and advantages of each biomarker at different molecular modalities (e.g. gene expression, DNA methylation, and DNA mutation). Given the existing low sensitivity of liquid biopsy assays for detection of early-stage tumors[31], this study could also be informative for the development of future liquid biopsy assays that could combine the power of highly prognostic biomarkers from different molecular modalities to increase the usefulness of current liquid biopsy assays.

This study aims to address these gaps by (1) curating a comprehensive list of liquid-based biomarkers for OPSCC through a systematic literature review; (2) assessing the prognostic value of these biomarkers using multi-omic data from TCGA; (3) investigating the biological pathways associated with diagnostic and prognostic biomarkers; and (4) determining whether these biomarkers can complement key established prognostic markers in OPSCC. We hypothesize that a subset of diagnostic liquid biopsy biomarkers also holds independent prognostic significance and, when analyzed across multiple molecular modalities, could improve the overall predictive power for patient outcomes. Ultimately, this work aims to inform future development of more sensitive and prognostically robust liquid-based diagnostic assays for OPSCC, with the potential to improve early detection, prognosis, and personalized treatment strategies.

Methods

This study used publicly available, de-identified data; therefore, no Institutional Review Board (IRB) approval was required.

In this study, we conducted a comprehensive systematic search to identify peer-reviewed articles that utilized liquid biopsy for the detection of HNSCC or OPSCC published up to February 2025, using key words selected based on the study objective. Where applicable, database-specific subject headings (e.g., Medical Subject Headings [MeSH] in PubMed) were incorporated to enhance the sensitivity and specificity of the search. The literature search was performed with the help of a research librarian in PubMed, EMBASE, Web of Science, and the Cochrane Library. Relevant systematic reviews were also screened for additional references. A complete list of search terms is provided in Appendix 1.

Although the primary focus of our prognostic analysis is OPSCC, we included HNSCC in our search strategy to capture the OPSCC cases that were included in those studies. This broader inclusion aimed to increase the probability of identifying diagnostic biomarkers in HNSCCs that would potentially be found in TCGA under relevant OPSCC biomarkers. Based on the findings, we compiled a list of liquid biopsy-based diagnostic biomarkers relevant to OPSCCs.

Study Selection and Data Extraction

All search results were imported into Covidence, a systematic review management platform, and duplicate records were removed.[32] Titles and abstracts were screened by the primary author (HS). Full-text review was then conducted independently by two reviewers (HS and HG), with discrepancies resolved through discussion to ensure consistency in study selection.

Based on the results of the literature search, we curated a list of liquid biopsy biomarkers reported across the included studies. Two reviewers (HS and HG) extracted data using a standard data extraction form. For each study, the following information was collected: PubMed ID, publication year, study type, biomarker name and type (e.g., protein, gene, miRNA, DNA mutation), the associated cancer type; and the biofluid source (e.g., saliva, serum, plasma, or urine).

Inclusion and Exclusion Criteria

Inclusion criteria consisted of English-language, primary, peer-reviewed clinical research studies involving adult (≥18 years old) human subjects with primary OPSCC that reported liquid-based biomarkers from saliva, blood, serum, plasma, or urine. We included studies reporting proteogenomic biomarkers such as microRNA (miRNA), messenger RNA (mRNA), proteins, cell-free DNA (cfDNA), DNA methylation markers, and DNA mutations. Studies on HNSCC that included OPSCC in their patient cohorts were also included. For studies that specifically identified OPC-related biomarkers, only those biomarkers were catalogued. In cases where the study did not specify which biomarkers were associated with a specific cancer subtype, the biomarkers were included provisionally and were later cross-referenced with OPSCC-specific datasets in TCGA and excluded when not found.

Exclusion criteria included case reports, editorials, letters, conference abstracts, studies with a sample size of fewer than five OPSCC individuals, and those that exclusively reported viral or metabolomic biomarkers.

Data Retrieval from The Cancer Genome Atlas

To identify tissue-based biomarkers associated with OPSCC, we utilized data from TCGA, a comprehensive and publicly available resource that provides multi-omic and clinical data for various cancer types. We accessed the TCGA-HNSCC dataset through the UCSC Xena Browser data portal.[33] While liquid biopsy biomarkers offer a minimally invasive window into tumor biology, these markers ultimately derive from tumor tissue. Therefore, mapping liquid-based biomarkers to their tissue-based counterparts in TCGA is essential to validate their biological relevance and better understand the molecular underpinnings of OPSCC. TCGA dataset includes genomic data (DNA mutation, DNA methylation, gene expression, miRNA expression, etc.) and associated clinical information such as primary diagnosis, cancer stage, site of biopsy and overall survival. We focused our analysis on individuals with primary HNSCC tumors and identified OPSCC cases with tumor resections from regions typically associated with OPSCC based on anatomical annotations (including tongue, base of tongue, tonsil, oropharynx, pharynx, palate, supraglottic, and overlapping lesions of the lip, oral cavity, and pharynx).

TCGA gene expression datasets report RNA expression of each biomarker according to its Ensemble gene ID, expression of each miRNA according to miRBase identifiers[34], and annotation of methylation probes and DNA mutations with gene symbols. We used Biomart v113 GRCh38.p14 annotations to convert ID of biomarkers to gene symbols as well as Ensembl gene ID.[35]

Survival Analysis

TCGA provides overall survival data, including the number of days from diagnosis to last follow-up and survival status (alive or deceased). For individuals still alive at the time of last follow-up, survival time is considered right censored, meaning the exact duration of survival is unknown but exceeds the recorded follow-up time. To evaluate the prognostic value of biomarkers identified, we used univariate Cox proportional hazards models, which appropriately handle right-censored data. We assessed gene mutations, gene expression, miRNA expression, and DNA methylation levels in OPSCC patients, using TCGA data. For each biomarker, we calculated the hazard ratio (HR) and p-value, with the HR representing the change in risk associated with a unit increase in the biomarker level. A concordance index (C-index), measuring how well model predictions match actual survival outcomes, was calculated. The C-index ranges from 0 to 1, with values above 0.5 indicating better-than-random prediction.[36] The CRAN project R statistical programming language (v4.2.0) and `survival` package (v3.4.0) was used for fitting the univariate and multi-variate Cox-proportional hazard models.

In this analysis, we are calculating the p-value for a large number of biomarkers. As a result, we would expect that due to multiple hypothesis testing, some of these biomarkers may have a small p-value but lack a replicable association with prognosis. Under the null hypothesis for no

association between biomarkers and survival outcomes, we would expect the p-values to follow a uniform distribution. Therefore, we expected that the subset of our hypotheses with a significant outcome to have lower values compared to a uniform distribution. To assess this, we investigated the quantile-quantile (Q-Q) plot for $-\log_{10}$ of the observed p-values plotted against the same number of p-values samples from a uniform distribution. In a Q-Q plot, p-values with a significant deviation above the diagonal are expected to have a smaller false discovery rate, and therefore more likely to be replicated in other studies. While statistical methods such as the Benjamini-Hochberg and Bonferroni corrections are commonly used to control for multiple hypothesis testing, our focus was on assessing the overall distribution of p-values and deviations from uniformity, rather than controlling a specific false discovery rate or family-wise error rate. To achieve this, we utilized a Q-Q plot, which allowed us to visually assess deviations from the expected uniform distribution and to report how each individual diagnostic biomarker is associated with prognosis on its own.

To establish if a given biomarker provides any additional prognostic value compared to well-established molecular biomarkers of OPSCC, multi-variate coxPH models adjusting for HPV status (positive or negative), *TP53* somatic mutation (positive or negative), *NOTCH1* gene expression, and p16 *(CDKN2A)* gene expression were used. Multi-variate coxPH models report a p-value and a hazard ratio for each of the inputs to the model, indicating if each of these independent variables can provide additional prognostic information when combined with other biomarkers. In this analysis, if a biomarker has a hazard ratio > 1 and a significant p-value in such a multi-variate coxPH model, it provides additional prognostic value compared to the established molecular biomarkers of OPSCC.

Pathway Analysis

Based on the univariate CoxPH analysis, biomarkers that were significantly associated with poorer survival outcomes were identified. For gene mutation, gene expression, and miRNA expression data, each biomarker could be directly mapped to its corresponding gene. However, this direct mapping does not apply to DNA methylation probes. Therefore, for methylation-based biomarkers, the nearest gene to each probe using probe annotation files from the TCGA methylation dataset available via the UCSC Xena browser was assigned.

To identify the biological pathways associated with these biomarkers, the Reactome pathway knowledgebase was used.[37] The list of gene symbols corresponding to these biomarkers were uploaded to the database and the pathway enrichment results which indicate the p-value for enrichment of each biological pathway in our gene set were retrieved. If there are k genes from the biomarker list which are present in each pathway, the p-value for pathway enrichment analysis represents the probability of observing an overlap of at least k genes between the biomarker list and genes of a given pathway under random chance.

Results

A total of 1,350 studies were identified in the literature search. After removing 741 duplicates, 609 studies underwent title and abstract screening, of which 409 were excluded. The remaining 207 articles were assessed in full-text review, leading to the exclusion of 108 studies for reasons detailed in Figure 1. Ultimately, 99 studies were included for data extraction. This screening process is illustrated in the PRISMA flow diagram (Figure 1).

