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ORIGINAL ARTICLE

Identifying predictive markers of head and neck squamous cell carcinoma in an umbrella trial (KCSG HN 15-16 TRIUMPH trial)

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Background: Recurrent or metastatic head and neck squamous cell carcinoma (HNSCC) is associated with a poor prognosis. Limited treatment options highlight the need for precision therapeutics.

Patients and methods: We investigated the correlation between diverse clinical features and genetic changes using next-generation sequencing data derived from our recent umbrella trial. We analyzed the targeted DNA and RNA sequencing data profiles of 419 patients with HNSCC enrolled in the recent genomic-based umbrella trial. Comprehensive analyses, including survival analysis, were conducted to assess the overall genetic landscape, mutational signature patterns, copy number variations, and their correlation with patient outcomes.

Results: Multiple genomic aberrations served as predictive factors in patients treated with targeted therapies. NOTCH1 mutations and MYC amplification were associated with worse prognosis ($P = 0.0037$ and $P = 0.0016$, respectively). CDKN2A mutations influenced the clinical outcome of patients treated with CDK4/6 inhibitors, with divergent effects based on mutation types (improved survival with deletions and poor survival with SNV/indels). p16 positivity was correlated with a favorable prognosis in patients who underwent immunotherapy during the TRIUMPH trial. Stratification of such groups revealed novel genomic characteristics, such as mutual exclusiveness

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between TP53 and PIK3CA SNV/indels in HPV-positive oropharyngeal cancer, along with a high prevalence of TP53 mutations in young patients with oral-cavity cancer, which were unrelated to germline predisposing mutations, smoking habits, or p16 expression.

Conclusion: Genomic profiling plays a significant role in the management of recurrent or metastatic HNSCC and may help identify potential targets for precision therapeutics.

Key words: head and neck squamous cell carcinoma, genomic profiling, umbrella trial

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) originates in the oral cavity, pharynx, and larynx, and accounts for 5.7% of total cancer-related mortality.[1] Despite advances in treatment, including chemotherapy, immune checkpoint inhibitors, and targeted therapy, managing recurrent/metastatic HNSCC (R/M HNSCC) remains challenging owing to the aggressive nature of the disease and limited treatment options, with a median overall survival of ∼1 year.[2] Currently, pembrolizumab is regarded as first-line therapy for R/M HNSCC, either as monotherapy or in combination with chemotherapy.[3,4]

Mirroring approaches in other cancers, the genomic profiling of HNSCC via multiple studies has identified various key mutations (*TP53*, *CDKN2A*, *PIK3CA*, *NOTCH1*, *HRAS*, and *CASP8*).[5-8] *TP53* is the most frequently mutated gene (60%-70%), followed by *CDKN2A* (40%-60%), *PICK3CA,* and *NOTCH1*, the former two of which are associated with an unfavorable prognosis.[9] Stratification by p16 status showed that HRAS mutations were more frequent in p16-negative tumors, especially those with wild-type TP53. CASP8 mutations co-occurred in some of these cases, suggesting a distinct subgroup.[5] These findings were molecularly and immunologically distinct from p16-positive, HPV-associated tumors.[10] Despite the comprehensive genetic profiling of HNSCC, the use of genetic information to guide treatment decisions remains unclear, necessitating further extensive research to determine the clinical significance and therapeutic implications of the identified mutations.

Herein, we comprehensively analyzed the association between various clinical characteristics and genetic alterations based on next-generation sequencing (NGS) data from our recent umbrella trial TRIUMPH (translational biomarker-driven umbrella project for head and neck and esophageal squamous cell carcinoma; ClinicalTrials.gov identifier: NCT03292250) (Supplementary Figure S1A, available at https://doi.org/10.1016/j.esmoop.2025.105772).[11] In this study, we therefore report the potential stratification and genetic characteristics of NGS-based precision medicine in HNSCC and analyze subgroups of interest for further investigation.

MATERIALS AND METHODS

Study design

The TRIUMPH study was a multicenter, multi-arm, non-randomized, open-label, phase II umbrella clinical trial of biomarker-matched target therapies. The TRIUMPH study delineated its targeted therapeutic strategies and specific molecular targets as follows: arm 1 utilized alpelisib (BYL719), targeting PIK3CA; arm 2 employed poziotinib, aimed at EGFR/HER2; arm 3 involved nintedanib, an inhibitor of FGFR; and arm 4 used abemaciclib, directed against CDK4/6. The detailed information on the study design was described in our previous publication.[12]

Sample and clinical data collection

Samples were collected from patients who provided written informed consent to participate in the TRIUMPH trial. The study was approved by the institutional review boards of 35 Korean institutions. Tumor specimens and matched peripheral blood were collected either at the time of initial diagnosis or at the time of biopsy-confirmed recurrence or metastasis during clinical follow-up, depending on availability. There were no specific restrictions regarding the timing of tissue acquisition to allow for real-world applicability and inclusivity of diverse clinical scenarios.

A total of 419 patients underwent molecular prescreening, among whom 180 had confirmed recurrent or metastatic (R/M) disease at the time of biopsy. Of these, 179 patients were subsequently enrolled into biomarker-driven therapeutic arms of the umbrella trial. The overall patient screening and enrollment process is illustrated in Supplementary Figure S1B, available at https://doi.org/10.1016/j.esmoop.2025.105772.

Comprehensive clinicopathologic data—including age, sex, tumor location, cigarette and alcohol use, clinical stage, prior treatment history, and survival data—were also collected for integrated genomic and clinical analysis. Smoking status (cigarette smoking only) was classified as current (active use at enrolment), former (prior use, stopped before enrolment), or never (<100 cigarettes lifetime). A summary of patient sample availability across different analyses is presented in Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2025.105772.

DNA extraction, RNA extraction, and immunohistochemistry

Formalin-fixed paraffin-embedded (FFPE) tumor sections (5 μm) were macro-dissected, and genomic DNA (gDNA; 0.2 μg per sample) was isolated using the QIAamp DNA FFPE Tissue Kit (QIAGEN, Hilden, Germany; Cat. # 56404). Libraries were prepared with the SureSelectXT HSQ Reagent Kit (Agilent Technologies, Santa Clara, CA; Cat. # G9611C) following the 'Low-Input' protocol and enriched with the

custom panel, which captures the complete coding exons of 244 cancer-relevant genes (1.12 Mb of non-redundant target, Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2025.105772). Sequencing was performed on an Illumina HiSeq 2500 in paired-end 2 × 100 bp mode, achieving a median on-target depth of >1000×.

Peripheral blood was collected in EDTA tubes. Germline DNA (1 μg input) was purified using the Maxwell 16 LEV Blood DNA Kit (Promega, Madison, WI; Cat. # AS1290) and sequenced.

Total RNA (typically >500 ng, adjusted according to extraction yield and RNA Integrity Number) was extracted from FFPE curls using the RNeasy FFPE Kit (QIAGEN; Cat. # 73504).

Immunohistochemistry for p16 was performed with the CINtec® p16 Histology Kit (Roche Diagnostics GmbH, Mannheim, Germany; Cat. # 825-4713) on Ventana Benchmark ULTRA instruments.

