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Original Article

Clinical Impact of *TP53* Mutations in Patients with Head and Neck Cancer Who Were Treated with Targeted Therapies or Immunotherapy

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Purpose Tumor suppressor p53 (*TP53*) mutations are common in head and neck squamous cell carcinoma (HNSCC). We evaluated their clinical impact in patients treated with targeted agents or immunotherapy in the KCSG HN15-16 TRIUMPH trial.

Materials and Methods We analyzed clinical characteristics and outcomes of patients with *TP53* mutations in the TRIUMPH trial, a multicenter, biomarker-driven umbrella trial in Korea. Patients were assigned to treatment groups based on genomic profiles: group 1, alpelisib; group 2, poziotinib; group 3, nintedanib; and group 4, abemaciclib. If there was no identifiable target, the patients were allocated to group 5 (durvalumab±tremelimumab).

Results *TP53* mutations were detected in 116/179 patients (64.8%), more frequently in human papillomavirus–negative and non-oropharyngeal cancers. Patients with *TP53* mutations exhibited shorter progression-free survival than *TP53* wild-type in all the patients (1.7 vs. 3.8 months, p=0.002) and in those who received targeted treatments (2.5 vs. 7.3 months, p=0.009). Furthermore, *TP53* mutations were strongly associated with poor overall survival than *TP53* wild-type in all the patients (11.1 vs. 28.8 months, p=0.005) and in group 5 (8.1 vs. 33.0 months, p=0.001).

Conclusion *TP53* mutations were associated with aggressive clinical characteristics and poor survival, particularly in HNSCC patients treated with immunotherapy.

Key words Head and neck neoplasms, Tumor suppressor protein P53, Next generation sequencing, Immunotherapy, Molecular targeted therapy

Introduction

The tumor suppressor p53 (*TP53*) gene, a crucial tumor suppressor gene that has been implicated in various cancers, encodes the p53 protein. This protein controls cell division and death at a low level in normal tissue [1,2]. However, mutations in the *TP53* gene due to numerous endogenous or exogenous stressors can cause a loss or gain of function of the wild-type *TP53*, which promotes the proliferation, invasion, and metastasis of cancer cells. The *TP53* mutations are frequently detected in head and neck squamous cell carcinomas (HNSCCs) and are associated with shorter survival times and resistance to chemotherapy or radiotherapy [3].

Genomic sequencing techniques are being increasingly integrated into routine diagnostic processes for precision medicine. Thus, clinicians can now readily identify patients with HNSCC who harbor the *TP53* mutations via next-generation sequencing (NGS) in a clinical setting. Concurrently, with the advancement of genomic alteration detection and availability of various targeted agents as well as immunooncology drugs, several patients with metastatic and advanced HNSCC can now receive individualized treatments. Therefore, there is a pressing need to thoroughly investigate the clinical significance of *TP53* mutations in patients with HNSCC who are being treated with novel drugs in the era of precision medicine.

In South Korea, a large-scale clinical trial, the Translational Biomarker-driven Umbrella Project for Head and Neck Squamous Cell Carcinoma (TRIUMPH), was conducted. In this multicenter, prospective, and umbrella trial, treatment regimens were allocated patients with recurrent and/or metastatic HNSCC on the basis of their individual NGS results. The clinical trial included the use of alpelisib, poziotinib, nintedanib, abemaciclib and durvalumab with or without

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tremelimumab. The TRIUMPH trial demonstrated the feasibility and applicability of NGS-based genomic profiling in precision medicine in patients with HNSCC. Using the database of the TRIUMPH trial, we aimed to further analyze the clinical characteristics of patients harboring the *TP53* mutations by utilizing the genomic profiling results of the participants in the TRIUMPH trial. Additionally, we evaluated the difference in the efficacy and prognostic implications of various drug treatments, by stratifying the patients according to the presence or absence of *TP53* mutations.