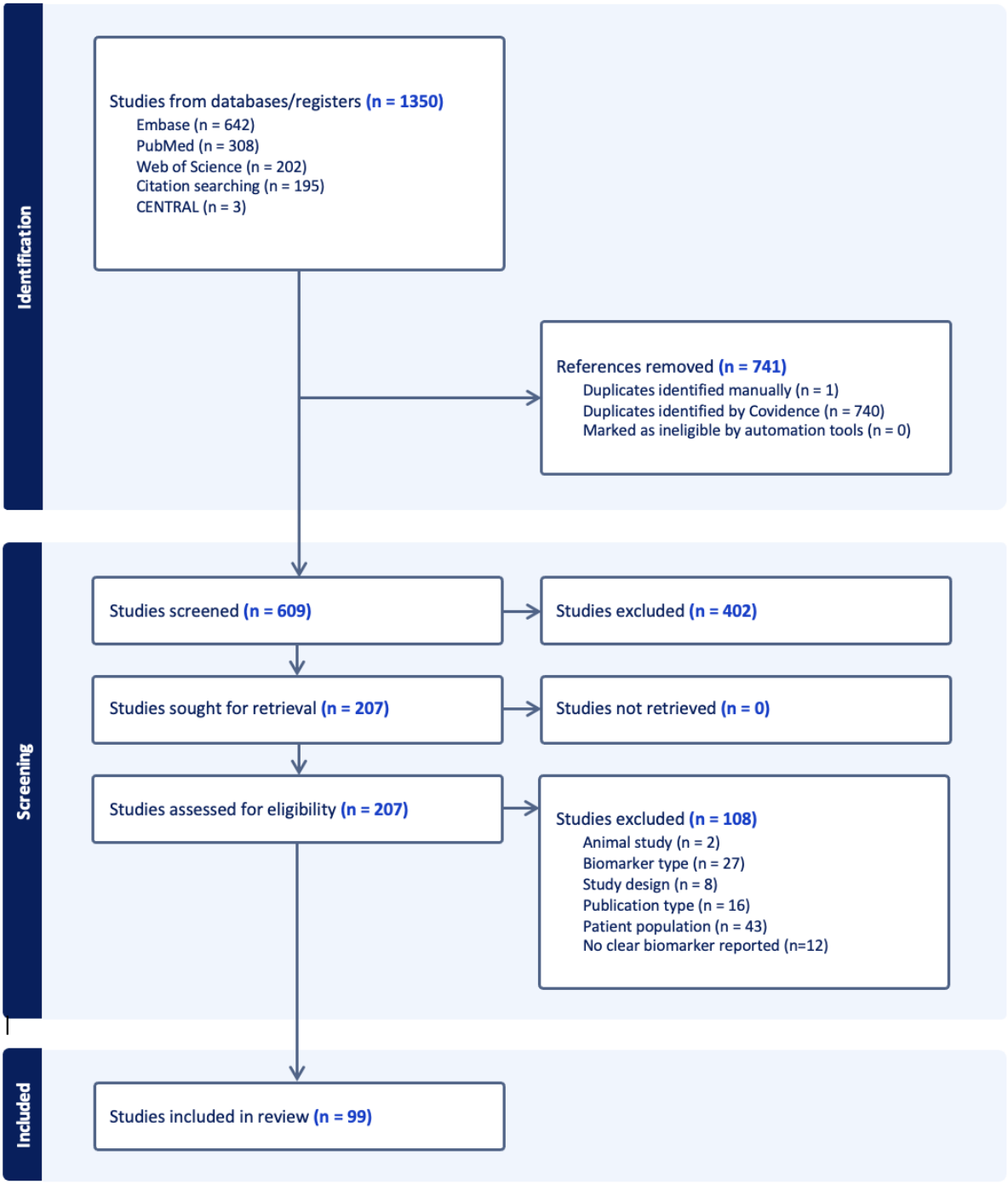

Figure 1. PRISMA chart describing identification, screening and included studies of the literature search

Of the included studies, 85 had biomarkers mappable to specific genes, resulting in a total of 1,544 biomarkers. Some of the most frequently reported biomarkers have established roles in oncogenesis and include *TP53* (19 studies)[14], *PIK3CA* (9 studies)[38], and *CDKN2A* (9 studies), seen in Figure 2.[39] Most biomarkers were from blood and blood products (serum, plasma) and involved DNA mutations (Figure 3). The full table containing information for each of the studies, the biomarkers identified from each study, and their gene symbol mapping is available in Appendix 2.

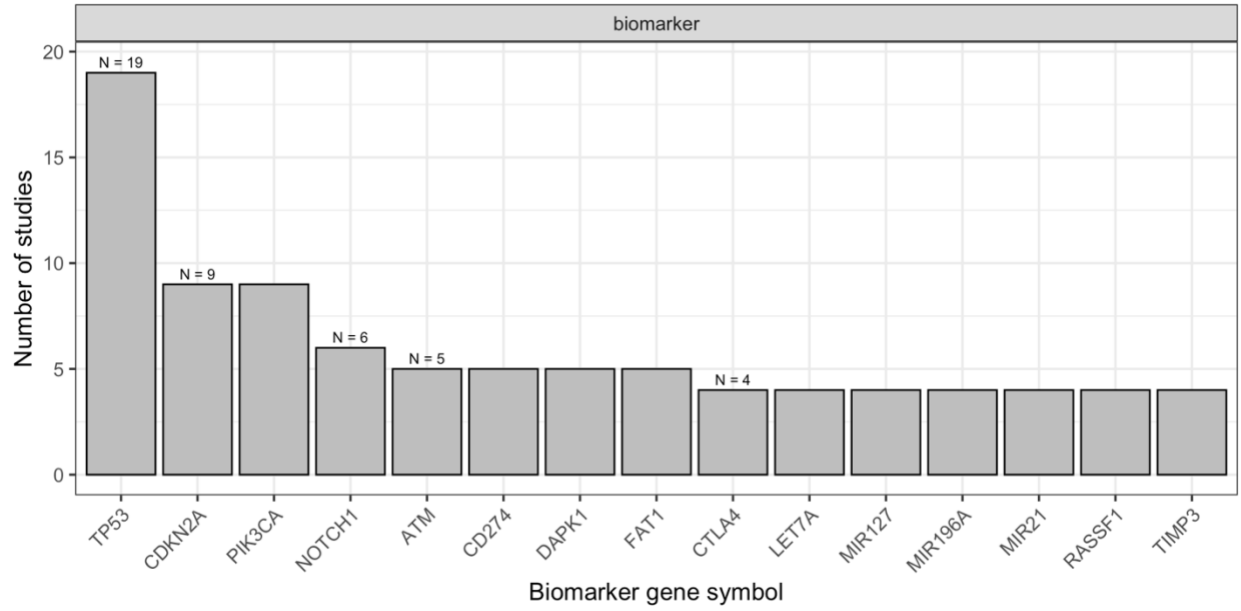

Figure 2. Biomarkers reported in more than 5 studies

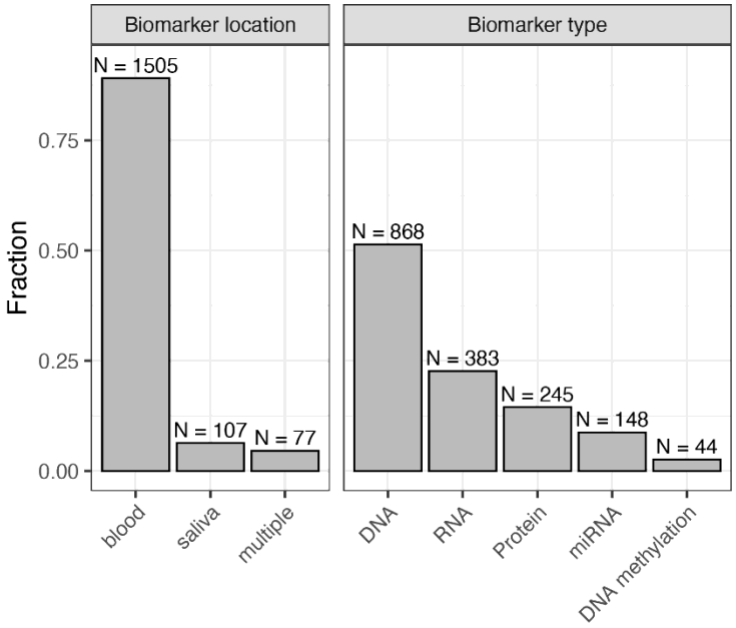

Figure 3. Biomarker location, type and PMID for studies reporting more than four biomarkers

Studies with the highest number of reported biomarkers included the LIONESS study which examined the ctDNA of a prospective cohort of 17 p16-negative HNSCC patients with deep exome sequencing[40], Huber et al. which examined platelet RNA of HNSCC patients[41] and Tuhkuri et al. which examined serum of HNSCC patients with ultra-definition mass spectrometry to identify protein biomarkers for early stage HNSCC detection.[42]

Liquid-based Biomarker Measurements in The Cancer Genome Atlas Datasets

1,544 genes obtained from liquid-based biomarkers were mapped to 51 miRNAs, 1,200 genes with somatic mutations, 2,257 Ensemble gene IDs with RNA expression, and 33,487 methylation probes in the TCGA datasets.

The TCGA head and neck cancer dataset included clinical and molecular data for 528 patients with primary head and neck tumors. Within this cohort, we identified 283 individuals with tumors classified as OPSCC based on the anatomical site of resection. Tumor locations in this subset included the palate (n=1), pharynx (n=1), posterior wall of the oropharynx n=1), supraglottic (n=1), oropharynx (n=9), tonsil (n=46), overlapping lesions of the lip, oral cavity, and pharynx (n=70), as well as the tongue and base of tongue n=154). Descriptive statistics were used to characterize clinical variables such as primary diagnosis, anatomical site, and clinical stage. (Figure 4)

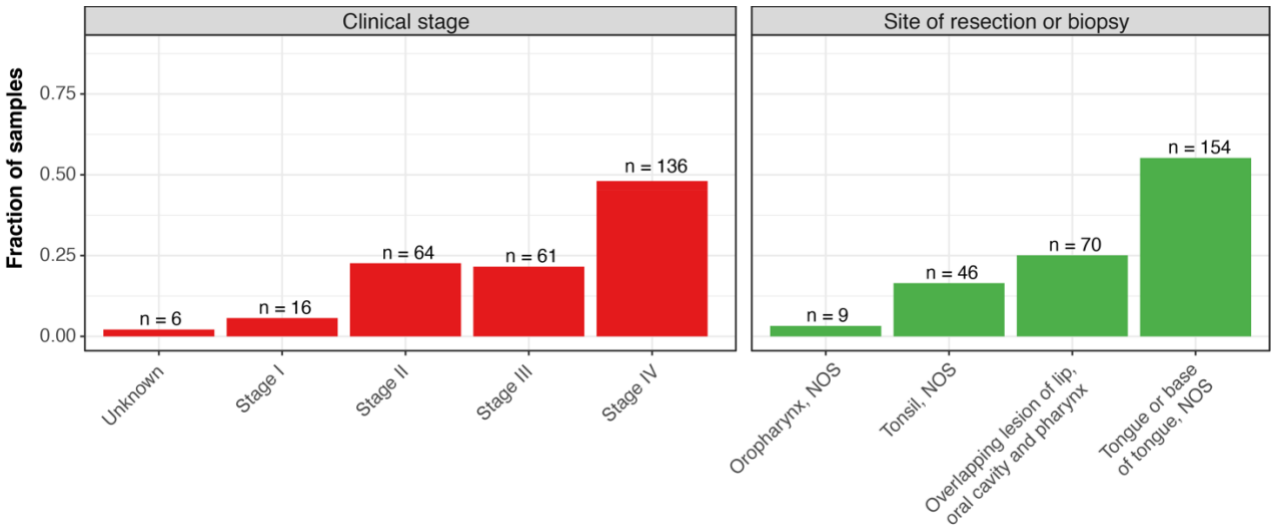

Figure 4. Cancer stage and site of biopsy for TCGA OPSCC individuals.