Bioinformatics pipeline

Data preprocessing and somatic and germline variant calling. Trimming was performed using fastq with several standards, including poly G, length, complexity, and front tail.[13] The Genome Analysis ToolKit (GATK) Best Practices methodology was used to detect somatic and germline variations. Hard filters were applied with the GATK HardFiltering workflow (Broad Institute, Cambridge MA; https://gatk.broadinstitute.org/hc/en-us/articles/360035890471). Germline variants with population allele frequency >0.001 were excluded. Annotations were generated using vcf2maf v1.6.20 (Cleveland Clinic Lerner Research Institute, USA; Zenodo DOI 10.5281/zenodo.1185418).[14] ENCODE blacklist regions were removed before downstream analysis.[15] Reads were aligned with BWA-MEM v0.7.17 to the GRCh38/hg38 reference genome (UCSC build), and all variant and copy-number analyses were conducted in this coordinate space.

Copy number variant calling, mutational signature analysis, visualization, oncogenic pathway, and microsatellite instability analysis. Copy number variants were analyzed using the CNVkit with the batch option.[16] The results were altered using the CNVkit filter cn option, which merges nearby values with the same called values. Genes with a copy number >4 were classified as amplified genes, whereas those with 0 copies were classified as deleted. Gene annotations were made based on the UCSC reference.

Mutational signatures were inferred with SigMA (v 0.3.6), a supervised machine-learning tool tailored for targeted panels.[17] Somatic SNVs called by GATK Mutect2 were converted to 96-channel profiles and compared with COSMIC v3 references using the HNSCC-optimized TCGA-MC3 model. Samples passing SigMA's multivariate-analysis (MVA) filter were assigned signatures on the basis of cosine similarity and cohort-level activities were computed.

Visualization including 'Oncoprint,' 'Heatmap,' and 'Lollipop plots' were drawn using the ComplexHeatmap (Gu et al., 2016, iMeta; https://onlinelibrary.wiley.com/doi/10.1002/imt2.43) and maftools packages (Mayakonda et al., 2018, Genome Medicine; PMID 30341162) in R.[18,19] Additional graphs were visualized using the ggplot2, ggsignif, ggradar, ggsci, gridtext, and gridExtra packages in R.[20,21] The oncogenic pathway was determined based on the findings of previous studies.[12,22] Microsatellite instability (MSI) was confirmed using MSIsensor, with a threshold of 3.5 distinguishing between MSI and microsatellite stable (MSS) statuses.[23]

NanoString assay and analysis

Extracted RNA was profiled on the nCounter Analysis System (NanoString Technologies, Seattle, WA) using a custom CodeSet (XT-GXA-P1CS-384; NanoString, supplied by Phil-Korea, Seoul, Republic of Korea) that measures the expression of 93 immune- and cancer-related genes. Counts were filtered using a negative probe in nSolver software ver. 4.0 to remove outliers. The geometric mean of the positive probe and housekeeping genes were used for data normalization. The expression levels in Supplementary Figure S3A, available at https://doi.org/10.1016/j.esmoop.2025.105772, were log2 normalized.

The annotation for each profile was provided by NanoString. T-cell function-related genes included *CD2, CD27, CD274, CD38, CD3E, CD3G, CD80, CD86, CD8A, CTLA4, CXCL10, CXCL9, CXCR5, IDO1, IFNG, IL18, IRF1, LAG3, LCK,* and *TIGIT*. Chemokine genes included *CCL5, CX3CR1, CXCL10, CXCL13, CXCL9, CXCR5, CXCR6, IL2RG, IRF1*, and *STAT1*.

Statistical and survival analysis

All statistical analyses were conducted in R (v4.3.3). Categorical variables were analyzed using Fisher's exact test or chi-square test, while continuous variables were evaluated with *t*-test, Wilcoxon rank sum test, or Kruskal—Wallis test. Multiple testing corrections were applied using the p.adjust function with Bonferroni correction. Survival analysis utilized the Kaplan—Meier method, log-rank test, and Cox regression analysis via the 'survival' package.

Ethics approval and consent to participate

In accordance with the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice, ethical approval for the use of human subjects was obtained from the Institutional Review Board of Severance Hospital (4-2017-0695), Institutional Review Board of Keimyung University Dongsan Hospital (DSMC 2017-09-043), National Cancer Center Institutional Review Board (NCC2017-0063), Institutional Review Board (IRB) of Samsung Medical Center (SMC 2017-09-076), Institutional Review Board at Uijeongbu St. Mary's Hospital, Catholic University of Korea (2017-5961-0002), Institutional Review Board of Chungnam National University Hospital (CNUH 2017-09-038), Institutional Review Board of Chonnam National University Hwasun Hospital (CNUHH-2017-138), Institutional Review Board of Chosun University

Hospital (CHOSUN 2017-10-009-001), Institutional Review Board of The Catholic University of Korea Yeouido St. Mary's Hospital (2017-5961-002), Ethics Committee of Kangdong Sacred Heart Hospital (KANGDONG 2017-09-006), Institutional Review Board of Korea University Guro Hospital (KUGH17269-001), Institutional Review Board of CHA Bundang Medical Center (CHAMC 2017-11-022-002), Ajou University Hospital Institutional Review Board (AJIRB-MED-CT2-17-303), Institutional Review Board of Kangbuk Samsung Hospital (KBSMC 2017-10-026-001), Institutional Review Board (IRB) of Gyeongsang National University Hospital (KYUH 2018-01-013-002), Institutional Review Board (IRB) of the Ewha Womans University Mokdong Hospital (EUMC 2017-09-030-002), Institutional Review Board (IRB) of Inha University Hospital (INHAUH 2017-10-007-001), Institutional Review Board in Inje University Busan Paik Hospital (18-0186), Institutional Review Board (IRB) of Gil Medical Center, Gachon University (GBIRB 2017-382), Institutional Review Board of Catholic Kwandong University, International St. Mary's Hospital (17YeonIRB055-1), Institutional Review Board (IRB) of the Dongnam Institute of Radiological & Medical Sciences (D-1710-002-001), Institutional Review Board of Bucheon St. Mary's Hospital, the Catholic University of Korea (2017-5690-0001), Institutional Review Board (IRB) of Seoul National University Bundang Hospital (B-1710/426-404), Institutional Review Board (IRB) for Clinical Research at Seoul National University Hospital (H-1704-087-846), Institutional Review Board of Asan Medical Center (S2017-1858-0001), Institutional Review Board of St. Vincent Hospital, the Catholic University of Korea (2017-5678-0001), Institutional Review Board of Soonchunhyang University Hospital Cheonan (SCHCA 2017-09-009-001), Pusan National University Hospital Institutional Review Board (IRB) (05-2017-143), Institutional Review Board (IRB) of Yeungnam University College of Medicine (YUMC 2017-10-035), Institutional Review Board of Wonju Severance Christian Hospital (2017-09-0274), Institutional Review Boards of Incheon St. Mary's Hospital, the Catholic University of Korea (2017-5786-0005), Institutional Review Board of Chung-Ang University Hospital (1791-008-298), Institutional Review Board of Wonkwang University Hospital (WKUH 2017-10-001), Institutional Review Board (IRB) of Kosin University Gospel Hospital (KUGH 2018-02-020), and Institutional Review Board/Ethics Committee of Hallym University Chuncheon Sacred Heart Hospital (2017-I131) in Korea. All participants provided written informed consent for genomic testing.

