pISSN 1598-2998, eISSN 2005-9256
https://doi.org/10.4143/crt.2024.1195 Cancer Res Treat. 2025;57(4):968-980

Original Article

Differential Efficacy of Alpelisib by *PIK3CA* Mutation Site in Head and Neck Squamous Cell Carcinoma: An Analysis from the KCSG HN 15-16 TRIUMPH Trial

Kyoo Hyun Kim[1], **Shinwon Hwang**[2], **Min Kyoung Kim**[3], **Keon-Uk Park**[4], **Tak Yun**[5], **Keun-Wook Lee**[6], **Joo Hang Kim**[7], **Bhumsuk Keam**[8], **Byoung Chul Cho**[1], **So Yeon Oh**[9], **Sang Hee Cho**[10], **Sangwoo Kim**[11,12], **Sung-Bae Kim**[13], **Min Hee Hong**[1], **Hye Ryun Kim**[1,12]
A list of author's affiliations appears at the end of the paper.

Purpose The TRIUMPH trial was a biomarker-driven umbrella trial for patients with recurrent or metastatic head and neck squamous cell carcinoma (R/M HNSCC). This analysis focuses on the PIK3CAα (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha) inhibitor alpelisib (arm 1) in patients with phosphoinositide 3-kinase (PI3K) pathway alterations.

Materials and Methods Patients with PI3K pathway altered tumors were enrolled in the alpelisib arm of the TRIUMPH study. We conducted a detailed analysis of the correlation between PI3K pathway mutations and treatment outcomes including disease control rate, overall survival (OS), and progression-free survival (PFS).

Results From October 2017 and August 2020, 203 were enrolled, with 42 treated with alpelisib. Response evaluation was possible for 33 patients. Genomic profiles revealed *PIK3CA* amplifications in 26.2%, and point mutations in E542K (26.2%), E545K (23.8%), and H1047R (9.5%). Neither *PIK3CA* amplification nor co-occurring *TP53* mutations had a notable influence on alpelisib response or survival outcomes. Although the overall response rates were similar between helical domain mutations (E542, E545) and kinase domain mutation (H1047), patients with H1047 mutation exhibited significantly poorer PFS compared to those with non-H1047 *PIK3CA* alterations (1.6 vs. 7.3 months, p=0.017). OS in patients with H1047 kinase domain mutation showed a trend toward being shorter compared to others, though this difference did not reach statistical significance.

Conclusion Alpelisib showed differential efficacy based on PI3K pathway alterations in patients with R/M HNSCC and was well-tolerated. These findings suggest the usefulness of next-generation sequencing testing-based decision-making when using the targeted agents in R/M HNSCC. We need to confirm results in larger cohorts.

Key words Squamous cell carcinoma of head and neck, PI3K pathway, Precision medicine, Umbrella trial, NGS, Alpelisib

Introduction

Recurrent and metastatic head and neck squamous cell carcinoma (R/M HNSCC) continues to present significant clinical challenges despite recent therapeutic progress. Unfortunately, patients with R/M HNSCC experience poor survival outcomes, with a median overall survival (OS) of approximately 1 year. Currently, first-line therapeutic options primarily involve pembrolizumab, administered either as monotherapy or in combination with cytotoxic agents [1]. In cases where immunotherapy is not deemed suitable, cetuximab combined with chemotherapy serves as an alternative treatment approach. While cetuximab stands as the sole approved targeted therapy for patients with R/M HNSCC, its efficacy remains unaffected by specific biomarkers [2].

Recent advancements in molecular profiling techniques, such as next-generation sequencing (NGS), genotyping, and mRNA expression analysis, have led to the discovery of multiple molecular targets for HNSCC. Some of these targets, including *PIK3CA* (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha), epidermal growth factor receptor (*EGFR*), *NOTCH*, *HRAS*, *NRAS*, *CCND*A, cyclin dependent kinase inhibitor 2A (*CDKN2A*), and fibroblast growth factor receptor (*FGFR*), offer actionable opportunities for potential treatments. The validation of these targets as predictive biomarkers are limited, however, emphasizing the ongoing need for continued investigation to establish their significance in guiding treatment decisions effectively [3,4].

Correspondence: Hye Ryun Kim
Division of Medical Oncology, Department of Internal Medicine, Yonsei Cancer Center, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea
Tel: 82-2-2228-8125 E-mail: nobelg@yuhs.ac

Received December 11, 2024 **Accepted** January 27, 2025
Published Online January 31, 2025

Co-correspondence: Min Hee Hong
Division of Medical Oncology, Department of Internal Medicine, Yonsei Cancer Center, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea
Tel: 82-2-2228-8129 E-mail: minhee_hong@yuhs.ac

Co-correspondence: Sung-Bae Kim
Department of Oncology, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, Korea
Tel: 82-2-3010-3217 E-mail: sbkim3@amc.seoul.kr

As a collaborative effort incorporating precision oncologic approach in HNSCC, the TRanslational bIomarker-driven Umbrella Project for Head and neck squamous cell carcinoma (TRIUMPH) study was launched. This multicenter, phase II trial, carried out in collaboration with the Korean Cancer Study Group (KCSG), encompassing various multiple targeted therapies for PI3K, EGFR, FGFR, CDK4/6, and programmed death-ligand 1/cytotoxic T-lymphocyte associated protein 4, marked the first umbrella trial designed for platinum-refractory HNSCC patients [5]. Here, we report in-depth results for a subset of patients whose tumor exhibit alterations in the PI3K pathway such as *PIK3CA* amplification, *PIK3CA* mutation, and *PIK3R1* mutation treated with alpelisib in the TRIUMPH trial.

The occurrence rates of *PIK3CA* amplification/mutation, *PTEN* deletion, and *PIK3R1* mutation in patients are reported to be 55%, 6%, and 3%, respectively, according to The Cancer Genomic Atlas (TCGA) data [6]. Aberrations in the PI3K pathway were found predominantly as activating events in *PIK3CA* (less commonly in *PIK3CB*) and inactivating events in *PTEN* or *PIK3R1*, with *PIK3CA* and *PTEN* alterations being most commonly found in head and neck cancer, breast cancer, gastrointestinal and gynecological cancers [7]. PI3Kα-targeted inhibitors for HNSCC are being investigated for use as standalone treatments or in combination in numerous clinical trials [8]. Nevertheless, the majority of ongoing trials are enrolling patients without stringent genetic criteria.

Alpelisib (BYL719) is an orally available, small-molecule inhibitor that selectively targets PI3Kα, demonstrating approximately 50 times greater inhibition of p110α compared to other isoforms [9]. Preclinical tumor models and a phase I trial in patients with advanced solid tumors have provided evidence of alpelisib efficacy in treating *PIK3CA*-mutated cancers, highlighting its potential as a promising treatment choice [10]. The combination of alpelisib and fulvestrant has received approval for the treatment of patients with *PIK3CA*-mutated, hormone receptor-positive, human epidermal growth factor receptor 2 (HER2)–negative advanced breast cancer following progression on prior endocrine therapy [11].

In this phase II, multicenter clinical trial conducted as part of a larger umbrella trial, we performed a comprehensive analysis to evaluate the association between PI3K pathway mutations and clinical outcomes, including disease control rate (DCR), OS, and progression-free survival (PFS).

Materials and Methods

1. Study design and treatment allocation

During the screening phase of TRIUMPH [5], each patient's genetic mutations were assessed using targeted NGS assay and Nanostring assay based on RNA expression. The key driver mutations examined included *PIK3CA*, *EGFR*, *FGFR*, and alterations related to the cell cycle pathway. Patients were then assigned to different treatment arms based on the recommendations made by the molecular tumor board (MTB). The treatment arms consisted of specific inhibitors such as alpelisib, poziotinib, nintedanib, abemaciclib, and, in cases where no matched targets were identified, durvalumab.