All 283 individuals with OPSCC in the TCGA have a measurement for DNA methylation, 280 have miRNA expression, 278 have gene expression, and 274 have somatic mutation measurements. And 266 individuals have a measurement for all 4 of the biomarker modalities. (Figure 5)

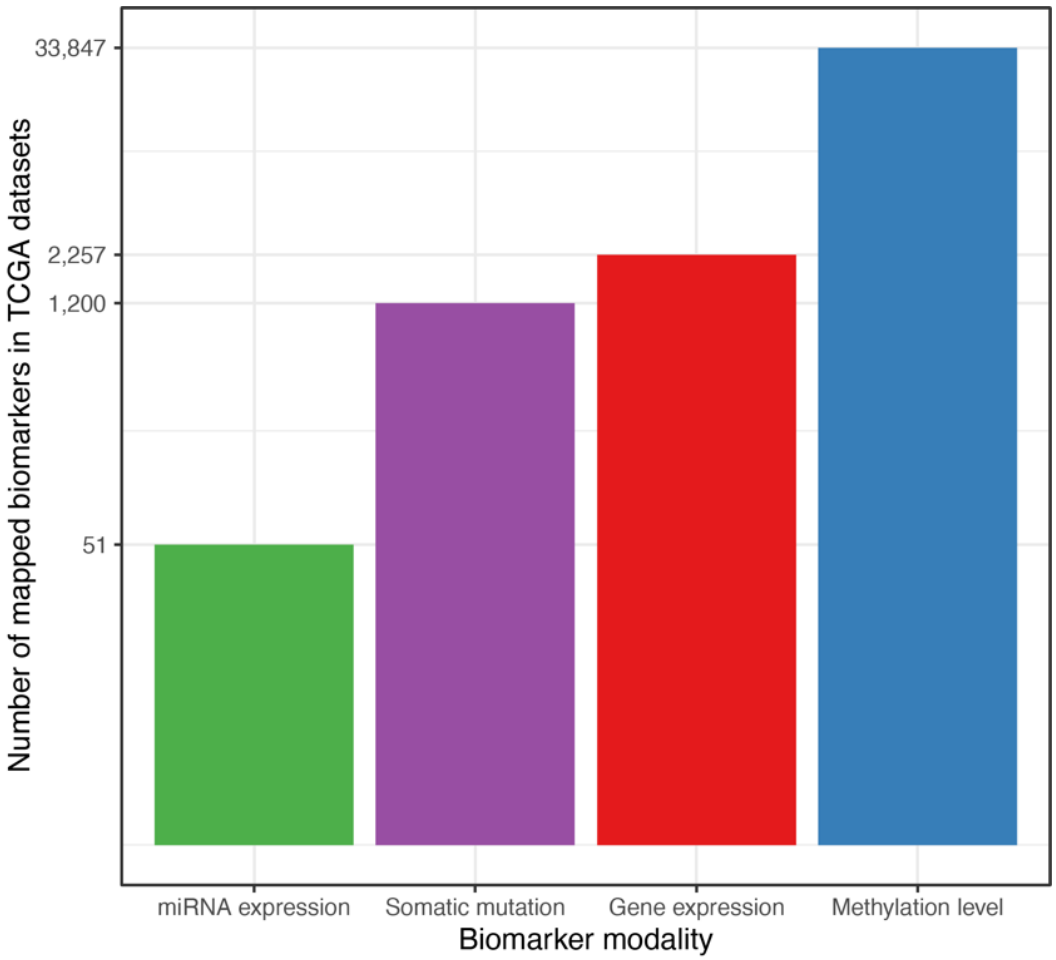

Figure 5. Number of biomarkers mapped to TCGA OPSCC miRNA expression, somatic mutation, gene expression, and methylation datasets.

Association of Liquid-based Biomarkers with Prognosis

As described above, the 1,544 genes corresponding to liquid-based biomarkers were mapped to a total of 37,355 molecular markers across 4 different data modalities. Excluding those with zero-variance biomarker measurements (e.g. identical measurement of the biomarker among all individuals, such as zero expression or no mutation), univariate coxPH statistics was measured for 33,620 (90%) of these biomarkers among the biomarker modalities.

The hazard ratios ranged from 10^{-7} to 5.45, p-valued ranges from 8.09×10^{-6} to 1, and concordance indices ranged from 0.45 to 0.72. See Table 1 for details.

Table 1. Range of test-statistics for univariate coxPH models of the liquid biopsy biomarkers within TCGA datasets.

Variable	Condition	N	Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
Hazard ratio	all	33,620	1.1e-07	0.951	0.998	1.01	1.05	5.45
Hazard ratio	p-value < 0.05	2,407	0.389	0.855	0.881	1.04	1.16	5.45
Hazard ratio	p-value < 0.05 & HR > 1	1,153	1.1	1.15	1.16	1.25	1.18	5.45
p-value	all	33,620	8.09e-06	0.21	0.463	0.472	0.725	1
p-value	p-value < 0.05	2,407	8.09e-06	0.0112	0.0227	0.0236	0.0359	0.05
p-value	p-value < 0.05 & HR> 1	1,153	8.09e-06	0.0121	0.0235	0.0243	0.0364	0.05
C-index	all	33,620	0.454	0.503	0.513	0.514	0.524	0.722
C-index	p-value < 0.05	2,407	0.479	0.532	0.539	0.539	0.547	0.722
C-index	p-value < 0.05 & HR > 1	1,153	0.479	0.531	0.539	0.538	0.547	0.61

From 37,620 biomarkers, 2,407 had a significant association with prognosis (p-value < 0.05). The direction of impact, however, was positive (presence or higher activity of biomarker translating to a worse survival outcome) for 1,153 (48%) of these biomarkers.

In this study, we focused on diagnostic liquid biopsy biomarkers—those whose presence in biofluids indicate the presence of disease. Our primary aim was to identify biomarkers associated with worse survival outcomes, as these may reflect more aggressive disease biology, making them particularly relevant for early detection and risk stratification. While biomarkers associated with better prognosis are also clinically valuable, our analysis prioritized those linked to poorer outcomes, since they may help identify high-risk individuals who would benefit most from intensified monitoring or treatment. Therefore, we limited the remainder of the analysis to these 1,153 biomarkers (3.43% of the 33,620 screened biomarkers). Q-Q plots suggest that these biomarkers have smaller p-values than expected by chance (Figure 6). These biomarkers corresponded to 8 miRNAs (15.68% of 51 screened miRNAs), 25 gene transcripts (1.15% of 2,178 screened transcripts), 54 somatic mutations (8.3% of 650 screened genes), 1,066 methylation markers (3.47% of 30,741 screened methylation markers).