RESULTS

Clinical characteristics of patients

After prescreening, the genomic and clinical information of 419 patients from the TRIUMPH trial was used in this study. The baseline characteristics of these patients are summarized in Table 1. Among the 419 patients, 276 (65.8%) were current or former cigarette somkers (see Methods). The median age was 61 years, and most patients (88%, n = 368/419) were men. The primary tumor sites were the oral cavity (35%, n = 145), hypopharynx (20%, n = 82), oropharynx (19%, n = 78), and larynx (18%, n = 75). p16 status was assessed in 200 patients, with 60 (30%) testing positive. The majority of p16-positive HNSCC cases were oropharyngeal cancer (76%, n = 42/55).

Genomic and transcriptomic landscape of HNSCC

We analyzed the overall patterns of somatic mutations in HNSCC, including somatic single nucleotide variants (SNVs), insertion/deletions (indels), and amplifications (Figure 1A). The most frequently mutated genes were consistent with those reported in previous studies,[7,24,25] including *TP53* (71%, n = 296/419), *CDKN2A* (27%, n = 112/419), *PIK3CA* (26%, n = 110/419), *FAT1* (23%, n = 95/419), and *EGFR* (16%, n = 69/419). We also observed recurrent mutations on hotspot sites, such as gain-of-function mutations in *PIK3CA* (p.E545K/A/G, p.E542K, and p.H1047R/L) and truncating mutations in *CDKN2A* (p.R80*, p.W110*, and p.

Table 1. Baseline characteristics of 419 patients prescreened for the TRIUMPH trial

Variable	Level	Overall	Oral cavity	Hypopharynx	Oropharynx	Larynx	Maxillary sinus	Nasal cavity
n		419	145	82	78	75	23	16
Age, years		60.9 (11.3)	57.7 (13.5)	64.3 (9.7)	60.5 (8.3)	64.9 (8.1)	58.7 (10.3)	57.8 (13.7)
Sex	F	64 (15.5)	46 (31.9)	5 (6.2)	5 (6.6)	1 (1.4)	4 (17.4)	3 (18.8)
	M	350 (84.5)	98 (68.1)	76 (93.8)	71 (93.4)	73 (98.6)	19 (82.6)	13 (81.2)
Stage	1	28 (7.8)	13 (10.1)	1 (1.3)	5 (7.4)	7 (12.7)	0 (0.0)	2 (18.2)
	2	23 (6.4)	9 (7.0)	6 (8.0)	4 (5.9)	3 (5.5)	0 (0.0)	1 (9.1)
	3	53 (14.8)	20 (15.5)	8 (10.7)	14 (20.6)	5 (9.1)	3 (14.3)	3 (27.3)
	4A	222 (61.8)	77 (59.7)	48 (64.0)	38 (55.9)	37 (67.3)	18 (85.7)	4 (36.4)
	4B	12 (3.3)	5 (3.9)	6 (8.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (9.1)
	4C	21 (5.8)	5 (3.9)	6 (8.0)	7 (10.3)	3 (5.5)	0 (0.0)	0 (0.0)
Smoking status	Current smoker	66 (15.8)	23 (15.9)	20 (24.4)	12 (15.4)	8 (10.7)	1 (4.3)	2 (12.5)
	Former smoker	210 (50.1)	63 (43.4)	39 (47.6)	50 (64.1)	44 (58.7)	9 (39.1)	5 (31.2)
	Never smoker	119 (28.4)	51 (35.2)	20 (24.4)	13 (16.7)	17 (22.7)	11 (47.8)	7 (43.8)
	Not available	24 (5.7)	8 (5.5)	3 (3.7)	3 (3.8)	6 (8.0)	2 (8.7)	2 (12.5)
p16 status	Positive	60 (14.3)	9 (6.2)	4 (4.9)	42 (53.8)	5 (6.7)	0 (0.0)	0 (0.0)
	Negative	140 (33.4)	55 (37.9)	37 (45.1)	13 (16.7)	23 (30.7)	7 (30.4)	5 (31.2)
	Not available	219 (52.3)	81 (55.9)	41 (50.0)	23 (29.5)	47 (62.7)	16 (69.6)	11 (68.8)

Numbers are *n* (%) unless otherwise noted. Smoking status (cigarette smoking only): current = active use at enrolment; former = prior use, stopped before enrolment; never = <100 cigarettes over a lifetime; not available = not recorded.