Materials and Methods

1. Study design

This retrospective collateral biomarker analysis utilized the genomic and clinical data from the TRIUMPH trial, a phase II umbrella study that evaluated targeted therapies for patients with recurrent or metastatic head and neck squamous cell carcinoma (R/M HNSCC) (NCT03292250). The detailed study design and results of the TRIUMPH trial have been previously published [4]. Patients with histologically confirmed, platinum-refractory, R/M HNSCC who underwent multiplexed targeted NGS were allowed to participate in the TRIUMPH trial. After consenting to participate in the trial, the identified gene mutations from the NGS were evaluated by the molecular tumor board (MTB) of the TRIUMPH trial. The MTB consisted of four medical oncologists, one pathologist, and three bioinformatic experts. High-confidence genetic alterations associated with the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PI3KCA*), epidermal growth factor receptor (*EGFR*), and fibroblast growth factor receptor (*FGFR*) genes and the cell cycle pathways (*CCND1*, cyclin dependent kinase inhibitor 2A/2B [*CDKN2A/B*], and cyclin dependent kinase 4/6 [*CDK4/6*]), including known hotspot mutations, were sourced from cancer knowledge databases such as COSMIC and OncoKB. Based on these results, the patients were allocated to one of five groups: group 1, alpelisib (a PIK3CA inhibitor); group 2, poziotinib (an EGFR/human epidermal growth factor receptor 2 inhibitor); group 3, nintedanib (an FGFR inhibitor); group 4, abemaciclib (a CDK4/6 inhibitor); and group 5, durvalumab±tremelimumab. If patients in groups 1-4 experienced disease progression, the patients were allowed to crossover to group 5. Therefore, group 5 consisted of patients without druggable alterations and those with druggable alterations who crossed over after targeted therapy.

We analyzed the overall somatic mutation patterns in HNSCC, including somatic single nucleotide variants, insertion/deletions (indels), and amplifications. Using genomic and clinical data, we compared the mutational profiles and clinical characteristics of the *TP53* mutant-type (*TP53* MT) and *TP53* wild-type (*TP53* WT) groups. Clinical characteristics included several age stratifications, sex, smoking status, primary tumor location, histological differentiation, human papillomavirus (HPV) status, number of the previous line of systemic therapy, and body mass index. In addition, we calculated the progression-free survival (PFS) and overall survival (OS) from the TRIUMPH clinical trial and compared the differences in PFS and OS between the *TP53* MT group and the WT group. Subsequently, we compared the survival between patients who were treated with targeted therapies (groups 1-4) and those who were treated immunotherapy (group 5).

2. Participants and clinical data

For the TRIUMPH trial, a total of 35 Korean Cancer Study Group–affiliated institutions in Korea participated. Patients who met the following criteria were eligible for the study: histologically confirmed HNSCC; recurrent and/or metastatic HNSCC of the oral cavity, oropharynx, hypopharynx, larynx, nasal cavity, or maxillary sinus; HNSCC not amenable to curative treatment; platinum-refractory HNSCC; patients aged ≥ 20 years; at least one measurable disease according to the Response Evaluation Criteria in Solid Tumors ver. 1.1; an Eastern Cooperative Oncology Group performance status of 0 or 1; and adequate organ function. Patients with nasopharyngeal carcinoma were excluded. Genomic data and clinical data of the participants, including age, sex, tumor location, tumor stage, treatment history, treatment response, and survival data, were collected.

3. Molecular profiling assays

NGS assays are described in detail in our previous report of the feasibility study done prior to the TRIUMPH study [5]. Briefly, genomic DNA was isolated from formalin-fixed paraffin-embedded (FFPE) samples using the QIAamp DNA FFPE Tissue Kit (Qiagen) for the targeted sequencing of 244 head and neck cancer–related genes. Library preparation was carried out using customized SureSelectXT Target Enrichement library generation kit (Agilent), then the libraries were sequenced using the high-throughput, Illumina HiSeq2500 platform with a depth of coverage > 1,000×.