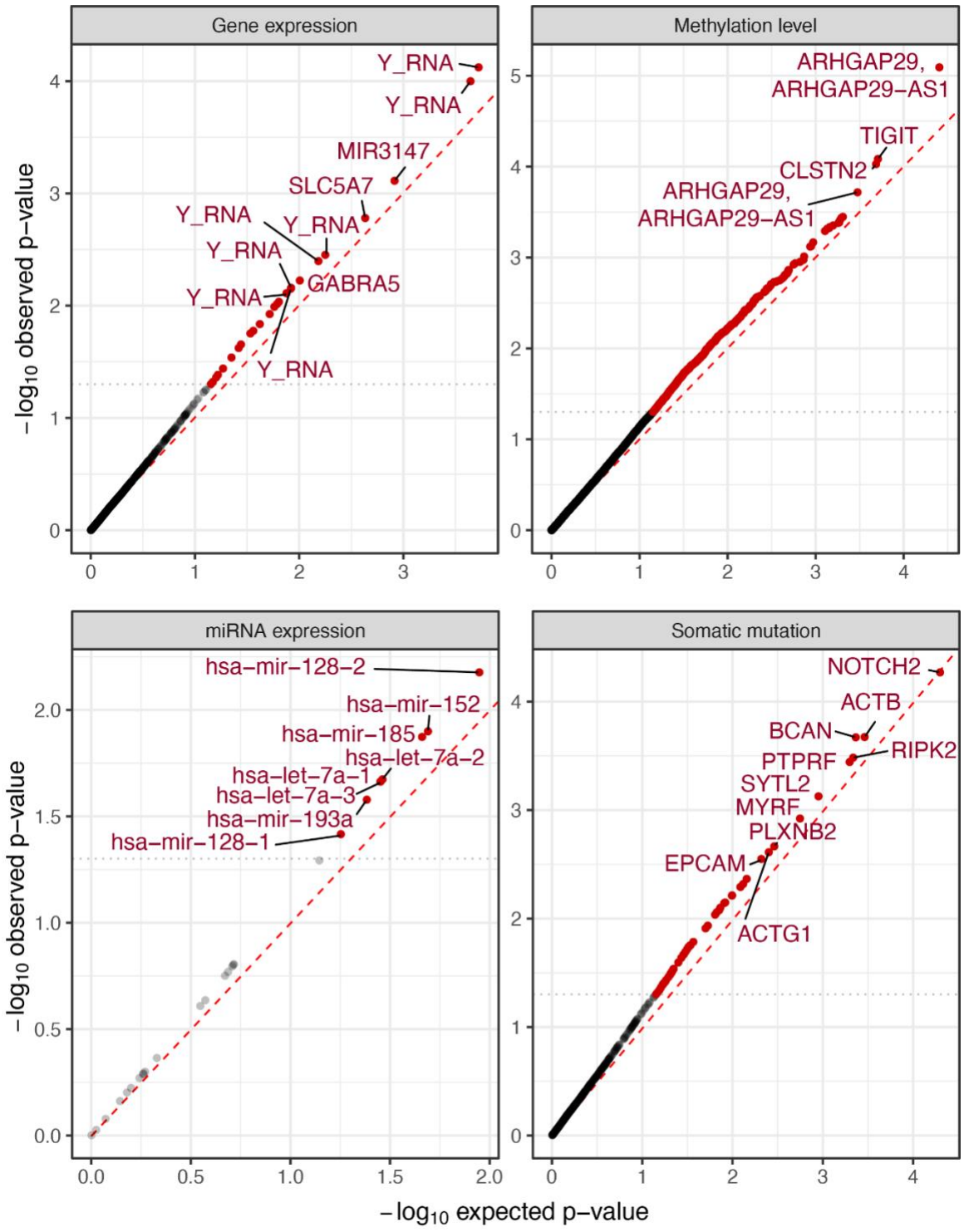

Figure 6. Q-Q plot for -log10 observed p-value (y-axis) compared to expected p-value under a uniform distribution. The red dashed line represents the diagonal. P-values < 0.05 (-log10 p-value > 1.3) are shown in red. Genes corresponding to some of the top biomarkers are annotated in the plot.

Biologically Relevant Diagnostic and Prognostic Liquid-based Biomarkers

Genes corresponding to certain biomarkers with a significant association with overall survival showed a well-established connection to HNSCC or OPSCC tumorigenesis. These included *NOTCH2*, *ACTB*, hsa-miR-128, hsa-miR-152, ARHGAP29, yRNAs, and *SLC5A7*.

To understand the role of the collective group of diagnostic and prognostic biomarkers in OPSCC, we performed a systematic pathway analysis. We mapped the 1,153 diagnostic and prognostic biomarkers to 524 unique gene symbols and queried the Reactome Pathway Knowledgebase to identify the biological pathways which are significantly enriched among these 524 genes.

We found that pathways associated with extracellular matrix (ECM) organization cell junction, and communication, are among the most impacted biological pathways (Table 2).

In addition to the main impacted pathways, other significantly impacted pathways (FDR < 0.05) indicate involvement of common hallmarks of cancer cell proliferation and include signaling by high-kinase activity *BRAF* mutants, *MITF-M*-dependent gene expression, *MAP2K* and *MAPK* activation, signaling by *BRAF* and *RAF1* fusions, signaling by *RAS* mutants, *TP53* regulates transcription of death receptors and ligands, *Pre-NOTCH t*ranscription and translation, Notch-HLH transcription pathway, and constitutive signaling by aberrant PI3K in cancer.

Table 2. Reactome biological pathways with significant over-representation of the diagnostic and prognostic genes.

Pathway name	#Genes found	#Total genes in pathway	p-value	FDR
Extracellular matrix organization	43	351	6.65E-10	1.02E-06
Degradation of the extracellular matrix	22	148	4.78E-07	3.65E-04
Intrinsic Pathway of Fibrin Clot Formation	9	26	2.00E-06	9.91E-04
Regulation of Insulin-like Growth Factor (IGF) transport and uptake by Insulin-like Growth Factor Binding Proteins (IGFBPs)	19	127	2.59E-06	9.91E-04
Cell-cell junction organization	16	99	6.16E-06	0.001643503
Regulation of MITF-M-dependent genes involved in DNA replication, damage repair and senescence	6	11	8.37E-06	0.001643503
Cell-Cell communication	21	165	9.13E-06	0.001643503
Regulation of CDH11 Expression and Function	8	35	1.33E-04	0.01011849

Post-translational protein phosphorylation	14	109	2.49E-04	0.017204553
Diseases of signal transduction by growth factor receptors and second messengers	40	536	3.45E-04	0.020029861
Signaling by Receptor Tyrosine Kinases	45	634	4.34E-04	0.023445861
Constitutive Signaling by Aberrant PI3K in Cancer	13	104	5.26E-04	0.027327339
Regulation of NFE2L2 gene expression	4	9	5.99E-04	0.029489829
Signaling by high-kinase activity BRAF mutants	8	44	6.02E-04	0.029489829
Signaling by PDGF	10	70	8.42E-04	0.039574967
TP53 Regulates Transcription of Death Receptors and Ligands	5	18	0.001036595	0.047683375
Signaling by BRAF and RAF1 fusions	10	73	0.001148846	0.047813413
Signaling by RAF1 mutants	8	49	0.001191232	0.047813413
MAP2K and MAPK activation	8	49	0.001191232	0.047813413
Pre-NOTCH Transcription and Translation	12	100	0.001195335	0.047813413

Liquid biopsy biomarkers with significant prognostic potential

Our results indicated that 1,153 biomarkers corresponding to 524 gene symbols are associated with worse prognosis. We next sought to address if any of these biomarkers could provide an added prognostic value when adjusting for the role of established prognostic biomarkers of OPSCC, i.e. presence of HPV infection, expression of *CDKN2A*, and mutations in *TP53* and *NOTCH1*.

To this end, for each of the 37,617 biomarkers (excluding *TP53*, *NOTCH1*, and *CDKN2A*), we fitted a multi-variate coxPH model by including established molecular biomarkers of HNSCC as co-variates (see Methods).

The multi-variate coxPH model found that 1,180 of the biomarkers provide additional prognostic value when adjusting for established prognostic biomarkers of OPSCC. These biomarkers had hazard ratios ranging up to 36.4 (average of 1.3) and concordance indices of up to 0.847 (average of 0.54).

Table 3. Summary statistics of the multi-variate coxPH model test statistics for different sets of biomarkers.

Variable	Condition	N	Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
HR	all	33,617	3.11e-11	0.952	0.996	73700	1.06	8.36e+09
HR	p-value < 0.05	2,091	0.129	0.857	1.14	1.09	1.17	36.4
HR	p-value < 0.05 & HR > 1	1,180	1.11	1.15	1.17	1.3	1.19	36.4
p-value	all	33,617	5.24e-06	0.246	0.461	0.509	0.788	1
p-value	p-value < 0.05	2,091	5.24e-06	0.0129	0.0256	0.0254	0.0381	0.05
p-value	p-value < 0.05 & HR > 1	1,180	5.24e-06	0.0133	0.0263	0.0256	0.038	0.05
C-index	all	33,617	0.483	0.512	0.517	0.519	0.525	0.872
C-index	p-value < 0.05	2,091	0.509	0.532	0.54	0.541	0.548	0.847
C-index	p-value < 0.05 & HR > 1	1,180	0.509	0.533	0.541	0.541	0.548	0.847

Several methylation biomarkers, particularly those around genes such as *MIR21, VMP1, KCNC1,* and *TEX15* had the largest concordance indices (c-index > 0.69). Among other modalities, we observed other biomarkers with added prognostic value as well (Figure 7). These included somatic mutations in *HUWE1* gene which is an E3 ligase with established roles in tumor development [43], miRNA-128 which regulated the activity of E2FA transcription factor and has established roles in HNSCC[44], and *SLC5A7* which also exhibited a strong prognostic association in a univariate coxPH model.[45,46]

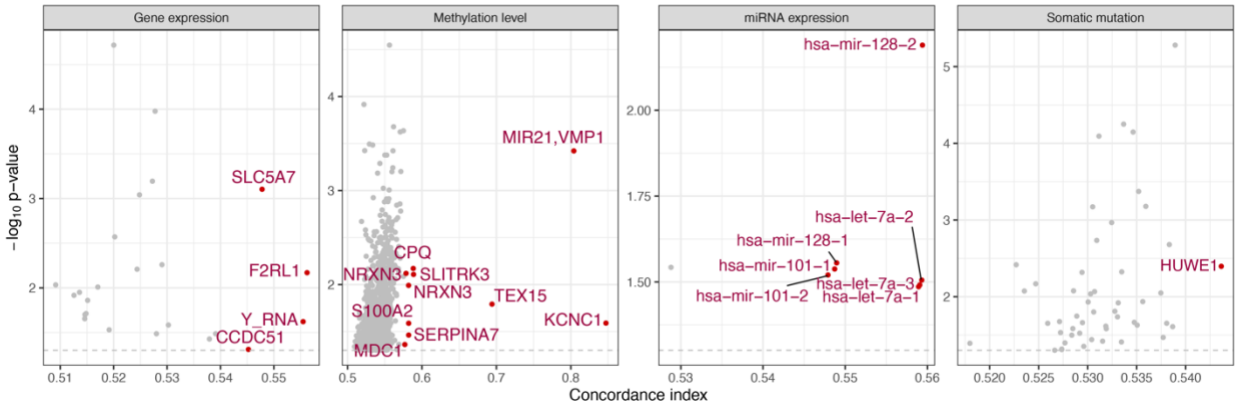

Figure 7. Concordance index (x-axis) and -log10 p-value (y-axis) of biomarkers with significant prognostic value after adjusting for established molecular biomarkers of OPSCC. The top biomarkers of modalities that have a concordance index larger than 0.54 are shown.