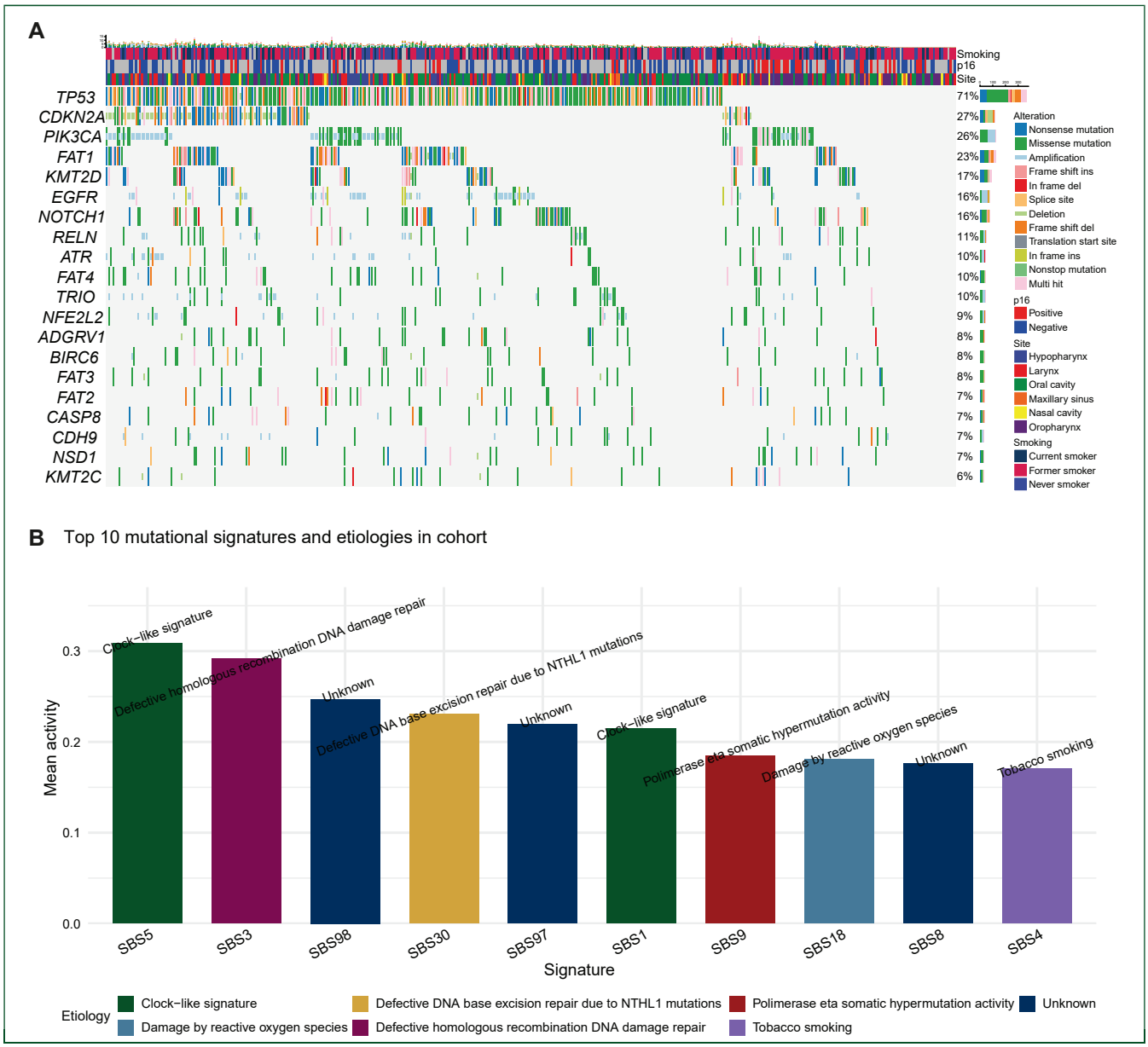

Figure 1. Overall genomic landscape and mutational signatures of HNSCC. (A) An oncoprint illustrating the overall mutational pattern of HNSCC, featuring the top 20 mutated genes. (B) Top 10 cohort-level mutational-signature profile. Stacked bar chart displaying the mean contribution of the 10 most prevalent COSMIC SBS signatures across the TRIUMPH cohort. Smoking status (cigarette smoking only): current = active use at enrolment; former = prior use, stopped before enrolment; never = <100 cigarettes lifetime. (Unknown = not recorded).

X51_splice) (Supplementary Figure S3, available at https://doi.org/10.1016/j.esmoop.2025.105772).[26]

We frequently detected amplifications in *PIK3CA* (15%), *EGFR* (13%), *FGFR1* (6%), and *CCND1* (25%). A clear correlation was observed between the copy number gain and increased expression of *EGFR* ($P = 2.20 \times 10^{-16}$), *ERBB2* ($P = 2.63 \times 10^{-9}$), and *PIK3CA* ($P = 0.0011$) (Supplementary Figure S4A, available at https://doi.org/10.1016/j.esmoop.2025.105772), except that of *FGFR1*. Notably, *EGFR* amplification resulted in a more substantial increase in expression than *PIK3CA* amplification (Supplementary Figure S4B, available at https://doi.org/10.1016/j.esmoop.2025.105772).

Mutational-signature analysis using Signature Multivariate Analysis (SigMA) revealed a dominance of SBS5 (clock-like), followed by SBS3 (homologous-recombination deficiency), SBS98 (unknown), SBS30 (base-excision-repair defect), SBS97 (unknown), SBS1 (clock-like), SBS9 (polymerase-η hypermutation), SBS18 (reactive-oxygen damage), SBS8 (unknown) and SBS4 (tobacco smoke) (Figure 1B). Per-sample signature contributions are presented in Supplementary Figure S5, available at https://doi.org/10.1016/j.esmoop.2025.105772. Collectively, this signature spectrum underscores the heterogeneous mutational processes active in HNSCC.

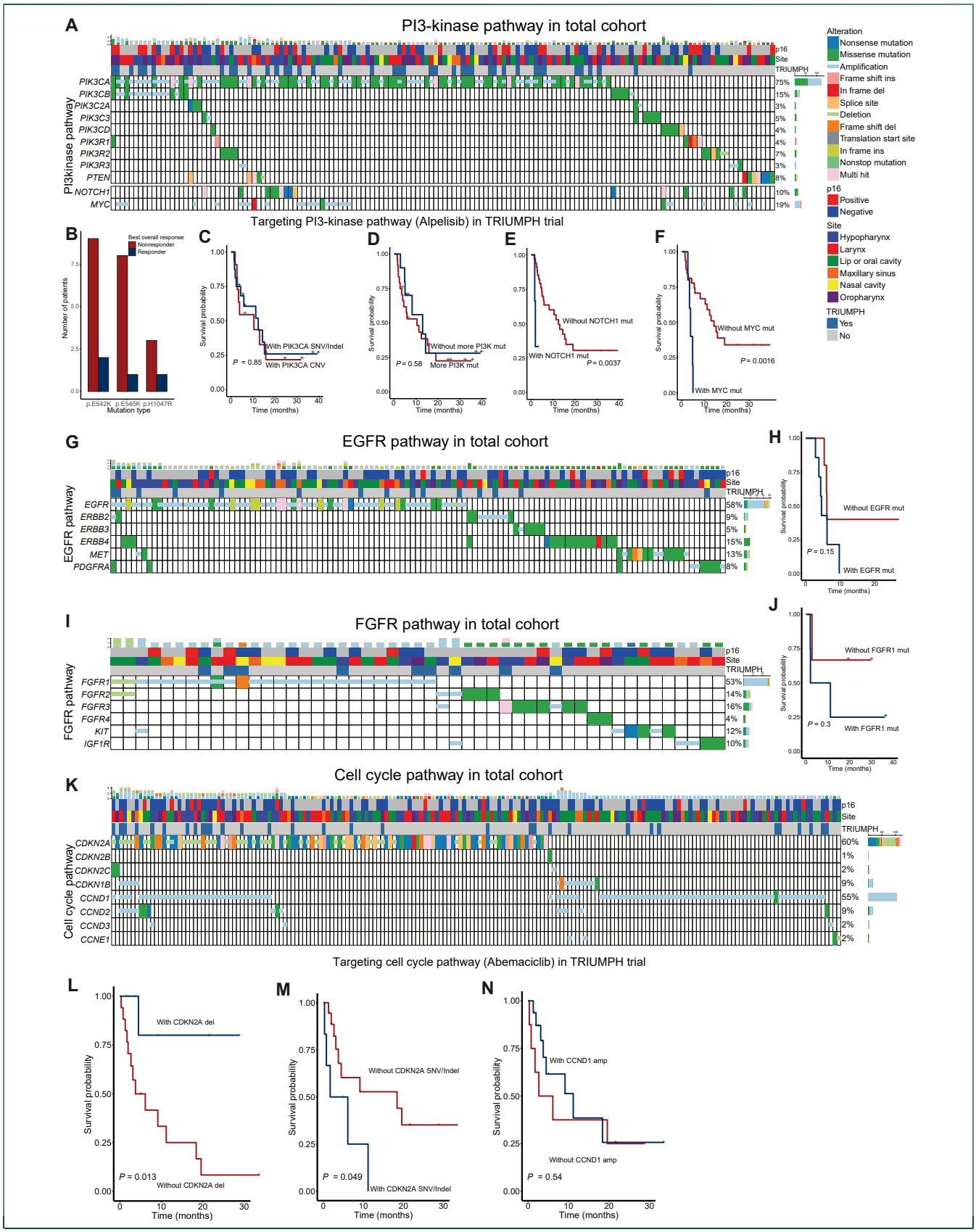

Figure 2. Potential therapeutic targeted pathways and genetic association with clinical outcome. The mutational patterns of potential therapeutic targeted pathways include (A) the PI3K, (G) EGFR, (I) FGFR, and (K) cell cycle pathways. (B) Best overall response of alpelisib according to PIK3CA mutation types. (C) Kaplan–Meier survival curves of patients with PIK3CA SNV/indel versus patients with PIK3CA amplification among individuals treated with alpelisib. (D) Kaplan–Meier survival curves of patients with PIK3CA mutations with or without concurrent mutations in the PI3K pathway among individuals treated with alpelisib. (E) Kaplan–Meier survival curves comparing patients with NOTCH1 mutations to those without among individuals treated with alpelisib. (F) Kaplan–Meier survival curves comparing patients with MYC mutations with those without among individuals treated with alpelisib. (H) Kaplan–Meier survival curves comparing patients with EGFR mutations with those without among individuals treated with poziotinib. (J) Kaplan–Meier survival curves comparing patients with FGFR mutations with

Genetic association with clinical outcomes

We analyzed 419 patients bearing genomic alterations [including SNVs, indels or copy number variations (CNVs)] in the PI3K, EGFR/HER2, FGFR, or cell cycle pathways. First, we characterized the overall frequency of these alterations across the cohort; next, we examined the survival data for patients who received the corresponding targeted therapy in the TRIUMPH trial. Although some individuals with the relevant genomic alteration did not ultimately enroll in that specific arm—and overlaps among pathways were also observed—this approach provides a broad overview of how certain coexisting mutations can significantly influence therapeutic outcomes. Patient-level details of qualifying alterations, matched treatment arms, oncogenic pathways and best overall RECIST responses are summarized in Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2025.105772.