2. Molecular profiling assays

In our previous feasibility study conducted before the TRIUMPH trial [12], we provided a comprehensive description of the NGS assays used. In summary, genomic DNA was extracted from formalin-fixed paraffin-embedded (FFPE) samples using the QIAamp DNA FFPE Tissue Kit (Qiagen) to target-sequence 244 genes associated with head and neck cancer. Library preparation was performed using the custom SureSelect Target Enrichment library generation kit (Agilent). Subsequently, the libraries were sequenced using the high-throughput Illumina HiSeq2500 platform, ensuring coverage depth of over 1,000×.

Following sequencing, the resulting short reads underwent quality trimming using Sickle [13] and were then aligned to the human reference genome using the Burrows-Wheeler Aligner [14]. The aligned reads were further processed using Genome Analysis ToolKit v3.5 [15]. For variant detection, MuTect v1.17 and Varscan2 v2.3.7 were employed, and ANNOVAR [16] was used to functionally annotate the identified high-confidence variants. Copy number alterations were identified using CNVkit, and genes with copy numbers of > 4 and 0 were considered amplified and deleted, respectively.

3. Patient selection

Patients with genetic alterations in the PI3K pathway were eligible to enroll in the alpelisib arm. Eligible patients were those with histologically confirmed HNSCC; R/M HNSCC of the oral cavity, oropharynx, hypopharynx, larynx, nasal cavity, maxillary sinus; not amenable to curative treatment and had progressed or recurred upon one or two systemic therapy regimens including platinum-based chemotherapy, or within 6 months after concurrent chemo-radiation (CCRT) administered as definitive treatment, with or without induction chemotherapy; aged ≥ 20 years; at least one measurable disease according to Response Evaluation Criteria in Solid Tumor (RECIST) ver. 1.1; an Eastern Cooperative Oncology

Group performance status of 0 or 1; and adequate organ function. Key exclusion criteria included patients previously treated with PI3K pathway inhibitors; nasopharyngeal carcinoma; active brain metastasis; previous surgery requiring general anesthesia within four weeks before enrollment, and history of ileus, airway obstruction, or active bleeding within 6 months of enrollment.

The eligibility for alpelisib arm allocation was evaluated for each patient according to the prespecified protocol criteria by the MTB [5]. The MTB consisted of an expert team including four medical oncologists, one pathologist, and three bioinformatics specialists. Each member of the MTB reviewed and prioritized the genetic alterations identified through sequencing and recommended the most appropriate treatment arm for patient allocation. In arm 1 (alpelisib), patients with well-established genetic mutations related to the PI3K pathway, such as *PIK3CA* hotspot mutations, amplifications, and *PIK3R1* mutations, were included based on information from two cancer knowledge databases: COSMIC [17] and OncoKB [18]. When similar levels of evidence supported multiple genetic alterations that could qualify for different trial arms, priority was given according to the protocol in the following order: arm 1, arm 2, arm 3, and arm 4.

Patients in the PI3K arm were treated with a single-agent alpelisib at a starting dose of 350 mg once daily. After progression on alpelisib, crossover to the durvalumab monotherapy arm (arm 5) of the TRIUMPH trial was allowed.

4. Treatment and assessment

Enrolled patients received a daily oral dose of 350 mg in 28-day cycles. Treatment continued until disease progression, unacceptable toxicity, or patient's withdrawal of consent. Adjustments or delays in dosage were made as needed, guided by the worst grade of toxicity according to the approved protocol.

Tumor response assessments were conducted following the RECIST ver. 1.1 at baseline and every 8 weeks thereafter. Continuing treatment after disease progression was not permitted, but patients had the option to cross over to the durvalumab arm upon progression, wherein 750 mg of durvalumab was administered intravenously every two weeks, for a maximum of 18 cycles. Safety evaluation was carried out for patients who received at least one dose of treatment, with adverse events (AEs) assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events ver. 4.03.

During each visit at the start of each treatment cycle, patients underwent laboratory tests including complete blood count, plasma biomarkers, and serum metabolic panel.

5. Statistical analysis

The primary endpoint was the investigator-assessed DCR at 8 weeks, representing the percentage of patients achieving complete response, partial response (PR), or stable disease at 8 weeks according to the RECIST ver. 1.1. Secondary endpoints included objective response rate (ORR), OS, measured from treatment initiation to the time of death, and PFS defined as the time from treatment initiation to disease progression, death, or last tumor assessment, whichever came first. Additional secondary endpoints included safety assessment and biomarker analysis.

To determine the sample size needed for response assessment, a one-sided binomial test was employed with 90% power to accept the hypothesis and a 10% significance level to reject the hypothesis. The null hypothesis posited a DCR at 8 weeks of $\leq 30\%$, against the alternative hypothesis of DCR $\geq 50\%$. To achieve adequate statistical power while accounting for potential dropouts, estimated at 10%, the final sample size was calculated to include 43 patients.

The efficacy set included patients who had undergone a baseline tumor assessment and had completed more than 4 weeks of the study treatment. To calculate 95% confidence intervals (CIs) for the proportion of patients with an objective response, the Clopper-Pearson estimation method was used. For median PFS and OS, along with their respective 95% CIs, we employed the Kaplan-Meier method in the efficacy analysis population.

Safety analyses were conducted on the safety set of all treated patients. AEs were summarized based on their frequency and the proportion of patients affected, according to the preferred term. The statistical analyses were carried out using SAS ver. 9.4 (SAS Institute Inc.) and R ver. 3.4.4 (R Foundation for Statistical Computing).

Results

1. Patients characteristics

From October 2017 to August 2020, a total of 419 HNSCC patients underwent genomic profiling during the pre-screening process, and 180 HNSCC patients were screened for enrollment onto the TRIUMPH trial. Ultimately, 203 patients were enrolled, including those in the crossover arm 5 (durvalumab) (S1 Fig.). According to the recommendations of the MTB, 45 patients were initially assigned to the alpelisib (arm 1), and out of them, 42 patients were enrolled (S2 Fig.) [5]. Subsequently, 21 patients, including the two patients who had initially crossed over during screening, later transitioned to receive durvalumab (arm 5). As of data cutoff date in December 2021, all patients had discontinued treatment. Among them, 32 experienced disease progression or died,

Table 1. Patient baseline characteristics

	BYL719 (arm 1) No. (%) (n=42)
Age (yr), median (range)	64.5 (32.0-80.0)
Sex	
Male	39 (92.9)
Female	3 (7.1)
Smoking	
Current	4 (9.5)
Former	28 (66.7)
Never	10 (23.8)
Unknown	0
Alcohol	
Yes	20 (47.6)
No	21 (50.0)
Unknown	1 (2.4)
Primary tumor location	
Lip/Oral cavity	8 (19.1)
Oropharynx	14 (33.3)
Hypopharynx	8 (19.1)
Larynx	8 (19.1)
Nasal cavity	1 (2.4)
Maxillary sinus	1 (2.4)
Unknown	2 (4.8)
HPV status[a]	
Positive	17 (40.4)
Negative	13 (30.9)
Not available	12 (28.6)
ECOG performance status	
0	14 (33.3)
1	28 (66.7)
Stage at diagnosis	
0	1 (2.4)
I	1 (2.4)
II	2 (4.8)
III	7 (16.7)
IV	30 (71.4)
Unknown	1 (2.4)
Histologic differentiation	
Well differentiated	8 (19.0)
Moderately differentiated	18 (42.9)
Poorly differentiated	11 (26.2)
Grade cannot be assessed	5 (11.9)
Previous lines of systemic therapy	
1	16 (38.1)
2	20 (47.6)
3	6 (14.3)

ECOG, Eastern Cooperative Oncology Group; HPV, human papillomavirus. [a]HPV status is determined either by p16 immunohistochemistry or HPV genotyping.

two discontinued treatment due to AEs (urticaria, pneumonitis), and eight chose to withdraw from the study. The median follow-up period from the start of treatment was 31.9 months (95% CI, 15.5 to 35.3).