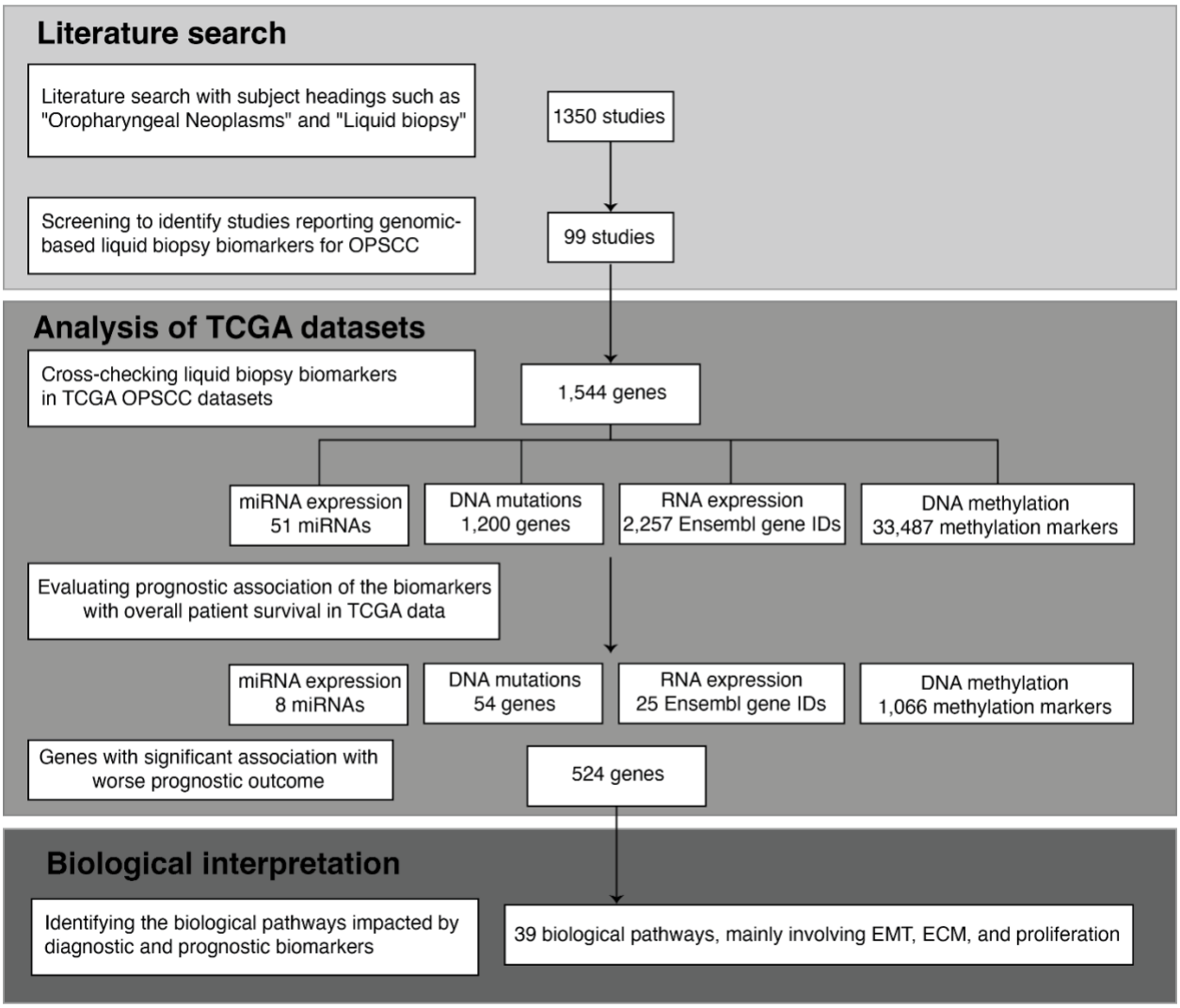

Figure 8. Study Summary. *Literature search identified 99 studies for biomarker extraction, resulting in 1,544 gene names. Biomarkers from different molecular modalities of these genes were assessed in the TCGA OPSCC studies. Prognostic significance of the different biomarkers was assessed in TCGA datasets, resulting in 524 genes with significant association with worse survival. Involvement of these genes were further assessed in biological pathways.*

Discussion

This study systematically assessed the prognostic relevance of liquid-based biomarkers in OPSCC using comprehensive multi-omic datasets from TCGA, expanding the potential use of liquid-based biomarkers in prognostication and personalized oncology.

Our main findings were: (1) 1,544 genes were identified from 99 included studies, corresponding to OPSCC liquid-based molecular biomarkers; (2) A significant portion of the investigated liquid-based biomarkers, corresponding to 524 unique gene symbols, were found to be associated with worse overall survival in individuals with OPSCC. Among the various molecular modalities assessed, DNA methylation markers demonstrated particularly strong prognostic performance; (3) Pathway enrichment analysis revealed that these prognostic biomarkers are predominantly involved in critical tumorigenic processes, including extracellular matrix (ECM) organization and degradation, cell-cell junction organization, and key oncogenic signaling pathways such as MAPK, Notch, and PI3K; (4) 1,180 biomarkers, retained their prognostic significance after adjusting for established molecular markers (HPV status, *TP53*/*NOTCH1* mutations, and *CDKN2A* expression), indicating their potential to offer independent prognostic value. Notably, multi-variate models with methylation biomarkers located near genes such as *MIR21*, *VMP1*, and *KCNC1* achieved concordance indices (C-index) greater than 0.69.

Our first aim was to curate a comprehensive list of liquid biopsy biomarkers reported for OPSCC. Through a systematic search, out of 99 included studies, we identified 1,544 gene symbols corresponding to diverse molecular biomarkers across multiple biofluids, primarily blood and saliva. These included DNA mutations, DNA methylation, gene expression, miRNA, and protein-based biomarkers.

Importantly, while numerous biomarkers have been proposed for diagnostic purposes, few had been systematically assessed for prognostic value. Many of these markers likely reflect non-tumor processes. Our study helps address this gap by rigorously evaluating the prognostic potential of this curated biomarker set using a robust multi-omic approach. This effort also highlights the need for further validation of diagnostic biomarkers to ensure their clinical relevance.

Our second aim was to systematically assess the prognostic value of these biomarkers in OPSCC using TCGA data. A significant portion of the investigated liquid-based biomarkers, corresponding to 524 unique gene symbols, were associated with worse overall survival in OPSCC individuals. This finding supports the growing evidence that liquid biopsy biomarkers offer unique prognostic insights in OPSCC that complement tissue-based markers.[15] While traditional tissue biomarkers derived from surgical specimens provide important prognostic information, they are limited by their invasive nature, representation of a single tumor region, and inability to monitor tumor evolution over time.[47]

Prioritizing biomarkers associated with poor outcomes, particularly those involved in invasion, metastasis, and treatment resistance, strengthens their clinical relevance and translational potential. Several of the significantly prognostic biomarkers are mapped to genes with

established roles in HNSCC or OPSCC tumorigenesis, further supporting their biological and clinical relevance. These included *NOTCH2* (regulator of key oncogenes such as *MYC* and *BCL-2*)[48], *ACTB* (actin beta, linked to cell motility and invasion)[49], *hsa-miR-128* (a microRNA that targets tumor suppressor genes and contributes to the regulation of cell proliferation and migration)[50], *hsa-miR-152* (microRNA, known for its role in modifying the epigenome through silencing of DNMT1 and acting as a tumor suppressor in various cancers, including HNSCC)[51], ARHGAP29 (a GTPase-activating protein that plays a role in regulating cell adhesion and migration, crucial for metastasis in OPSCC)[52], *yRNA*s (small non-coding RNAs involved in RNA stability and transcriptional regulation, which may contribute to chemoresistance in HNSCC)[53], and *SLC5A7* (mediates the interaction between p53 and MDM2 and has established roles in tumorigenesis).[46] The involvement of these genes in core cellular processes such as proliferation, survival, migration, and apoptosis reinforce their relevance as prognostic indicators and potential therapeutic targets.

The superior prognostic performance of methylation markers compared to mutation-based biomarkers suggests a potential shift in focus for liquid biopsy strategies, moving beyond traditional emphasis on circulating tumor DNA (ctDNA) mutations or HPV DNA detection.[15,54] Our findings advocate for incorporating epigenetic profiling —particularly of DNA methylation patterns—into prognostic workflows to provide more informative, robust, and clinically actionable insights. DNA methylation changes occur early in tumorigenesis and are reversible making them ideal biomarkers for risk stratification and therapeutic targeting in OPSCC.[55] In other malignancies such as breast, lung and colorectal cancer, the integration of epigenetic biomarkers with clinical and genomic data has improved prognostic stratification and informed personalized treatment strategies.[56, 57] Our results suggest that adopting similar integrative approaches in OPSCC could enhance individualized risk assessment and guide therapeutic decisions.

Most previous investigations into liquid biopsy for HNSCCs have focused predominantly on diagnostic applications, particularly ctDNA mutation detection or HPV DNA quantification.[29] However, they offer limited insight into prognosis. Our multi-modal analysis, integrating DNA methylation, mRNA, and miRNA profiles provide a more comprehensive view of OPSCC biology. The gain in predictive accuracy compared to the baseline model underscores the prognostic potential of these biomarkers.

Our third aim was to explore the biological pathways enriched among prognostic biomarkers. Pathway enrichment analysis revealed that prognostic biomarkers are involved in key molecular circuits known to drive OPSCC pathogenesis. These include ECM remodeling, cell-cell adhesion dynamics, canonical oncogenic signaling pathways such as MAPK and Notch[58,59], and EMT which is a hallmark of tumorigenesis.[60] The biological relevance of ECM remodeling, EMT and oncogenic signaling (MAPK, Notch) is especially notable as it aligns with known mechanisms of tumor invasion and metastasis in OPSCC.[60] ECM degradation and EMT facilitate detachment and dissemination of tumor cells, contributing to metastasis.[61] Methylation alterations in genes such as *MIR21* (regulating EMT)[61,62] and *KCNC1* (implicated in tumor aggressiveness)[63] suggest that epigenetic dysregulation of these pathways may drive aggressive disease phenotypes and that targeting these alterations could yield novel therapeutic opportunities.

These insights offer a biological rationale for their prognostic relevance and point to potential future therapeutic opportunities.