Among 146 patients (34.8%) with PI3K-pathway alterations (*PIK3CA, PIK3CB, PIK3C2A, PIK3C3, PIK3CD*, or *PIK3R1*), 110 (75%) harbored *PIK3CA* mutations, most frequently E545K (18.2%), E542K (14.5%), or H1047R (6.4%) (Figure 2A). However, in those treated with a *PIK3CA* inhibitor in the TRIUMPH trial, neither these hotspot variants ($P = 1.00$, Fisher's exact test; Figure 2B), nor alteration type (SNV/indel versus amplification; Figure 2C), nor co-occurrence of multiple PI3K-pathway mutations (Figure 2D) correlated with survival. By contrast, *NOTCH1* mutations ($P = 0.0037$, log-rank test; Figure 2E) and *MYC* amplification ($P = 0.0016$, log-rank test; Figure 2F) were associated with worse survival, indicating that non-PI3K-pathway factors may impact outcomes.

Of the 119 patients (28.4%) with EGFR/HER2-pathway alterations (*EGFR, ERBB2, ERBB3, ERBB4, MET, PDGFRA*), *EGFR* was the most frequently altered gene (58%, $n = 69$), followed by *ERBB4* (15.1%, $n = 18$), *ERBB2* (9.2%, $n = 11$), and *ERBB3* (5%, $n = 6$) (Figure 2G). None of these individual alterations significantly influenced survival in patients who received EGFR/HER2-targeted therapy ($P = 0.15$; Figure 2H). Similarly, among the 49 patients (11.7%) carrying *FGFR1-4* alterations, *FGFR1* was most common (53.1%, $n = 26$), followed by *FGFR3* (16.3%, $n = 8$), *FGFR2* (14.3%, $n = 7$), and *FGFR4* (4.1%, $n = 2$) (Figure 2I). In those treated with FGFR inhibitors, none of these specific alterations were associated with survival (Figure 2J).

In the cell cycle pathway, 187 individuals (44.6%) exhibited alterations in *CDKN2A* (59.9%, $n = 112$) or *CCND1* (54.5%, $n = 102$) (Figure 2K). Although *CCND1* amplification did not correlate with clinical outcome (Figure 2N), *CDKN2A* deletions were associated with prolonged survival ($P = 0.013$), whereas *CDKN2A* SNV/indels were linked to poorer survival ($P = 0.049$) in patients treated with CDK4/6 inhibitors (Figure 2L and M). Multivariable Cox regression analyses (Supplementary Table S3A-D, available at https://doi.org/10.1016/j.esmoop.2025.105772) corroborated these findings, underscoring the importance of assessing both the presence and subtype of genetic alterations to refine therapeutic decision-making.

Genomic and clinical characteristics of p16 status in oropharyngeal cancer

HPV status is the primary factor in the classification of oropharyngeal cancer. The expression of the p16 protein can be utilized to determine HPV status in these cancers.[27,28] We further supplemented HPV status by conducting HPV genotyping. We analyzed the genetic and clinical characteristics between HPV-positive and -negative subgroups of patients with oropharyngeal cancer. As previously reported, the *TP53* mutation rate was higher in HPV-negative (11/16, 68.8%) than in HPV-positive (4/43, 9.3%) patients (Figure 3A). Notably, we observed mutual exclusiveness between *TP53* and *PIK3CA* SNV/indel in p16-negative patients (Fisher's exact test, $P = 0.0018$, Figure 3B), confirming the role of *PIK3CA* as a driver in *TP53*-negative, HPV-negative oropharyngeal cancer.[29] In contrast, we did not observe any such pattern in HPV-positive patients ($P = 0.43$, Figure 3C).

Transcriptomic patterns were also associated with HPV status (Figure 3D). In HPV-positive patients, we identified the upregulation of genes associated with immune processes and chemokines (Figure 3D and E), including *IL2RG*, *BTN1A2*, and *PTPN7*. Similarly, interferon-gamma (*IFNG*) was upregulated in p16-positive patients across the total cohort (Supplementary Figure S6A, available at https://doi.org/10.1016/j.esmoop.2025.105772), as previously reported.[11,30] The geometric mean expression of T-cell function-related genes and cytotoxic cytokines was increased in p16-positive patients (Supplementary Figure S6B-D, available at https://doi.org/10.1016/j.esmoop.2025.105772), suggesting a favorable response to immunotherapy. HPV-positive patients with oropharyngeal cancer who were initially treated with immunotherapy in arm 5 showed prolonged survival outcomes compared with those treated with other targeted therapies in the umbrella trial (Figure 3F, log-rank test, $P = 0.064$).

Genomic characteristics of young patients with HNSCC

The etiology of oral-cavity cancer in young individuals remains unclear.[31] We investigated the clinical and genomic characteristics of young patients with HNSCC (age $\leq$40 years) who participated in the trial. Of the 21 patients studied, 16 (76%) were diagnosed with oral-cavity cancer. The prevalence of *TP53* mutations in young patients (11/16, 69%) was comparable to that in the older oral-cavity cancer group (101/129, 78%) (Fisher's exact test, $P = 0.28$, Figure 4A and C). Additionally, we did not detect any

those without among individuals treated with nintedanib. (L) Kaplan—Meier survival curves comparing patients with CDKN2A deletions with those without among individuals treated with abemaciclib. (M) Kaplan—Meier survival curves comparing patients with CDKN2A SNV/indels with those without among individuals treated with abemaciclib. (N) Kaplan—Meier survival curves comparing patients with CCND1 copy number alterations with those without among individuals treated with abemaciclib.