As described in prior study [5] (Table 1), the primary tumor sites included oropharynx (33.3%), lip/oral cavity (19.1%), hypopharynx (19.1%), and larynx (19.1%). The majority of patients had a history of previous surgery or radiotherapy and had undergone up to three prior lines of systemic therapy. All patients enrolled in this arm met the eligibility criteria and were part of the safety analysis, with 33 out of the 42 included in the efficacy analysis.

2. Mutation profiling of PI3K pathway

We conducted a further analysis of the mutation profiles of the patients initially screened and assigned to the alpelisib arm. The prevalence of the genetic aberrations related with the signaling pathway of alpelisib included the *PIK3CA* mutation (28/42, 66.7%), *PIK3CA* amplification (21/42, 50.0%), *PIK3C2A* mutation (2/42, 4.8%) and *PIK3R1* mutation (2/42, 4.8%), as well as *PIK3C3* and *PIK3R2* mutations (Fig. 1A).

The *PIK3CA* mutations of the 28 patients were E542K (11/42, 26.2%), E545A/K (10/42, 23.8%), and H1047R (4/42, 9.5%) at "hotspot" locations in the p110α subunit, which are collectively referred to as canonical mutations. Other non-canonical mutations, such as *PIK3CA* Q75E, R88Q, G263A, K567_I571delinsN, or C604R were found (Fig. 1B).

Among the total of 42 patients, p53 mutation co-occurrence was identified in 48% (20/42) of the patients. Additionally, *EGFR* amplification (4/42, 9.5%), *CCND1* amplification (11/42, 26.2%), *CCND2* amplification (1/42, 2.4%), and *CDKN2A* alterations (10/42, 23.8%) were detected, respectively (Fig. 2A).

3. Treatment outcome

The primary endpoint of DCR was 65.6% (95% CI, 46.8 to 81.4), meeting the prespecified criteria (DCR, 50% or more). Among the 33 patients in the efficacy set, seven achieved PR, resulting in an ORR of 21.2% (95% CI, 9.3 to 40.0). Of the seven patients with PR, five patients showed durable response of more than 6 months (range, 1.6 to 12.9 months) [5].

The median PFS was 3.4 months (95% CI, 1.9 to 7.5 months) (S3A Fig.), and the median duration of response (DoR) was 6.0 months (range, 1.6 to 12.9). Median OS reached 12.4 months (95% CI, 4.9 to 19.0) (S3B Fig.). Fig. 2A displays swimmer's plot for patients included in the efficacy analysis, illustrating the distribution of DoR in relation to PI3K pathway mutations and *TP53* mutations. A waterfall plot of the patients in the efficacy analysis, as previously reported [5], is provided in S4 Fig. The presence of a co-occurring *TP53* mutation did

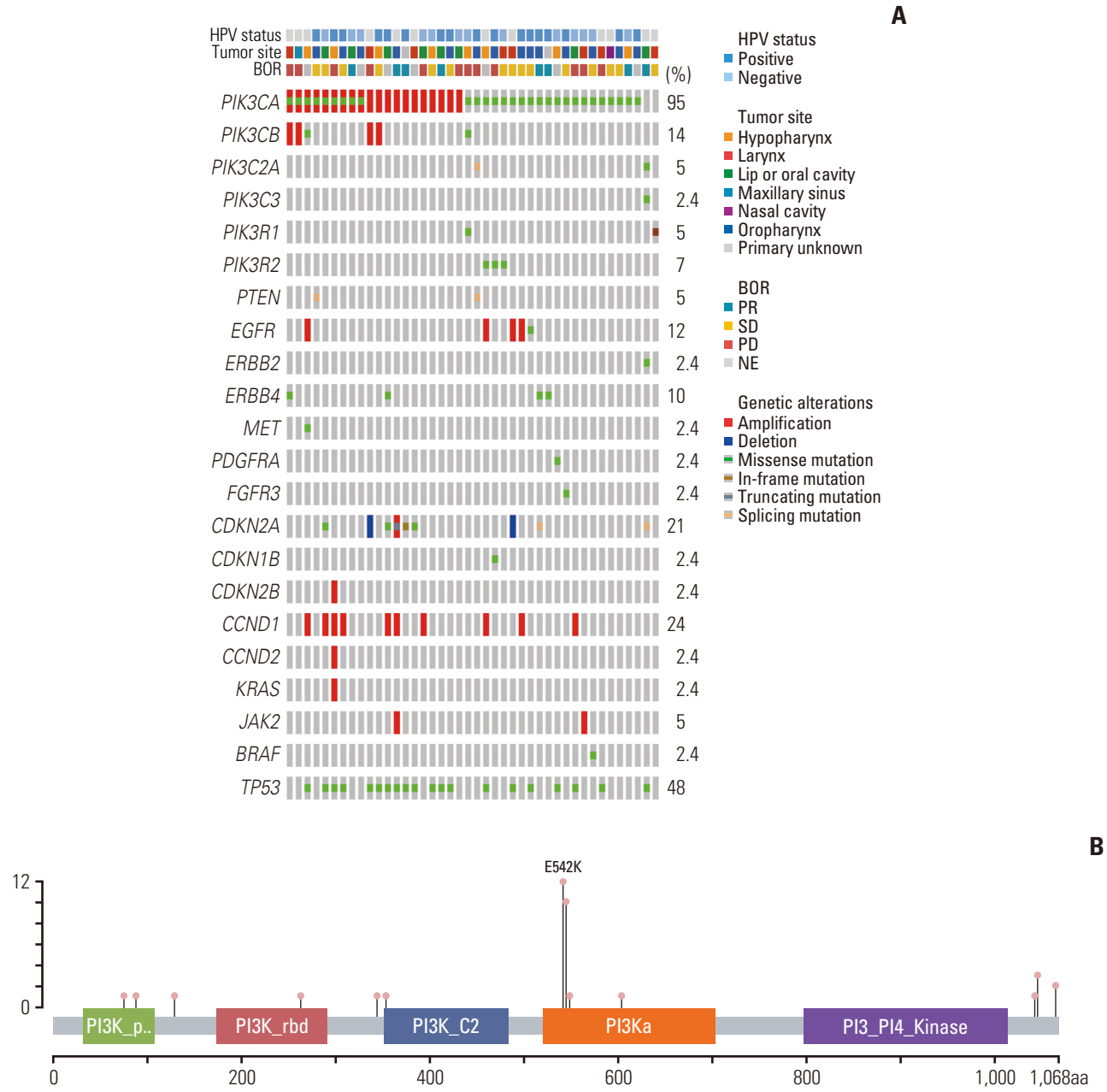
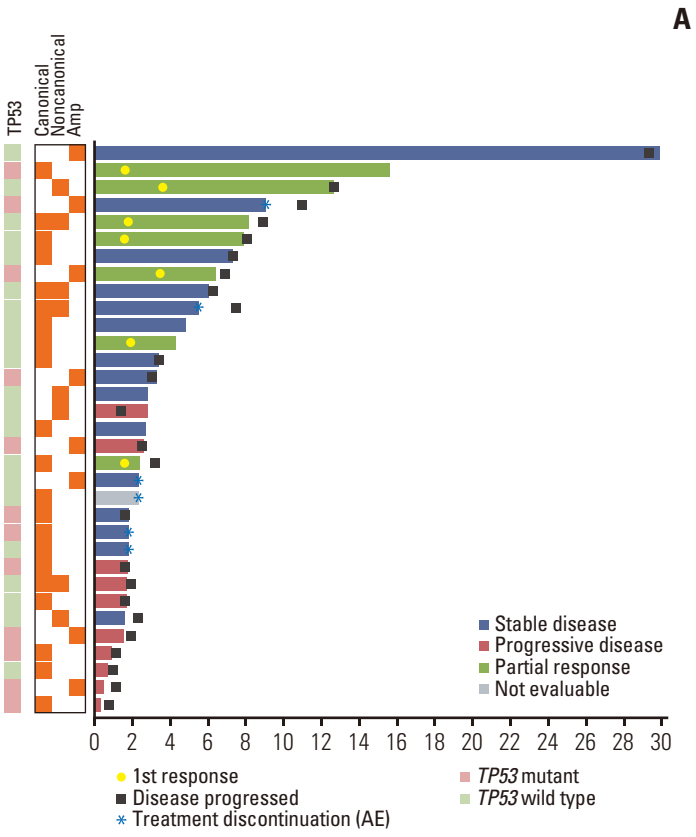