Our fourth aim was to determine whether liquid-based biomarkers provide prognostic value beyond established markers such as HPV status, *TP53/NOTCH1* mutations, and *CDKN2A* expression. A subset of 1,180 biomarkers demonstrated robust prognostic value, even after adjusting for known prognostic indicators. Our analysis identified a multi-modal panel of biomarkers—including DNA methylation, mRNA, miRNA, and somatic mutations—outperformed traditional markers like *CDKN2A* expression and *TP53/NOTCH1* mutations in predicting poorer prognosis.

Moreover, DNA methylation markers demonstrated particularly strong prognostic performance. For example, in a multi-variate model, methylation markers near *MIR21, VMP1*, and *KCNC1* exhibited C-indices greater than 0.69. These methylation events are linked to key biological processes such as apoptosis, immune regulation, and epithelial-mesenchymal transition (EMT), underscoring their relevance to cancer progression.[29]

The high prevalence of HPV-positive OPSCC, particularly in North America where up to 63% of cases are associated with HPV16 infection[2], has significantly influenced risk stratification and treatment de-intensification strategies.[64] Although HPV positivity generally correlates with a favorable prognosis, clinical heterogeneity persists.[65] Our analysis identified biomarkers retaining prognostic significance independent of HPV status, suggesting their potential to further refine risk stratification in OPSCC and within HPV-positive subgroups. Future studies could leverage these biomarkers to identify individuals who might benefit from intensified therapy or enhanced surveillance. Similar multi-modal biomarker approaches are gaining traction in other cancers. For instance, integrating ctDNA, circulating tumor cells (CTCs), and protein biomarkers has demonstrated enhanced diagnostic and prognostic performance in cancers such as metastatic breast cancer and pancreatic cancer.[66, 67] In OPSCC, recent studies highlight the promise of combining assays (e.g., ctDNA, CTCs, methylation markers) to improve the sensitivity and specificity of minimal residual disease (MRD) detection and risk stratification.[68] However, the adoption of multi-modal panels also presents challenges. The costs of multiplexed testing can be significant, especially when assays require high-throughput sequencing, digital PCR, or highly sensitive analytical platforms.[69] Technical challenges include assay standardization, harmonization of bioinformatic pipelines, and ensuring reproducibility across laboratories and patient populations.[69] Furthermore, regulatory hurdles and reimbursement policies could delay widespread clinical implementation. Despite these challenges, multi-modal panels could offer substantial benefits. They capture the tumor's biological heterogeneity more comprehensively than single-analyte tests, providing a dynamic and systemic snapshot of tumor burden and evolution.[15]

Limitations and Future Directions

Despite the strengths of our multi-modal analysis, several limitations should be acknowledged. First, the TCGA dataset is retrospective in nature, includes an over-representation of late-stage samples, and may not fully represent real-world clinical variables, particularly sample processing variations, treatment protocols, and missing data on relevant clinical variables (e.g., smoking status, detailed treatment regimens, etc.) which could confound survival analyses. Second, although the OPSCC subset (n=266) analyzed here is one of the most comprehensive molecularly characterized cohorts available, the sample size may still limit statistical power to detect weaker but potentially relevant associations. Third, survival outcomes in TCGA reflect overall rather than disease-specific survival, which could dilute the impact of cancer-specific prognostic signals.

Prospective validation of these biomarkers in independent and clinically diverse cohorts will be essential to confirm their prognostic utility. Future studies should consider the development of multi-modal liquid biopsy panels that integrate top-performing markers from various molecular classes. Such panels could enable early identification of individuals with higher risk of worse prognosis, guide risk-adapted treatment strategies, and inform post-treatment surveillance protocols. Moreover, the biological pathways identified here suggest novel therapeutic targets—for instance, drugs modulating ECM remodeling or EMT signaling could be explored as adjuncts to current therapies.

Conclusion

In conclusion, this study demonstrates that a subset of liquid-based biomarkers possesses robust prognostic value in OPSCC, independent of established molecular markers. Our findings highlight the promise of multi-omic liquid biopsy strategies to refine risk stratification, augment current diagnostic frameworks, and guide future personalized oncology approaches for OPSCC.

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Appendix 1:

Search Strategy:

((((*Liquid Biopsy*) AND (*Biomarkers*)) OR ((blood OR serum OR urine OR saliva OR salivary) NEAR/2 biomarker*)) **AND (***Molecular Diagnostic Techniques***))** OR "saliva* proteomics") **AND (***Cancers***)**

PubMed

	Concept: Liquid Biopsy	**Concept: Biomarkers**
Subject Headings (MeSH)	"Early Detection of Cancer"[Mesh] "Liquid Biopsy"[Mesh]	"Biomarkers, Tumor"[Mesh]
Free text terms (searched in title, abstract, and author keywords [Title/Abstract])	"liquid biops*" "fluid biops*"	"biomarker*"
	"blood biomarker"[tiab:~2] "serum biomarker"[tiab:~2] "urine biomarker"[tiab:~2] "saliva biomarker"[tiab:~2] "salivary biomarker"[tiab:~2] "blood biomarkers"[tiab:~2] "serum biomarkers"[tiab:~2] "urine biomarkers"[tiab:~2] "saliva biomarkers"[tiab:~2] "salivary biomarkers"[tiab:~2]	

"saliva* proteomics"

	Concept: Molecular Diagnostic Techniques	**Concept: Cancers**
Subject Headings (MeSH)	"Molecular Diagnostic Techniques"[Mesh:noexp] "MicroRNAs"[Mesh] "Cell-Free Nucleic Acids"[Mesh] "Proteomics"[Mesh] "Metabolomics"[Mesh] "Genomics"[Mesh] "Transcriptome"[Mesh]	"Head and Neck Neoplasms"[Mesh:noexp] "Pharyngeal Neoplasms"[Mesh:noexp] "Oropharyngeal Neoplasms"[Mesh] "Squamous Cell Carcinoma of Head and Neck"[Mesh]

	"Exosomes"[Mesh]	
Free text terms (searched in title, abstract, and author keywords [Title/Abstract])	"molecular diagnostic techniques" "microRNA*" "micro-RNA*" miRNA* mRNA* "small temporal RNA*" stRNA* "cell free nucleic acid*" "circulating nucleic acid*" "cell-free DNA" "circulatory DNA" "cell-free deoxyribonucleic acid*" "circulating DNA*" cfDNA cirDNA "cell-free RNA*" "cell-free ribonucleic acid*" "circulating RNA*" cirRNA cfRNA "proteomics" "metabolomics" "genomics" "transcriptom*" "exosom*"	"pharyngeal neoplasm*" "pharyngeal cancer*" "pharyngeal carcinoma"[Title/Abstract:~2] "pharyngeal carcinomas"[Title/Abstract:~2] "cancer pharynx"[Title/Abstract:~2] "neoplasm pharynx"[Title/Abstract:~2] "carcinoma pharynx"[Title/Abstract:~2] "cancers pharynx"[Title/Abstract:~2] "neoplasms pharynx"[Title/Abstract:~2] "carcinomas pharynx"[Title/Abstract:~2] "oropharyngeal neoplasm*" "oropharyngeal cancer*" "oropharyngeal carcinoma"[Title/Abstract:~2] "oropharyngeal carcinomas"[Title/Abstract:~2] "cancer oropharynx"[Title/Abstract:~2] "neoplasm oropharynx"[Title/Abstract:~2] "carcinoma oropharynx"[Title/Abstract:~2] "cancers oropharynx"[Title/Abstract:~2] "neoplasms oropharynx"[Title/Abstract:~2] "carcinomas oropharynx"[Title/Abstract:~2] "throat neoplasm"[Title/Abstract:~2] "throat cancer"[Title/Abstract:~2] "throat carcinoma"[Title/Abstract:~2] "throat neoplasms"[Title/Abstract:~2] "throat cancers"[Title/Abstract:~2] "throat carcinomas"[Title/Abstract:~2] "tonsil* neoplasm*"

		"tonsil* cancer*" "tonsils neoplasm"[Title/Abstract:~2] "tonsils cancer"[Title/Abstract:~2] "tonsils neoplasms"[Title/Abstract:~2] "tonsils cancers"[Title/Abstract:~2] "tonsil carcinoma"[tiab:~2] "tonsil carcinomas"[tiab:~2] "tonsils carcinoma"[tiab:~2] "tonsils carcinomas"[tiab:~2] "cancer base tongue"[Title/Abstract:~3] "cancers base tongue"[Title/Abstract:~3] "neoplasm base tongue"[Title/Abstract:~3] "neoplasms base tongue"[Title/Abstract:~3] "carcinoma base tongue"[Title/Abstract:~3] "carcinomas base tongue"[Title/Abstract:~3])

((((("early detection of cancer"[Mesh] OR "Liquid Biopsy"[Mesh] OR "liquid biops*"[Title/Abstract]) AND ("biomarker*"[Title/Abstract] OR "biomarkers, tumor"[MeSH Terms])) OR "blood biomarker"[tiab:~2] OR "serum biomarker"[tiab:~2] OR "urine biomarker"[tiab:~2] OR "saliva biomarker"[tiab:~2] OR "salivary biomarker"[tiab:~2] OR "blood biomarkers"[tiab:~2] OR "serum biomarkers"[tiab:~2] OR "urine biomarkers"[tiab:~2] OR "saliva biomarkers"[tiab:~2] OR "salivary biomarkers"[tiab:~2])

AND

("Molecular Diagnostic Techniques"[Mesh:noexp] OR "MicroRNAs"[Mesh] OR "Cell-Free Nucleic Acids"[Mesh] OR "Proteomics"[Mesh] OR "Metabolomics"[Mesh] OR "Genomics"[Mesh] OR "Transcriptome"[Mesh] OR "Exosomes"[Mesh] OR "molecular diagnostic techniques"[Title/Abstract] OR "microRNA*"[Title/Abstract] OR "micro-RNA*"[Title/Abstract] OR miRNA*[Title/Abstract] OR mRNA*[Title/Abstract] OR "small temporal RNA*"[Title/Abstract]