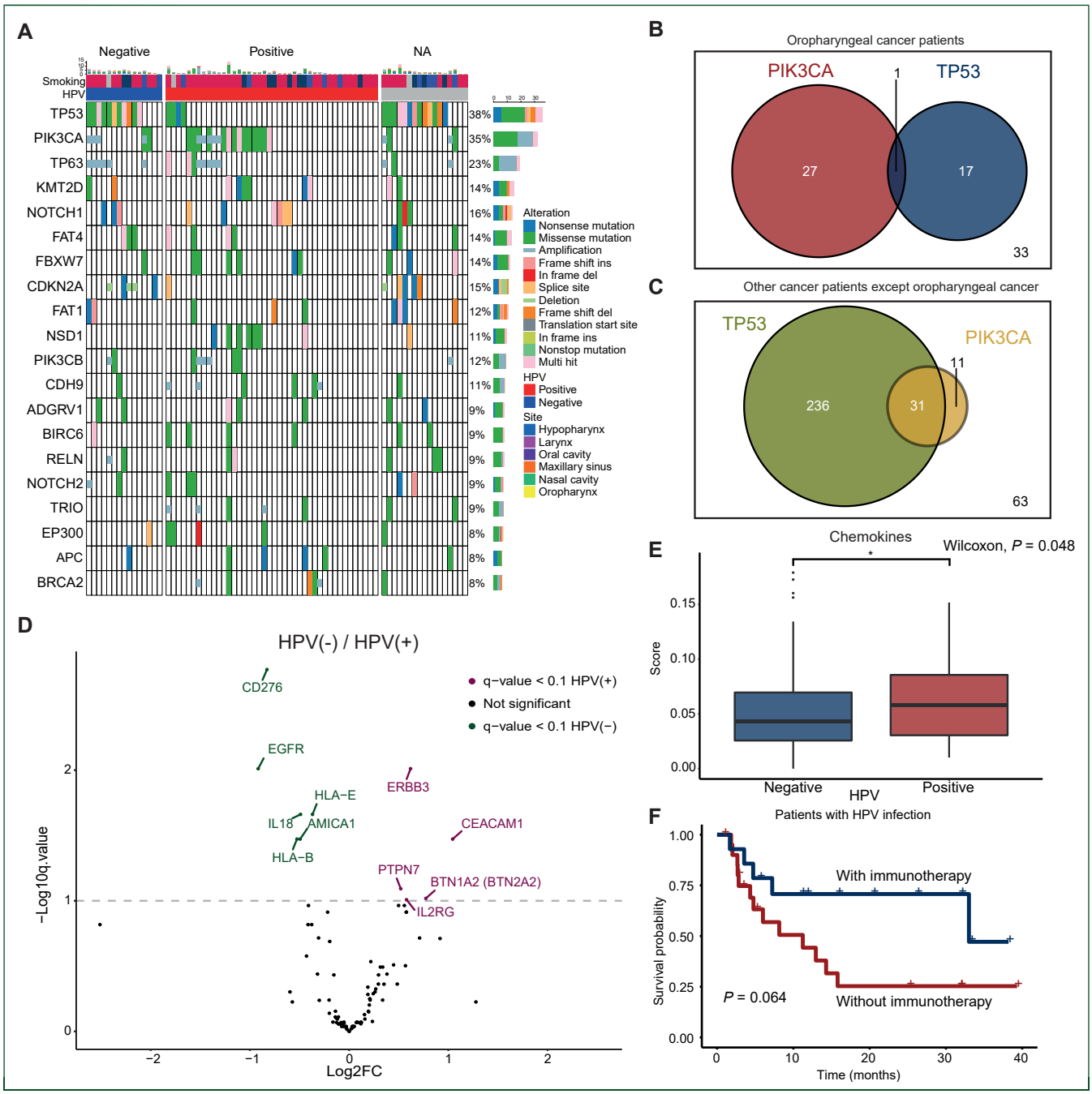

Figure 3. Characteristics of HPV-related HNSCC. (A) Comparison of mutational patterns between HPV-positive and -negative patients. (B) Venn diagram illustrating the co-occurrence of SNV/indel mutations in PIK3CA and TP53 in patients with oropharyngeal cancer. (C) Venn diagram showing the relationship between SNV/indel mutations in PIK3CA and TP53 in patients with other types of cancer. (D) Volcano plot representation of differential expression analysis between HPV-positive and -negative patients with HNSCC. (E) Geometric mean of chemokine expression between HPV-positive and -negative patients with HNSCC. (F) Kaplan—Meier survival curves of HPV-positive and -negative patients with HNSCC who initially underwent immunotherapy.

significant difference in smoking history between the young (6/14, 43%) and older (78/121, 64%) groups; in fact, the young group included a higher proportion of nonsmokers than the older group (Fisher's exact test, $P = 0.15$, Figure 4D). None of the patients exhibited notable recurrent germline variants (Figure 4B), thereby ruling out the presence of congenital genetic susceptibility factors. In addition, all patients who underwent p16 status evaluation were negative (6 of 6, Figure 4E). Microsatellite instability (MSI) was absent in all evaluable tumors from young patients (0/5) and present in 13 of 79 tumors from older patients (16%), although this difference was not statistically significant (Fisher's exact test, $P = 1.00$; Figure 4F).[17] The high prevalence of oral-cavity cancer in young patients implies heterogeneous origins beyond known genetic and other risk factors, warranting further exploration.