Fig. 1. Integrated clinical and genomic profile of patients screened for the alpelisib (BYL719) arm. (A) Oncoplot showing both patient-specific mutations in phosphoinositide 3-kinase (PI3K) pathway as well as other, concurrent mutations in the major tumor-related molecular pathways. Upper columns show human papillomavirus (HPV) status, tumor primary site, and best of response (BOR) for each patient. NE, not evaluable; PD, progressive disease; PR, partial response; SD, stable disease. (B) Lollipop plot showing sites of mutation for PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha).

not significantly affect treatment outcomes, including ORR and DoR.

Among the efficacy group, 12 patients with human papillomavirus–mediated (p16-positive) oropharyngeal cancer were included. While p16+ oropharyngeal cancer patients had numerically longer PFS (6.9 vs. 2.7 months; hazard ratio [HR], 0.56; 95% CI, 0.25 to 1.24) and OS (15.8 vs. 10.4 months; HR, 0.49; 95% CI, 0.20 to 1.21), survival analysis showed no



B

Clinical/Genetic variables	Hazard ratio (95% CI)	p-value
Age (< 60 vs. ≥ 60 yr)	1.32 (0.56-3.09)	0.52
Sex (female vs. male)	1.13 (0.26-4.86)	0.88
Smoking (never vs. current/Ex-)	0.52 (0.20-1.36)	0.18
Tumor stage at diagnosis (I-III vs. IV)	1.25 (0.45-3.48)	0.67
Tumor differentiation (well/moderate vs. poor)	2.10 (0 71-6.23)	0.18
Previous lines of therapy (1 vs. 2-3)	0.82 (0.37-1.79)	0.62
PIK3CA canonical alterations	0.93 (0.42-2.05)	0.85
Helical domain mutations (E542 or E545)	0.72 (0.32-1.59)	0.42
Kinase domain mutation (H1047)	3.53 (0.99-12.63)	0.05
PIK3CA noncanonical alterations	1.18 (0.49-2.87)	0.71
PIK3CA dual canonical/noncanonical alterations	1.13 (0.38-3.36)	0.83
PIK3CA amplification	1.01 (0.42-2.42)	0.99
TP53 mutations	1.50 (0.69-3.26)	0.30
Other PI3K/AKT pathway alterations	1.23 (0.45-3.40)	0.69
Concurrent[a] major pathway alterations	1.26 (0.54-2.91)	0.59

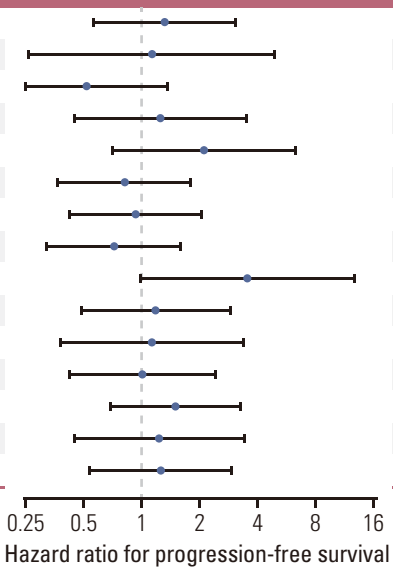


Fig. 2. Tumor response according to *PIK3CA* (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha) or phosphoinositide 3-kinase (PI3K) pathway alterations and concurrent alterations. (A) Swimmer plot for time on treatment by patient. *TP53* status, as well as type of *PIK3CA* mutation categorized into canonical, noncanonical, or amplification(amp) for each patient is shown on the left columns. (B) Forest plot illustrating the outcomes of Cox proportional hazard ratio assessment for progression-free survival based on clinical and genetic factors, including different types of PI3K pathway mutations. AE, adverse event; CI, confidence interval. [a]Concurrent major pathways alterations include mutations in the cell cycle pathway, *JAK2*, *FGFR3*, *ERBB4*, and *KRAS* alterations.