OR stRNA*[Title/Abstract] OR "cell free nucleic acid*"[Title/Abstract] OR "circulating nucleic acid*"[Title/Abstract] OR "cell-free DNA"[Title/Abstract] OR "circulatory DNA"[Title/Abstract] OR "cell-free deoxyribonucleic acid*"[Title/Abstract] OR "circulating DNA*"[Title/Abstract] OR cfDNA[Title/Abstract] OR cirDNA[Title/Abstract] OR "cell-free RNA*"[Title/Abstract] OR "cell-free ribonucleic acid*"[Title/Abstract] OR "circulating RNA*"[Title/Abstract] OR cirRNA[Title/Abstract] OR cfRNA[Title/Abstract] OR "proteomics"[Title/Abstract] OR "metabolomics"[Title/Abstract] OR "genomics"[Title/Abstract] OR "transcriptom*"[Title/Abstract] OR "exosom*"[Title/Abstract])) OR "saliva* proteomics"[tiab])

AND

("Head and Neck Neoplasms"[Mesh:noexp] OR "Pharyngeal Neoplasms"[Mesh:noexp] OR "Oropharyngeal Neoplasms"[Mesh] OR "Squamous Cell Carcinoma of Head and Neck"[Mesh] OR "pharyngeal neoplasm*"[Title/Abstract] OR "pharyngeal cancer*"[Title/Abstract] OR "pharyngeal carcinoma"[Title/Abstract:~2] OR "pharyngeal carcinomas"[Title/Abstract:~2] OR "cancer pharynx"[Title/Abstract:~2] OR "neoplasm pharynx"[Title/Abstract:~2] OR "carcinoma pharynx"[Title/Abstract:~2] OR "cancers pharynx"[Title/Abstract:~2] OR "neoplasms pharynx"[Title/Abstract:~2] OR "carcinomas pharynx"[Title/Abstract:~2] OR "oropharyngeal neoplasm*"[Title/Abstract] OR "oropharyngeal cancer*"[Title/Abstract] OR "oropharyngeal carcinoma*"[Title/Abstract] OR "oropharyngeal carcinoma"[Title/Abstract:~2] OR "oropharyngeal carcinomas"[Title/Abstract:~2] OR "cancer oropharynx"[Title/Abstract:~2] OR "neoplasm oropharynx"[Title/Abstract:~2] OR "carcinoma oropharynx"[Title/Abstract:~2] OR "cancers oropharynx"[Title/Abstract:~2] OR "neoplasms oropharynx"[Title/Abstract:~2] OR "carcinomas oropharynx"[Title/Abstract:~2] OR "throat neoplasm"[Title/Abstract:~2] OR "throat cancer"[Title/Abstract:~2] OR "throat carcinoma"[Title/Abstract:~2] OR "throat neoplasms"[Title/Abstract:~2] OR "throat cancers"[Title/Abstract:~2] OR "throat carcinomas"[Title/Abstract:~2] OR "tonsil* neoplasm*"[Title/Abstract] OR "tonsil* cancer*"[Title/Abstract] OR "tonsils neoplasm"[Title/Abstract:~2] OR "tonsils cancer"[Title/Abstract:~2] OR "tonsils neoplasms"[Title/Abstract:~2] OR "tonsils cancers"[Title/Abstract:~2] OR "tonsil carcinoma"[tiab:~2] OR "tonsil carcinomas"[tiab:~2] OR "tonsils carcinoma"[tiab:~2] OR "tonsils carcinomas"[tiab:~2] OR "cancer base tongue"[Title/Abstract:~3] OR "cancers base tongue"[Title/Abstract:~3] OR "neoplasm base tongue"[Title/Abstract:~3] OR "neoplasms base tongue"[Title/Abstract:~3] OR "carcinoma base tongue"[Title/Abstract:~3] OR "carcinomas base tongue"[Title/Abstract:~3])

AND

("Molecular Diagnostic Techniques"[Mesh:noexp] OR "MicroRNAs"[Mesh] OR "Cell-Free Nucleic Acids"[Mesh] OR "Proteomics"[Mesh] OR "Metabolomics"[Mesh] OR "Genomics"[Mesh] OR "Transcriptome"[Mesh] OR "Exosomes"[Mesh] OR "molecular diagnostic techniques"[Title/Abstract] OR "microRNA*"[Title/Abstract] OR "micro-RNA*"[Title/Abstract] OR miRNA*[Title/Abstract] OR mRNA*[Title/Abstract] OR "small temporal RNA*"[Title/Abstract]
OR stRNA*[Title/Abstract] OR "cell free nucleic acid*"[Title/Abstract] OR "circulating nucleic acid*"[Title/Abstract] OR "cell-free DNA"[Title/Abstract] OR "circulatory DNA"[Title/Abstract] OR "cell-free deoxyribonucleic acid*"[Title/Abstract] OR "circulating DNA*"[Title/Abstract] OR cfDNA[Title/Abstract] OR cirDNA[Title/Abstract] OR "cell-free RNA*"[Title/Abstract] OR "cell-free ribonucleic acid*"[Title/Abstract] OR "circulating RNA*"[Title/Abstract] OR cirRNA[Title/Abstract] OR cfRNA[Title/Abstract] OR "proteomics"[Title/Abstract] OR "metabolomics"[Title/Abstract] OR "genomics"[Title/Abstract] OR "transcriptom*"[Title/Abstract] OR "exosom*"[Title/Abstract])

Limits: none

157 results as of 2/3/25

Embase; Elsevier

	Concept: Liquid Biopsy	**Concept: Biomarkers**
Subject Headings (Emtree)	'early cancer diagnosis'/exp OR 'liquid biopsy'/exp	'tumor marker'/exp
Free text terms (searched in title, abstract, and keyword (:ti,ab,kw))	"liquid biops*" "fluid biops*"	"biomarker*"
	((blood OR serum OR urine OR saliva OR salivary) NEAR/3 biomarker*)	

'saliva* proteomics'

	Concept: Molecular Diagnostic Techniques	**Concept: Cancers**
Subject Headings (Emtree)	'molecular diagnosis'/exp 'microRNA'/exp 'cell free nucleic acid'/exp 'proteomics'/exp 'metabolomics'/exp 'genomics'/exp 'transcriptome'/exp 'exosome'/exp	'head and neck cancer'/de 'head and neck carcinoma'/de 'head and neck squamous cell carcinoma'/de 'pharynx squamous cell carcinoma'/de 'pharynx tumor'/de 'pharynx cancer'/de 'pharynx carcinoma'/de 'oropharynx tumor'/exp 'oropharynx carcinoma'/exp 'hypopharynx tumor'/exp 'hypopharynx carcinoma'/exp
Free text terms (searched in title, abstract, and keyword (:ti,ab,kw))	"molecular diagnostic techniques" "microRNA*" "micro-RNA*" miRNA* mRNA* "small temporal RNA*" stRNA* "cell free nucleic acid*" "circulating nucleic acid*" "cell-free DNA*" "circulatory DNA*" "cell-free deoxyribonucleic acid*" "circulating DNA*" cfDNA cirDNA "cell-free RNA*"	"pharyngeal neoplasm*" "pharyngeal cancer*" "oropharyngeal neoplasm*" "oropharyngeal cancer*" "tonsil* neoplasm*" "tonsil* cancer*" ((pharyngeal OR oropharyngeal) NEAR/3 carcinoma*) ((cancer OR cancers OR neoplasm OR neoplasms OR carcinoma OR carcinomas) NEAR/3 (pharynx OR oropharynx OR throat OR tonsil*))

	"cell-free ribonucleic acid*" "circulating RNA*" cirRNA cfRNA "proteomics" "metabolomics" "genomics" "transcriptom*" "exosom*"	

***Running note**: This can be run from Quick Search, Advanced Search, or the Results screen. If you run it from Advanced Search, make sure to **uncheck** the orange boxes.*

((((('early cancer diagnosis'/exp OR 'liquid biopsy'/exp OR ("liquid biops*" OR "fluid biops*"):ti,ab,kw) **AND** ('tumor marker'/exp OR "biomarker*":ti,ab,kw)) OR ((blood OR serum OR urine OR saliva OR salivary) NEAR/3 biomarker*):ti,ab,kw)

AND

('molecular diagnosis'/exp OR 'microRNA'/exp OR 'cell free nucleic acid'/exp OR 'proteomics'/exp OR 'metabolomics'/exp OR 'genomics'/exp OR 'transcriptome'/exp OR 'exosome'/exp OR ("molecular diagnostic techniques" OR "microRNA*" OR "micro-RNA*" OR miRNA* OR mRNA* OR "small temporal RNA*" OR stRNA* OR "cell free nucleic acid*" OR "circulating nucleic acid*" OR "cell-free DNA*" OR "circulatory DNA*" OR "cell-free deoxyribonucleic acid*" OR "circulating DNA*" OR cfDNA OR cirDNA OR "cell-free RNA*" OR "cell-free ribonucleic acid*" OR "circulating RNA*" OR cirRNA OR cfRNA OR "proteomics" OR "metabolomics" OR "genomics" OR "transcriptom*" OR "exosom*"):ti,ab,kw)) OR 'saliva* proteomics':ti,ab,kw)

AND

('head and neck cancer'/de OR 'head and neck carcinoma'/de OR 'head and neck squamous cell carcinoma'/de OR 'pharynx squamous cell carcinoma'/de OR 'pharynx tumor'/de OR 'pharynx cancer'/de OR 'pharynx carcinoma'/de OR 'oropharynx tumor'/exp OR 'oropharynx carcinoma'/exp OR 'hypopharynx carcinoma'/exp OR ("pharyngeal neoplasm*" OR "pharyngeal cancer*" OR "oropharyngeal neoplasm*" OR "oropharyngeal cancer*" OR "tonsil* neoplasm*" OR "tonsil* cancer*" OR ((pharyngeal OR oropharyngeal) NEAR/3 carcinoma*) OR ((cancer OR cancers OR neoplasm OR neoplasms OR carcinoma OR carcinomas) NEAR/3 (pharynx OR oropharynx OR throat OR tonsil*)) OR ((cancer* OR neoplasm* OR carcinoma*) NEAR/4 base NEAR/4 tongue)):ti,ab,kw)

Limits: none

325 results as of 2/3/25

Note: to download more than 500 results, you must log into Embase using an Embase/Elsevier account. *This is separate from your UW NetID.* The account is free, but does involve sharing your data with them.