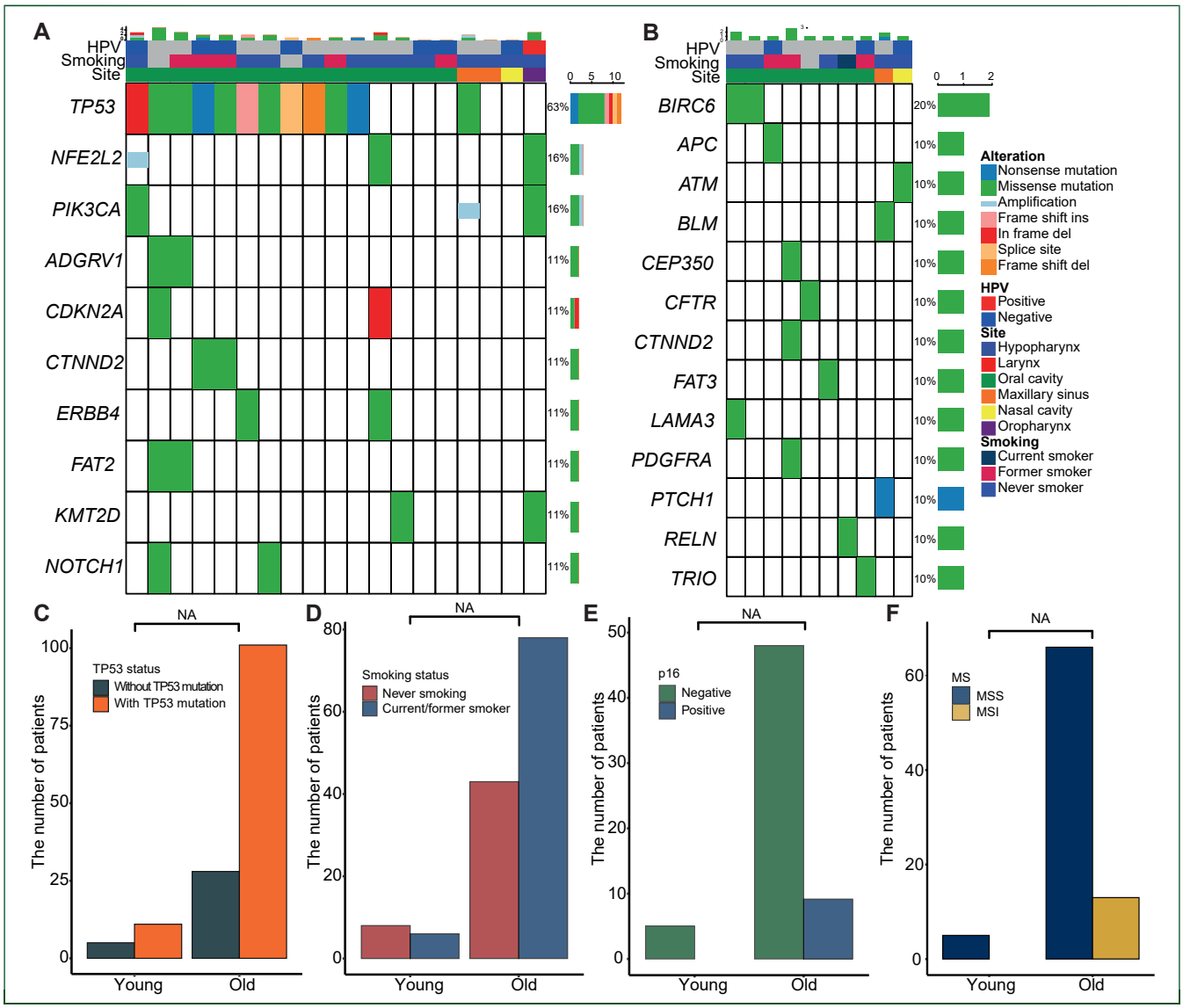

Figure 4. Patients with HNSCC with young age and oral squamous cell carcinoma. (A) Somatic mutations among patients aged <40 years. (B) Germline variants among patients aged <40 years. (C) Number of patients categorized by TP53 mutation status, differentiated between young and old patients. (D) Number of patients categorized by smoking status, differentiated between young and old patients. (E) Number of patients categorized by p16 status, differentiated between young and old patients. (F) Number of patients categorized by microsatellite instability status (MSS versus MSI) differentiated between young and old patients. Smoking status (cigarette smoking only): current = active use at enrolment; former = prior use, stopped before enrolment; never = <100 cigarettes lifetime. (Unknown = not recorded).

DISCUSSION

This study presents the comprehensive genomic analysis of patients with HNSCC using NGS data. Our study, involving the genetic analysis of 419 patients with HNSCC recruited from our umbrella study, revealed clinically relevant targeted pathways and identified potential therapeutic candidates. In addition to the prespecified arms, recurrent alterations were observed in the AKT-mTOR, NOTCH, RAS-RAF, and TP53 pathways, which may represent candidate axes for future targeted strategies (Supplementary Figure S7, available at https://doi.org/10.1016/j.esmoop.2025.105772).

To determine the variables influencing patient outcomes, we examined both survival data and the molecular status. We identified a link between *EGFR* amplification and elevated *EGFR* expression, which was associated with worse patient survival. The strong association between *EGFR* amplification and expression suggested a potential association with the efficacy of cetuximab in recurrent or metastatic HNSCC.[32]

We established a connection between the genomic landscape and clinical outcomes of patients receiving targeted therapies in our umbrella trial, revealing several significant findings. Notably, *NOTCH1* mutations and *MYC* amplifications correlated with worse survival in patients treated with PIK3CA-targeted agents. These alterations have previously been implicated in conferring resistance to PI3K inhibitors,[33,34] although they may also modulate responsiveness to other therapeutic modalities, including chemotherapy. Our data thus underscore that mutations outside the designated target pathway can substantially influence clinical outcomes.

By contrast, although *CDKN2A* mutations did not predict poor survival in multivariable Cox regression across the entire TRIUMPH cohort, patients harboring *CDKN2A* SNVs/indels exhibited unfavorable outcomes when treated with CDK4/6 inhibitors. Collectively, these observations emphasize the complexity of treatment responses in HNSCC and highlight that both on-target and off-target genetic changes can shape patient prognosis. Consequently, comprehensive mutation profiling—beyond the pathway of interest—remains essential for optimizing therapeutic strategies in precision oncology.

HPV infection plays a pivotal role in HNSCC pathogenesis, particularly in oropharyngeal cancers. Genetic variations in HNSCC can be identified through *TP53* mutations, closely related to p16 status. *TP53* mutations are frequently absent in p16-positive HNSCC tumors, reducing heterogeneity. Somatic mutations in *PIK3CA* appear to be more important in the etiology of p16-positive HNSCC in the absence of *TP53* mutation. In our cohort, p16-positive HNSCC tumors showed increased expression of *IFNG,* cytotoxic cytokine genes, and chemokine-related cytokines. In addition, immunotherapy has demonstrated good prognosis for patients with p16-positive HNSCC.

In our examination of germline variants, no distinct patterns or recurrent variants were observed according to the primary sites or within the subgroup of young patients. However, the prevalence of *TP53* mutations remained high in young patients with oral-cavity cancer. Notably, these young patients did not exhibit a high prevalence of p16 positivity, nor did they have a history of current/former smoking. Mutational-signature analysis revealed a diverse palette of genomic processes, underscoring the heterogeneous etiology of HNSCC. Notably, neither microsatellite instability, pathogenic germline variants, nor a history of tobacco use was enriched among young patients with oral-cavity cancer. This paucity of canonical risk factors highlights the complex and as-yet-unresolved origins of oral tumors in this age group. Comprehensive, integrative studies will therefore be required to clarify the unique pathogenic mechanisms driving young-onset oral-cavity cancer.

Our study had several limitations. First, the analysis was restricted to targeted gene sequencing and expression data, which may have limited the scope of our genomic landscape and mutational signature analysis compared with whole-exome or whole-genome approaches. Second, although most tumor specimens were obtained at the time of initial diagnosis, some patients may have received systemic therapy before enrollment, and comprehensive treatment histories were not uniformly available. Third, because sequencing was performed only once, it provided a single molecular snapshot, and subsequent tumor evolution may have gone undetected. Finally, the number of subgroups analyzed was relatively small, resulting in limited statistical power for certain comparisons.

In conclusion, in this study, we conducted a large-scale genomic analysis of patients with HNSCC and identified several genetic traits associated with clinical features. Despite the challenges posed by HNSCC heterogeneity, our findings provide valuable insights into the treatment of real-world patients through detailed genomic profiling.

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DISCLOSURE

SK is a cofounder of AIMA Inc., which seeks to develop techniques for early cancer diagnosis based on circulating tumor DNA. SBK has received institutional research funding from Novartis and Sanofi-Aventis; has served in consultancy and advisory roles for Novartis, AstraZeneca, Lilly, Dae Hwa Pharmaceutical Co. Ltd, ISU Abxis, Daiichi-Sankyo, and Ensol Biosciences; holds equity ownership in Genopeaks. HRK received honoraria from AstraZeneca, Bristol Myers Squibb, Genentech/Roche, stock ownership in Bridgebio Therapeutics; served as a consultation or advisory role for Bayer, AstraZeneca, Bristol Myers Squibb, Takeda, and Yuhan; and received research funding from the Yonsei Lee Youn Jae Fellowship outside of the current work. MHH received honoraria from AstraZeneca, Amgen, BMS, MSD, Ono Pharmaceutical, Takeda, and Roche; served in a

consulting or advisory role for AstraZeneca, BMS, MSD, Pfizer, Takeda, Roche, and Yuhan; acted as an investigator or coinvestigator of trials for Abbvie, AstraZeneca, BMS, IMPACT Therapeutics, Ignyta, Loxo Oncology, Merck Sereno, MSD, Novartis, ORIC, Roche, Pfizer, and Yuhan; and received research support from AstraZeneca, MSD, Novartis, and Yuhan. All other authors have declared no conflicts of interest.