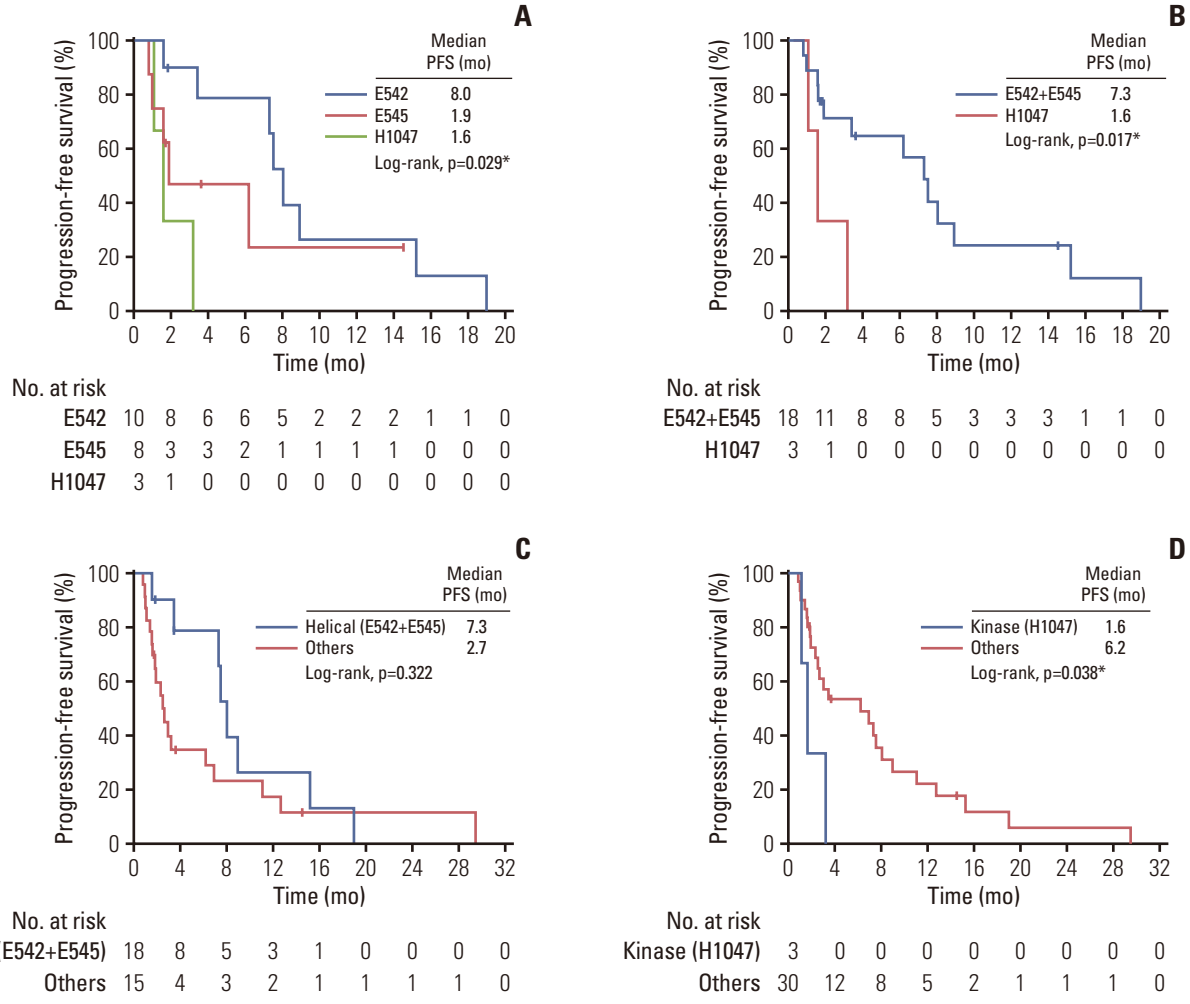


Fig. 3. Survival comparison according to *PIK3CA* (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha) canonical mutation site. (A) Progression-free survival (PFS) for patients with *PIK3CA* canonical mutations (n=21), including hotspot mutations at E542, E545, and H1047. (B) PFS comparison between helical domain mutations (E542, E545, n=18) and kinase domain mutation H1047 (n=3). (C) PFS comparison in the total efficacy set (n=33): helical domain mutations (E542, E545, n=18) vs. others (n=15). (D) PFS comparison between kinase domain (H1047) mutation (n=3) and all other mutations (n=30). *p < 0.05. *(Continued to the next page)*

significant differences compared to other patients (S3C and S3D Fig.).

Using a Cox proportional hazards model, we evaluated various clinical and genetic factors for their impact on PFS. As shown in the forest plot (Fig. 2B), none of the clinical factors—including age, sex, smoking history, initial tumor stage, differentiation, or previous lines of therapy—significantly influenced PFS.

Notably, the *PIK3CA* H1047 kinase domain mutation showed a trend toward worse PFS (HR, 3.53; 95% CI, 0.99 to 12.63; p=0.053). Although this result does not reach the conventional level of statistical significance, it suggests a potential association that calls for further investigation. No other genetic variables, such as *PIK3CA* amplification, dual canonical/noncanonical alterations, or other concurrent pathway alterations, reached statistical significance.

4. Efficacy comparison between *PIK3CA* mutation sites and co-occurring mutations

In this study, we specifically focused on the three enriched, "hotspot" mutation sites within the *PIK3CA* gene: namely, E542K, E545A/K, and H1047R. E542 and E545 mutations are located within the helical domain, while H1047R is within the kinase domain. Our analysis aimed to investigate whether the response to alpelisib varied depending on the mutation site.

In terms of the best overall response, no significant difference was observed when comparing helical and kinase

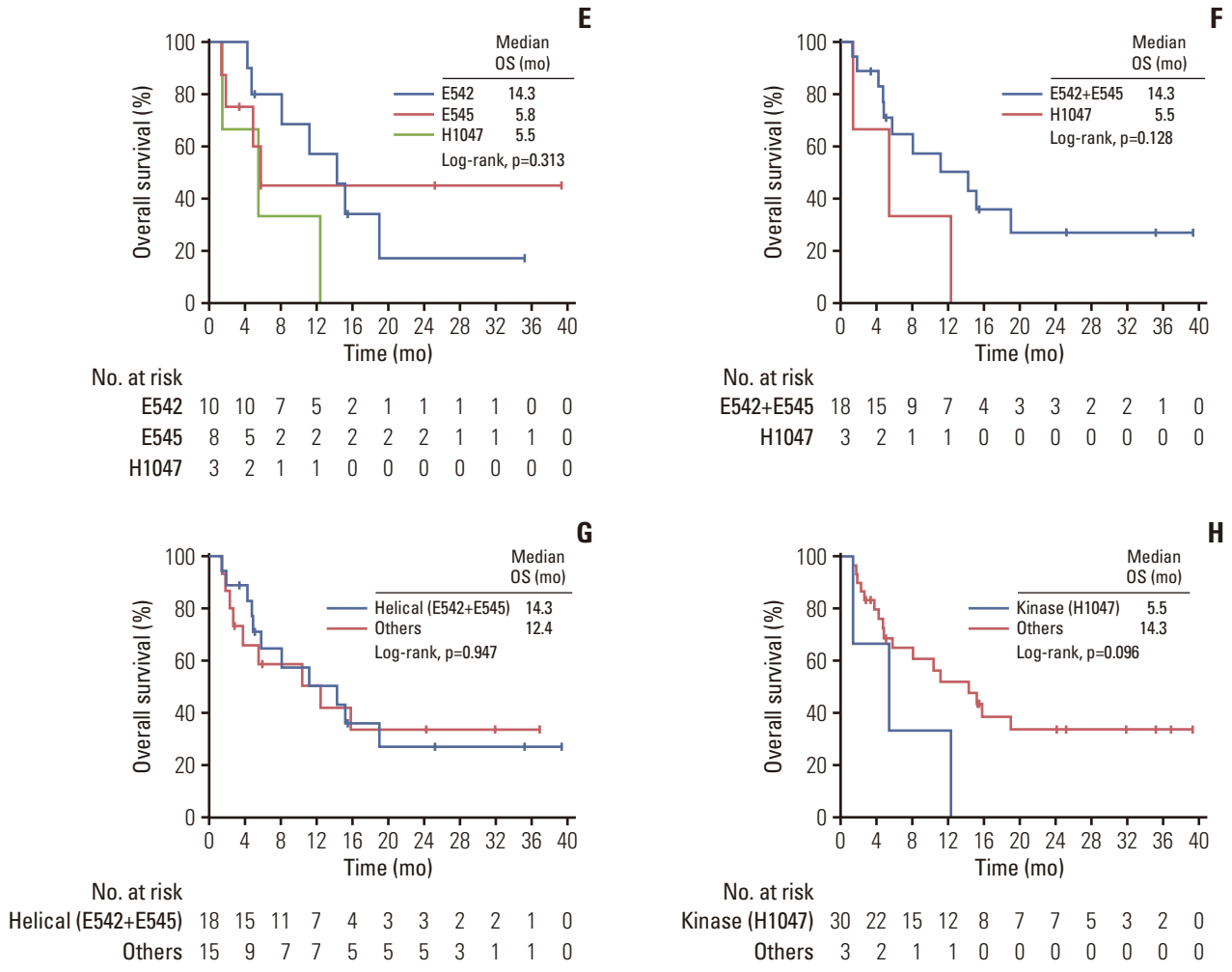


Fig. 3. *(Continued from the previous page)* (E) Overall survival (OS) for patients with PIK3CA canonical mutations (n=21), including hotspot mutations at E542, E545, and H1047. (F) OS comparison between helical domain mutations (E542, E545, n=18) and kinase domain mutation H1047 (n=3). (G) OScomparison in the total efficacy set (n=33): helical domain mutations (E542, E545, n=18) vs. others (n=15). (H) OS comparison between kinase domain (H1047) mutation (n=3) vs. all other mutations (n=30).