Cochrane Central Register of Controlled Trials; Wiley
Cochrane Database of Systematic Reviews; Wiley

	Concept: Liquid Biopsy	**Concept: Biomarkers**
Subject Headings [mh]	[mh "Early Detection of Cancer"] [mh "Liquid Biopsy"]	[mh "Biomarkers, Tumor"]
Free text terms :ti,ab,kw	"liquid biopsy" "liquid biopsies" "fluid biopsy" "fluid biopsies"	biomarker*
Free text terms :ti,ab,kw	((blood OR serum OR urine OR saliva OR salivary) NEAR/2 biomarker*)	

(saliva* NEXT proteomics)

	Concept: Molecular Diagnostic Techniques	**Concept: Cancers**
Subject Headings [mh]	[mh ^"Molecular Diagnostic Techniques"] [mh "MicroRNAs"] [mh "Cell-Free Nucleic Acids"] [mh "Proteomics"] [mh "Metabolomics"] [mh "Genomics"] [mh "Transcriptome"] [mh "Exosomes"]	[mh ^"Head and Neck Neoplasms"] [mh ^"Pharyngeal Neoplasms"] [mh "Oropharyngeal Neoplasms"] [mh "Squamous Cell Carcinoma of Head and Neck"]
Free text terms :ti,ab,kw	"molecular diagnostic techniques" "microRNA" "micro-RNA" "micro-RNAs" miRNA* mRNA* "small temporal RNA" "small temporal RNAs" stRNA* "cell free nucleic acid" "cell free nucleic acids" "circulating nucleic acid" "circulating nucleic acids" "cell-free DNA" "cell-free DNAs" "circulatory DNA" "circulatory DNAs"	((pharyngeal OR oropharyngeal OR tonsil*) NEXT (neoplasm* OR cancer*)) ((pharyngeal OR oropharyngeal) NEAR/2 carcinoma*) ((cancer OR cancers OR neoplasm OR neoplasms OR carcinoma OR carcinomas) NEAR/2 (pharynx OR oropharynx OR throat OR tonsil*)) ((cancer* OR neoplasm* OR carcinoma*) NEAR/3 base NEAR/3 tongue) (tongue NEAR/3 base NEAR/3 (cancer* OR neoplasm* OR carcinoma*))

	"cell-free deoxyribonucleic acid" "cell-free deoxyribonucleic acids" "circulating DNA" "circulating DNAs" cfDNA cirDNA "cell-free RNA" "cell-free RNAs" "cell-free ribonucleic acid" "cell-free ribonucleic acids" "circulating RNA" "circulating RNAs" cirRNA cfRNA proteomics metabolomics genomics transcriptom* exosom*	

Search Name: Liquid biopsy biomarkers - Feb 3 2025
Date Run: 03/02/2025 15:43:14
Comment: Contact: Hedyeh Samady

ID	Search	Hits
#1	[mh "Early Detection of Cancer"] OR [mh "Liquid Biopsy"]	2715
#2	("liquid biopsy" OR "liquid biopsies" OR "fluid biopsy" OR "fluid biopsies"):ti,ab,kw	348
#3	#1 OR #2	3039
#4	[mh "Biomarkers, Tumor"]	8045
#5	(biomarker*):ti,ab,kw	60998
#6	#4 OR #5	65169
#7	#3 AND #6	524
#8	((blood OR serum OR urine OR saliva OR salivary) NEAR/2 biomarker*):ti,ab,kw	18834
#9	#7 OR #8	19282
#10	[mh ^"Molecular Diagnostic Techniques"] OR [mh "MicroRNAs"] OR [mh "Cell-Free Nucleic Acids"] OR [mh "Proteomics"] OR [mh "Metabolomics"] OR [mh "Genomics"] OR [mh "Transcriptome"] OR [mh "Exosomes"]	2574
#11	("molecular diagnostic techniques" OR "microRNA" OR "micro-RNA" OR "micro-RNAs" OR miRNA* OR mRNA* OR "small temporal RNA" OR "small temporal RNAs" OR stRNA* OR "cell free nucleic acid" OR "cell free nucleic acids" OR "circulating nucleic acid" OR "circulating nucleic acids" OR "cell-free DNA" OR "cell-free DNAs" OR "circulatory DNA" OR "circulatory DNAs" OR "cell-free deoxyribonucleic acid" OR "cell-free deoxyribonucleic acids" OR "circulating DNA" OR "circulating DNAs" OR cfDNA OR cirDNA	14627

	OR "cell-free RNA" OR "cell-free RNAs" OR "cell-free ribonucleic acid" OR "cell-free ribonucleic acids" OR "circulating RNA" OR "circulating RNAs" OR cirRNA OR cfRNA OR proteomics OR metabolomics OR genomics OR transcriptom* OR exosom*):ti,ab,kw	
#12	#10 OR #11	14882
#13	#9 AND #12	876
#14	(saliva* NEXT proteomics):ti,ab,kw	0
#15	#13 OR #14	876
#16	[mh ^"Head and Neck Neoplasms"] OR [mh ^"Pharyngeal Neoplasms"] OR [mh "Oropharyngeal Neoplasms"] OR [mh "Squamous Cell Carcinoma of Head and Neck"]	3999
#17	(((pharyngeal OR oropharyngeal OR tonsil*) NEXT (neoplasm* OR cancer*)) OR ((pharyngeal OR oropharyngeal) NEAR/2 carcinoma*) OR ((cancer OR cancers OR neoplasm OR neoplasms OR carcinoma OR carcinomas) NEAR/2 (pharynx OR oropharynx OR throat OR tonsil*)) OR ((cancer* OR neoplasm* OR carcinoma*) NEAR/3 base NEAR/3 tongue) OR (tongue NEAR/3 base NEAR/3 (cancer* OR neoplasm* OR carcinoma*))):ti,ab,kw	1429
#18	#16 OR #17	4778
#19	#15 AND #18	3
#20	#19 in Trials	3

Web of Science Core Collection – SCI-EXPANDED, SSCI, AHCI, ESCI

	Concept: Liquid Biopsy	**Concept: Biomarkers**
Topic (searches title, abstract, author keywords, Keywords Plus®)	"liquid biops*" "fluid biops*"	"biomarker*"
	((blood OR serum OR urine OR saliva OR salivary) NEAR/2 biomarker*)	

"saliva* proteomics"

	Concept: Molecular Diagnostic Techniques	**Concept: Cancers**
Topic (searches title, abstract, author keywords, Keywords Plus®)	"molecular diagnostic techniques" "microRNA*" "micro-RNA*" miRNA* mRNA* "small temporal RNA*" stRNA* "cell free nucleic acid*" "circulating nucleic acid*" "cell-free DNA*" "circulatory DNA*" "cell-free deoxyribonucleic acid*" "circulating DNA*" cfDNA cirDNA "cell-free RNA*" "cell-free ribonucleic acid*" "circulating RNA*" cirRNA cfRNA "proteomics" "metabolomics" "genomics" "transcriptom*" "exosom*"	"pharyngeal neoplasm*" "pharyngeal cancer*" "oropharyngeal neoplasm*" "oropharyngeal cancer*" "tonsil* neoplasm*" "tonsil* cancer*" ((pharyngeal OR oropharyngeal) NEAR/2 carcinoma*) ((cancer OR cancers OR neoplasm OR neoplasms OR carcinoma OR carcinomas) NEAR/2 (pharynx OR oropharynx OR throat OR tonsil*)) ((cancer* OR neoplasm* OR carcinoma*) NEAR/3 base NEAR/3 tongue) (tongue NEAR/3 base NEAR/3 (cancer* OR neoplasm* OR carcinoma*))

(
(
((TS=("liquid biops*" OR "fluid biops*") **AND** TS=("biomarker*")) OR TS=((blood OR serum OR urine OR saliva OR salivary) NEAR/2 biomarker*))

AND TS=("molecular diagnostic techniques" OR "microRNA*" OR "micro-RNA*" OR miRNA* OR mRNA* OR "small temporal RNA*" OR stRNA* OR "cell free nucleic acid*" OR "circulating nucleic acid*" OR "cell-free DNA*" OR "circulatory DNA*" OR "cell-free deoxyribonucleic acid*" OR "circulating DNA*" OR cfDNA OR cirDNA OR "cell-free RNA*" OR "cell-free ribonucleic acid*" OR "circulating RNA*" OR cirRNA OR cfRNA OR "proteomics" OR "metabolomics" OR "genomics" OR "transcriptom*" OR "exosom*"))
OR TS=("saliva* proteomics"))
AND
TS=("pharyngeal neoplasm*" OR "pharyngeal cancer*" OR "oropharyngeal neoplasm*" OR "oropharyngeal cancer*" OR "tonsil* neoplasm*" OR "tonsil* cancer*" OR ((pharyngeal OR oropharyngeal) NEAR/2 carcinoma*) OR ((cancer OR cancers OR neoplasm OR neoplasms OR carcinoma OR carcinomas) NEAR/2 (pharynx OR oropharynx OR throat OR tonsil*)) OR ((cancer* OR neoplasm* OR carcinoma*) NEAR/3 base NEAR/3 tongue) OR (tongue NEAR/3 base NEAR/3 (cancer* OR neoplasm* OR carcinoma*)))
Limits: none
31 results as of 1/28/25

Running note: Run string from the Advanced Search. Set date limits on Advanced Search page or in the results sidebar.

Full names of collections in UW's Web of Science subscription. Include these in appendix/supplementary material.:
Science Citation Index Expanded, 1900-present
Social Sciences Citation Index, 1900-present
Arts & Humanities Citation Index, 1975-present
Emerging Sources Citation Index, 2005-present