DATA SHARING

The genomic and clinical datasets generated during this study are stored in a controlled-access repository maintained by the Korea Cancer Study Group (KCSG). De-identified data will be provided for academically sound proposals that receive approval from the KCSG Data-Sharing Committee and the requester's institutional-review board. Inquiries should be directed to the corresponding author.

DECLARATION OF GENERATIVE AI IN SCIENTIFIC WRITING

The authors used ChatGPT during the preparation of the manuscript to improve the readability and language of the manuscript. After using this tool/service, the authors have reviewed and edited the content as needed and take full responsibility for the content of the published article.

REFERENCES

1. Patterson RH, Fischman VG, Wasserman I, et al. Global burden of head and neck cancer: economic consequences, health, and the role of surgery. *Otolaryngol Head Neck Surg*. 2020;162(3):296-303.
2. Burtness B, Harrington KJ, Greil R, et al. Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): a randomised, open-label, phase 3 study. *Lancet*. 2019;394 (10212):1915-1928.
3. Ferris RL, Blumenschein G Jr, Fayette J, et al. Nivolumab for recurrent squamous-cell carcinoma of the head and neck. *N Engl J Med*. 2016;375(19):1856-1867.
4. Cohen EEW, Soulières D, Le Tourneau C, et al. Pembrolizumab versus methotrexate, docetaxel, or cetuximab for recurrent or metastatic head-and-neck squamous cell carcinoma (KEYNOTE-040): a randomised, open-label, phase 3 study. *Lancet*. 2019;393(10167):156-167.
5. The Cancer Genome Atlas Network. Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature*. 2015;517(7536):576-582.
6. Agrawal N, Frederick MJ, Pickering CR, et al. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. *Science*. 2011;333(6046):1154-1157.
7. Stransky N, Egloff AM, Tward AD, et al. The mutational landscape of head and neck squamous cell carcinoma. *Science*. 2011;333(6046): 1157-1160.
8. Pickering CR, Zhang J, Yoo SY, et al. Integrative genomic characterization of oral squamous cell carcinoma identifies frequent somatic drivers. *Cancer Discov*. 2013;3(7):770-781.
9. Zhou G, Liu Z, Myers JN. TP53 mutations in head and neck squamous cell carcinoma and their impact on disease progression and treatment response. *J Cell Biochem*. 2016;117(12):2682-2692.
10. Bodelon C, Untereiner ME, Machiela MJ, Vinokurova S, Wentzensen N. Genomic characterization of viral integration sites in HPV-related cancers. *Int J Cancer*. 2016;139(9):2001-2011.
11. Hu T, Yang P, Zhu H, et al. Accumulation of invariant NKT cells with increased IFN-γ production in persistent high-risk HPV-infected high-grade cervical intraepithelial neoplasia. *Diagn Pathol*. 2015;10(1):20.
12. Keam B, Hong MH, Shin SH, et al. Personalized biomarker-based umbrella trial for patients with recurrent or metastatic head and neck squamous cell carcinoma: KCSG HN 15-16 TRIUMPH trial. *J Clin Oncol*. 2024;42(5):507-517.
13. Chen S, Zhou Y, Chen Y, Gu J. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*. 2018;34(17):i884-i890.
14. Kandoth C, Gao JJ, Mattioni M, et al. mskcc/vcf2maf: vcf2maf v1.6.19. 2020. Available at https://zenodo.org/records/1185418. Accessed September 8, 2025.
15. Amemiya HM, Kundaje A, Boyle AP. The ENCODE blacklist: identification of problematic regions of the genome. *Sci Rep*. 2019;9(1). 9354-9354.
16. Talevich E, Shain AH, Botton T, Bastian BC. CNVkit: genome-wide copy number detection and visualization from targeted DNA sequencing. *PLOS Comput Biol*. 2016;12(4):e1004873.
17. Gulhan DC, Lee JJ, Melloni GEM, Cortés-Ciriano I, Park PJ. Detecting the mutational signature of homologous recombination deficiency in clinical samples. *Nat Genet*. 2019;51(5):912-919.
18. Gu Z. Complex heatmap visualization. *Imeta*. 2022;1(3):e43.
19. Mayakonda A, Lin DC, Assenov Y, Plass C, Koeffler HP. Maftools: efficient and comprehensive analysis of somatic variants in cancer. *Genome Res*. 2018;28(11):1747-1756.
20. Ahlmann-Eltze C, Patil I. ggsignif: R package for displaying significance brackets for 'ggplot2'. *PsyArXiv*. 2021.
21. Xiao N. *ggsci: scientific journal and Sci-Fi themed color palettes for 'ggplot2'* 2021.
22. Sanchez-Vega F, Mina M, Armenia J, et al. Oncogenic signaling pathways in the cancer genome atlas. *Cell*. 2018;173(2):321-337.e310.
23. Niu B, Ye K, Zhang Q, et al. MSIsensor: microsatellite instability detection using paired tumor-normal sequence data. *Bioinformatics*. 2014;30(7):1015-1016.
24. Dubot C, Bernard V, Sablin MP, et al. Comprehensive genomic profiling of head and neck squamous cell carcinoma reveals FGFR1 amplifications and tumour genomic alterations burden as prognostic biomarkers of survival. *Eur J Cancer*. 2018;91:47-55.
25. Lim SM, Cho SH, Hwang IG, et al. Investigating the feasibility of targeted next-generation sequencing to guide the treatment of head and neck squamous cell carcinoma. *Cancer Res Treat*. 2019;51(1):300-312.
26. Martínez-Sáez O, Chic N, Pascual T, et al. Frequency and spectrum of PIK3CA somatic mutations in breast cancer. *Breast Cancer Res*. 2020;22(1):45.
27. Klussmann JP, Gültekin E, Weissenborn SJ, et al. Expression of p16 protein identifies a distinct entity of tonsillar carcinomas associated with human papillomavirus. *Am J Pathol*. 2003;162(3):747-753.
28. Westra WH. Detection of human papillomavirus (HPV) in clinical samples: evolving methods and strategies for the accurate determination of HPV status of head and neck carcinomas. *Oral Oncol*. 2014;50(9):771-779.
29. Nichols AC, Palma DA, Chow W, et al. High frequency of activating PIK3CA mutations in human papillomavirus—positive oropharyngeal cancer. *JAMA Otolaryngol Head Neck Surg*. 2013;139(6):617-622.
30. Garrido F, Wild CM, Mittelberger J, et al. The role of chemokines in cervical cancers. *Medicina (Kaunas, Lithuania)*. 2021;57(11):1141.
31. Majchrzak E, Szybiak B, Wegner A, et al. Oral cavity and oropharyngeal squamous cell carcinoma in young adults: a review of the literature. *Radiol Oncol*. 2014;48(1):1-10.
32. Vermorken JB, Mesia R, Rivera F, et al. Platinum-based chemotherapy plus cetuximab in head and neck cancer. *N Engl J Med*. 2008;359(11): 1116-1127.
33. Dail M, Wong J, Lawrence J, et al. Loss of oncogenic Notch1 with resistance to a PI3K inhibitor in T-cell leukaemia. *Nature*. 2014;513 (7519):512-516.
34. Bhin J, Yemelyanenko J, Chao X, et al. MYC is a clinically significant driver of mTOR inhibitor resistance in breast cancer. *J Exp Med*. 2023;220(11):e20211743.