domain mutations, with response rates of 22.2% and 33.3%, respectively. However, survival analysis revealed that individuals with mutations at different canonical sites exhibited significantly different PFS (p=0.029) (Fig. 3A). Notably, those with the H1047 kinase domain mutation (n=3) had significantly worse PFS compared with individuals with helical domain mutations E542/E545, with a median PFS of 1.6 months (95% CI, 0.8 to 2.4) versus 7.3 months (95% CI, 5.2 to 9.4) (p=0.017) (Fig. 3B). Additionally, patients with H1047 mutation experienced significantly worse PFS compared to all other patients in the alpelisib arm, with a median PFS of 1.6 months (95% CI, 0.8 to 2.4) versus 6.2 months (95% CI, 0.3 to 12.1) (p=0.038) (Fig. 3D). While the mutations within the helical domain showed a trend towards longer PFS compared to other mutations, these differences did not reach statistical significance (Fig. 3C). OS did not significantly differ between canonical and noncanonical mutation sites, although the kinase domain mutation H1047 did show a trend towards worse OS, similar to the pattern observed for PFS (Fig. 3E-H).

Patients with *TP53* mutations had shorter PFS (2.5 vs. 7.3 months) and OS (10.4 vs.14.3 months), but these differences were not statistically significant (S3E and S3F Fig.). Similarly, the coexistence of *TP53* mutations with *PIK3CA* canonical mutations did not significantly affect PFS or OS (S3G and S3H Fig.).

5. Safety

Safety analysis included all 42 alpelisib-treated patients. The most common AEs were hyperglycemia (26/42, 61.9%), anorexia (20/42, 47.6%), and fatigue (14/42, 33.3%). Serious

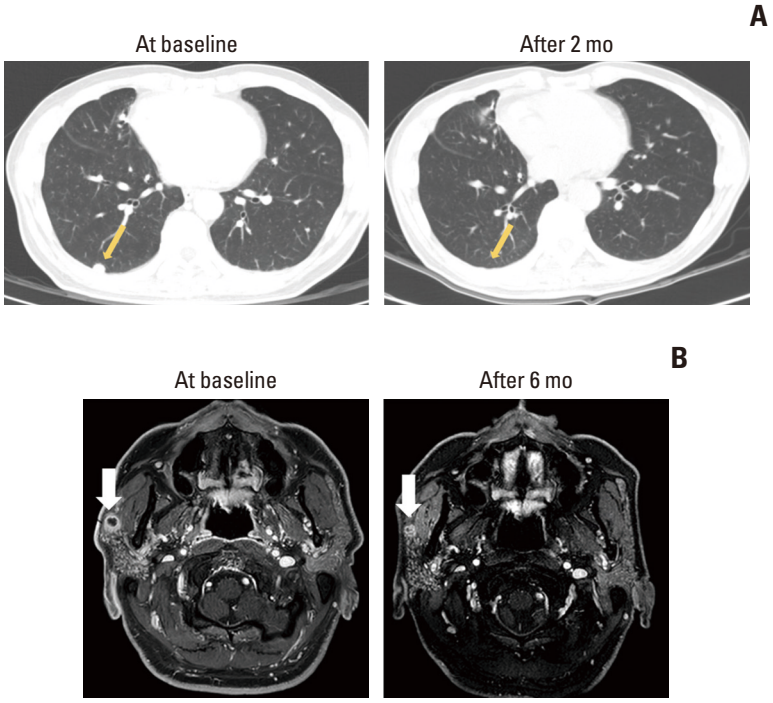


Fig. 4. Tumor shrinkage of patients showing good responses to alpelisib. (A) Lung computed tomography scans at baseline and at 1st response assessment (at 8 weeks) of a patient with oral cavity cancer. (B) Neck magnetic resonance imaging at baseline and at 6 months after alpelisib treatment of a patient with parotid gland cancer, showing shrinkage of primary lesion at anterior portion of the right parotid gland.

Grade 3 or higher AEs occurred in 33 patients, including hyperglycemia (45.2%), fatigue (9.5%), dyspnea (9.5%), and hypokalemia (9.5%) (S5 Table). Dose delays and reductions occurred in 24 (57.1%) and 16 (38.1%) patients, respectively.

No new significant safety signals were identified beyond prior alpelisib trials. Most AEs were manageable with adequate treatment, although 10 patients (23.8%) permanently discontinued alpelisib due to AEs. Three patients died during the study, one due to disease progression, and two from pneumonia.

6. Patient cases

Here we highlight two patient cases who have shown good responses to alpelisib.

1) Case 1

The first case involved a 61-year-old male diagnosed with p16-positive oropharyngeal cancer, stage II (cT2N2cM0). The patient initially received cisplatin-based CCRT. However, the disease recurred with lung metastasis within a year. He then underwent first-line palliative platinum-doublet (FP) chemotherapy and received radiation therapy to the mediastinum. NGS revealed a *PIK3CA* amplification with a copy number of 4 and a mutation (E542K), along with an *ATM* mutation (R2719H) and a *CBL* frameshift deletion (P625fs) mutation. The patient was subsequently assigned to the alpelisib treatment arm, receiving the drug for 8 months. This treatment resulted in a PR, with tumor shrinkage of 54.5%. Fig. 4A illustrates the near-complete disappearance of the target lung lesion in this patient. The patient experienced grade 2 hyperglycemia, which was managed by reducing the alpelisib dose. Additionally, the patient experienced grade 3 pericardial effusion deemed unlikely related to alpelisib, leading to a temporary suspension of the drug, which was resumed after the event resolved.

2) Case 2

The second case involved a 68-year-old male diagnosed with p16-positive oropharyngeal cancer with parotid gland metastases as well as multiple lung metastases. The patient underwent an initial treatment of 6 cycles of platinum-doublet chemotherapy. However, local disease progression occurred, with neck lymph node metastases. Upon screening for the TRIUMPH trial, the patient was found to harbor a canonical *PIK3CA* mutation (E545A) along with concurrent *CCND1*, *CCND2*, *KRAS*, and *TP53* mutations. The patient was subsequently assigned to the alpelisib arm, receiving treatment for 14.5 months and exhibiting a PR with a 41%

shrinkage of the lesion at anterior portion of the right parotid gland (Fig. 4B). Patient experienced grade 1 fatigue and grade 1 hyperglycemia, with the latter managed through the initiation of diabetic medications, including metformin and empagliflozin.

Discussion

In this report, we present the findings from the administration of the oral selective p110α inhibitor alpelisib within a biomarker-driven subset cohort, as part of a larger precision-oncology, multi-arm umbrella trial in HNSCC [5]. Among the patients who received alpelisib, the trial mets its primary endpoint, with a DCR of 65.6%, surpassing the initial hypothesis of 30%. Additionally, ORR reached 21.9%. Alpelisib demonstrated distinct efficacy based on the type of PI3K pathway alterations in patients with R/M HNSCC and was generally well-tolerated.

The results of this trial emphasize the growing significance of targeted therapies in the management of R/M HNSCC. Alpelisib's efficacy in this study highlights the potential of precision oncology to tailor treatments based on specific genetic aberrations and the importance of molecular profiling in developing patient-specific treatment strategies [19]. The use of NGS in our study further demonstrates the feasibility of incorporating genomic data into clinical decision-making processes.

PIK3CA stands out as the most frequently mutated oncogene in HNSCC, with mutations found in approximately 13.7% of cases according to TCGA [6]. Notably, in the TRIUMPH prescreened patients (n=419), the prevalence of *PIK3CA* mutation was higher, accounting for 26% of the patients (n=110). *PIK3CA* encodes p110α catalytic subunit of class 1A PI3K, and when abnormally activated, PI3K initiates a cascade of downstream pathways that lead to uncontrolled cell growth, enhanced cell survival, and increased cell motility—factors that contribute significantly in cancer progression [20,21].

Among the 97 HNSCC tumors with *PIK3CA* mutations reported in TCGA, a significant majority, constituting 63%, are clustered in specific "hotspot" regions within the p110α subunit, including the E542, E545, and H1047 [22]. These canonical mutations are known to activate the PI3K/AKT/mammalian target of rapamycin pathway, driving cancer progression. The remaining 37% of *PIK3CA* mutations identified in HNSCC are dispersed throughout the p110α subunit and are termed noncanonical mutations. Although the exact biological and biochemical effects of the noncanonical mutations detected in HNSCC are not yet fully understood, a study by Jin et al. [23] demonstrated using preclinical models that the majority (22 out of 32, 68.8%) of these noncanonical *PIK3CA* mutations, such as Q75E, produce oncogenically activated proteins. These mutations, in turn, may potentially serve as predictive biomarkers for PI3Kα-targeted therapies [23].

Previous studies have documented an association between alpelisib treatment and interstitial lung disease (ILD), extending beyond the well-documented ILD related to immunotherapy [11,24,25]. In our cohort, four cases of pneumonitis were observed during alpelisib therapy, while none occurred among the patients who subsequently crossed over to receive durvalumab. These findings suggest that prior exposure to alpelisib may not substantially increase the risk of ILD during subsequent durvalumab treatment. However, the relatively small number of crossover patients limits the conclusiveness of these observations, emphasizing the need for further studies and continued vigilance for pneumonitis in this population.

Clinical trials have investigated the potential therapeutic benefits of PI3Kα-targeted agents in treating tumors harboring *PIK3CA* mutations. For instance, in patients with hormone receptor-positive/HER2-negative breast cancer, the combination of alpelisib and fulvestrant, an estrogen receptor antagonist, showed prolonged PFS in patients whose tumors contained *PIK3CA* mutations in exons 7,9, and 20 (HR, 0.65; 95% CI, 0.50 to 0.85; $p < 0.001$), but not in patients without these mutations, highlighting the specificity of alpelisib's efficacy in mutation-driven cancers [11,26]. Similar findings were observed in a phase II study in *PIK3CA* mutated breast cancer patients who had progressed after CDK4/6 inhibitor treatment [9].

In our study, we further explored whether alpelisib response varied according to different *PIK3CA* mutation sites. In our study, *PIK3CA* amplification or co-occurring *TP53* mutations did not significantly impact alpelisib response or survival outcomes. While no significant difference in overall response was observed between helical domain (E542, E545) and kinase domain (H1047) mutations, H1047 mutations indicated significantly worse PFS compared to all other individuals with non-H1047 PIK3A alterations. These findings align with the observations from a phase I trial of alpelisib in advanced solid cancers with *PIK3CA* mutations, wherein responses were seen in patients with helical domain mutations or non-canonical mutations, while no responses were observed in patients with kinase mutations, specifically those at H1047 [10]. Although previous report have suggested that double *PIK3CA* mutations in cis enhance oncogenicity and sensitivity to PI3Kα inhibitors [27], our analysis did not demonstrate a significant impact on survival in patients harboring such double mutations.

The H1047 mutations, positioned near the C-terminus of

PI3Kα, lead to an expanded positive charge distribution, enhancing the mutant's ability to bind membranes rich in anionic lipids [28]. Additionally, the H1047 mutation disrupts the auto-inhibitory function of the C-terminal tail by eliminating critical intermolecular interactions. Structural and functional analyses indicate that the H1047R mutation modifies the kinase domain's conformation, altering the twisting motion between the N-lobe and C-lobe and repositioning conserved P-loop residue near the active site, thereby influencing enzyme activity [28].

Recent cryo–electron microscopy studies have identified two distinct allosteric inhibitor binding sites unique to PI3Kα^{H1047R} [29]. Because alpelisib acts as an ATP-competitive inhibitor, targeting the orthosteric site to block PI3Kα activation rather than the aforementioned allosteric sites, it may result in a less favorable response in H1047R mutants compared to mutations in other regions.

In the context of HNSCC patients, most trials have not preselected patients based on PI3K pathway alterations, making our study unique in its biomarker-driven research [30,31]. While our study provides promising results, it also has several limitations. The sample size, although sufficient for initial observations, calls for larger trials to validate these findings. Additionally, the single-arm design limits comparative efficacy analysis with standard treatments. Future research should focus on randomized controlled trials comparing alpelisib with existing treatment modalities. Furthermore, exploring combination therapies involving alpelisib and other agents may reveal synergistic effects, potentially enhancing treatment efficacy and patient outcomes.

As the first biomarker-driven umbrella trial for HNSCC patients who have experienced disease progression after prior platinum-based therapy, our study offers valuable insights into the clinical applicability of precision oncology approach based on NGS. Alpelisib demonstrated varying efficacy depending on specific PI3K pathway alterations, highlighting the need for further investigation into targeting the PI3K pathway and refining patient selection strategies using NGS-based profiling to optimize treatment outcomes.

Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (https://www.e-crt.org).

Ethical Statement

The TRIUMPH study was conducted following the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice. The institutional review board of each trial center approved the protocol. Patients provided written informed consent before participation and the index patient included in the manuscript agreed on providing the photography. This study was registered at ClinicalTrials.gov (NCT03292250).

Author Contributions

Conceived and designed the analysis: Kim KH, Keam B, Kim S, Kim SB, Hong MH, Kim HR.
Collected the data: Kim MK, Park KU, Yun T, Lee KW, Kim JH, Keam B, Cho BC, Oh SY, Cho SH, Hong MH, Kim HR.
Contributed data or analysis tools: Hwang S, Kim MK, Park KU, Yun T, Lee KW, Kim JH, Keam B, Cho BC, Oh SY, Cho SH, Kim S, Hong MH, Kim HR.
Performed the analysis: Kim KH.
Wrote the paper: Kim KH, Hwang S, Kim MK, Park KU, Yun T, Lee KW, Kim JH, Keam B, Cho BC, Oh SY, Cho SH, Kim S, Kim SB, Hong MH, Kim HR.

ORCID iDs

Kyoo Hyun Kim : https://orcid.org/0000-0002-7871-2142
Sung-Bae Kim : https://orcid.org/0000-0001-5588-8332
Min Hee Hong : https://orcid.org/0000-0003-3490-2195
Hye Ryun Kim : https://orcid.org/0000-0002-1842-9070

Conflicts of Interest

Alpelisib (BYL719) was provided by Novartis. The authors declare that Novartis had no role in the design, conduct, or interpretation of the study.

Funding

The TRIUMPH study was supported by grants from the National R&D Program for Cancer Control, Ministry of Health and Welfare, Republic of Korea (grant numbers HA16C0015 and RS-2022-CC125144). This research was also supported by the Bio&Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MSIT) (No. RS-2023-00261820). The funder had no role in the study design, data collection, analyses, and interpretation of data, in the writing of the report, or in the decision to submit the article for publication.

Author Details

[1]Division of Medical Oncology, Department of Internal Medicine, Yonsei Cancer Center, Yonsei University College of Medicine, Seoul, [2]Department of Dermatology, Yonsei University College of Medicine, Seoul, [3]Division of Hematology-Oncology, Department of Internal Medicine, Yeungnam University Hospital, Yeungnam University College of Medicine, Daegu, [4]Department of Internal Medicine, Keimyung University Dongsan Medical Center, Daegu, [5]Rare Cancers Clinic, Center for Specific Organs Center, National Cancer Center, Goyang, [6]Department of Internal Medicine, Seoul National University Bundang Hospital, Seoul National University College of Medicine, Seongnam, [7]Department of Internal Medicine, CHA Bundang Medical Center, CHA University, Seongnam, [8]Department of Internal Medicine, Seoul National University Hospital, Seoul,

[9]Department of Internal Medicine, Pusan National University Yangsan Hospital, Pusan National University School of Medicine, Yangsan, [10]Department of Hemato-Oncology, Chonnam National University Hwasun Hospital, Chonnam National University Medical School, Hwasun, [11]Department of Biomedical Systems Informatics, Yonsei University College of Medicine, Seoul, [12]Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul, [13]Department of Oncology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

References

1. Mody MD, Rocco JW, Yom SS, Haddad RI, Saba NF. Head and neck cancer. Lancet. 2021;398:2289-99.
2. Vermorken JB, Trigo J, Hitt R, Koralewski P, Diaz-Rubio E, Rolland F, et al. Open-label, uncontrolled, multicenter phase II study to evaluate the efficacy and toxicity of cetuximab as a single agent in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck who failed to respond to platinum-based therapy. J Clin Oncol. 2007;25:2171-7.
3. Morris LGT, Chandramohan R, West L, Zehir A, Chakravarty D, Pfister DG, et al. The molecular landscape of recurrent and metastatic head and neck cancers: insights from a precision oncology sequencing platform. JAMA Oncol. 2017;3:244-55.
4. Marret G, Bieche I, Dupain C, Borcoman E, du Rusquec P, Ricci F, et al. Genomic alterations in head and neck squamous cell carcinoma: level of evidence according to ESMO scale for Clinical Actionability of Molecular Targets (ESCAT). JCO Precis Oncol. 2021;5:215-26.
5. Keam B, Hong MH, Shin SH, Heo SG, Kim JE, Ahn HK, 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;42:507-17.
6. Cancer Genome Atlas Network. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature. 2015;517:576-82.
7. Zhang Y, Kwok-Shing Ng P, Kucherlapati M, Chen F, Liu Y, Tsang YH, et al. A pan-cancer proteogenomic atlas of PI3K/AKT/mTOR pathway alterations. Cancer Cell. 2017;31:820-32.
8. Marquard FE, Jucker M. PI3K/AKT/mTOR signaling as a molecular target in head and neck cancer. Biochem Pharmacol. 2020;172:113729.
9. The PI3Kalpha inhibitor alpelisib has activity in PIK3CA-altered tumors. Cancer Discov. 2018;8:OF7.
10. Juric D, Rodon J, Tabernero J, Janku F, Burris HA, Schellens JH, et al. Phosphatidylinositol 3-kinase alpha-selective inhibition with alpelisib (BYL719) in PIK3CA-altered solid tumors: results from the first-in-human study. J Clin Oncol. 2018;36:1291-9.
11. Andre F, Ciruelos EM, Juric D, Loibl S, Campone M, Mayer IA, et al. Alpelisib plus fulvestrant for PIK3CA-mutated, hormone receptor-positive, human epidermal growth factor receptor-2-negative advanced breast cancer: final overall survival results from SOLAR-1. Ann Oncol. 2021;32:208-17.
12. Lim SM, Cho SH, Hwang IG, Choi JW, Chang H, Ahn MJ, 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;51:300-12.
13. Joshi NA, Fass JN. Sickle: a sliding-window, adaptive, quality-based trimming tool for FastQ files (version 1.33) [Internet]. GitHub Inc.; 2011 [cited 2023 Mar 1]. Available from: https://github.com/najoshi/sickle
14. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25:1754-60.
15. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010;20:1297-303.
16. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 2010;38:e164.
17. Tate JG, Bamford S, Jubb HC, Sondka Z, Beare DM, Bindal N, et al. COSMIC: the Catalogue Of Somatic Mutations In Cancer. Nucleic Acids Res. 2019;47:D941-7.
18. Chakravarty D, Gao J, Phillips SM, Kundra R, Zhang H, Wang J, et al. OncoKB: a precision oncology knowledge base. JCO Precis Oncol. 2017;2017:1-16.
19. Reardon B, Moore ND, Moore NS, Kofman E, AlDubayan SH, Cheung AT, et al. Integrating molecular profiles into clinical frameworks through the Molecular Oncology Almanac to prospectively guide precision oncology. Nat Cancer. 2021;2:1102-12.
20. Gabelli SB, Huang CH, Mandelker D, Schmidt-Kittler O, Vogelstein B, Amzel LM. Structural effects of oncogenic PI3Kalpha mutations. Curr Top Microbiol Immunol. 2010;347:43-53.
21. Zhao L, Vogt PK. Hot-spot mutations in p110alpha of phosphatidylinositol 3-kinase (pI3K): differential interactions with the regulatory subunit p85 and with RAS. Cell Cycle. 2010;9:596-600.
22. Cochicho D, Esteves S, Rito M, Silva F, Martins L, Montalvao P, et al. PIK3CA gene mutations in HNSCC: systematic review and correlations with HPV status and patient survival. Cancers (Basel). 2022;14:1286.
23. Jin N, Keam B, Cho J, Lee MJ, Kim HR, Torosyan H, et al. Therapeutic implications of activating noncanonical PIK3CA mutations in head and neck squamous cell carcinoma. J Clin Invest. 2021;131:e150335.
24. Guo XJ, Cai XT, Rong ZX, Zhang YP, Wen YX, Bai X, et al. Interstitial pneumonitis associated with combined regimen of immunotherapy and conventional therapies-pharmacovig-

ilance database analysis with real-world data validation. BMC Med. 2023;21:6.
25. Isiklar A, Basaran G, Sepin B, Gumusay O, Kocagoz AS, Cuhadaroglu C. Alpelisib induced interstitial lung disease in a patient with advanced breast cancer. J Oncol Pharm Pract. 2023;29:484-8.
26. Andre F, Ciruelos E, Rubovszky G, Campone M, Loibl S, Rugo HS, et al. Alpelisib for PIK3CA-mutated, hormone receptor-positive advanced breast cancer. N Engl J Med. 2019;380:1929-40.
27. Vasan N, Razavi P, Johnson JL, Shao H, Shah H, Antoine A, et al. Double PIK3CA mutations in cis increase oncogenicity and sensitivity to PI3Kalpha inhibitors. Science. 2019;366:714-23.
28. Gkeka P, Evangelidis T, Pavlaki M, Lazani V, Christoforidis S, Agianian B, et al. Investigating the structure and dynamics of the PIK3CA wild-type and H1047R oncogenic mutant. PLoS Comput Biol. 2014;10:e1003895.
29. Huang X, Wang K, Han J, Chen X, Wang Z, Wu T, et al. Cryo-EM structures reveal two allosteric inhibition modes of PI3Kalpha(H1047R) involving a re-shaping of the activation loop. Structure. 2024;32:907-17.
30. Tsang ES, Aggarwal RR, Dhawan MS, Bergsland EK, Alvarez EA, Calabrese S, et al. A phase IB trial of the PI3K inhibitor alpelisib and weekly cisplatin in patients with solid tumor malignancies. Cancer Res Commun. 2022;2:570-6.
31. Razak AR, Wang HM, Chang JY, Ahn MJ, Munster P, Blumenschein G Jr, et al. A phase 1b/2 study of alpelisib in combination with cetuximab in patients with recurrent or metastatic head and neck squamous cell carcinoma. Target Oncol. 2023;18:853-68.