We evaluated the quality of FASTQ files by examining the base quality, GC content, and total base throughput. The reads were trimmed using FASTQ, considering various criteria such as poly G, length, complexity, and front tail. The somatic and germline variations were identified using the Genome Analysis ToolKit's (GATK) Best Practices protocol [6]. Germline variations were called using the HaplotypeCaller in GATK v4.2.3.0, while somatic variants were identified using Mutect2 with the default settings. Hardfilter was

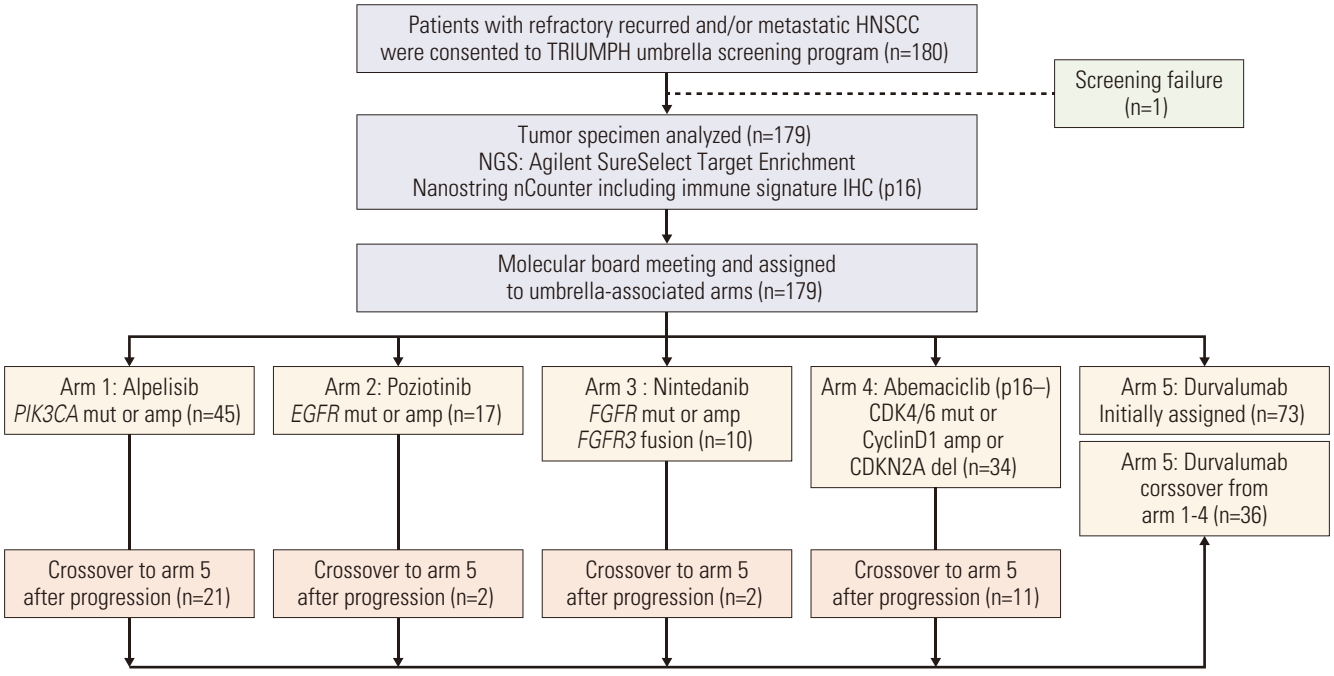


Fig. 1. Consort diagram. CDK, cyclin dependent kinase; *CDKN2A*, cyclin dependent kinase inhibitor 2A; *EGFR*, epidermal growth factor receptor; *FGFR*, fibroblast growth factor receptor; HNSCC, head and neck squamous cell carcinoma; IHC, immunohistochemistry; NGS, next-generation sequencing; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha.

applied to filter the variations, and germline variations that are not present in typical samples were eliminated [7]. Furthermore, variations with minor allele frequencies exceeding 0.001 were eliminated [8]. Variant annotation was performed using vcf2maf v1.6.20, and blacklist genes were filtered using the ENCODE blacklist [9]. Based on the annotations, deleterious variants were selected for further analysis [9]. Copy number variations were examined using the CNVkit with the batch option [10]. These results were refined using the CNVkit filter to merge adjacent values with identical called values. Genes with > 4 copies were categorized as amplified genes, while those with 0 copies were classified as deleted gene.

Additionally, Nanostring assay for RNA expression analysis was performed by first isolating total tumor RNA with the RNeasy kit (Qiagen). Using nCounter Analysis System (Nanostring Technologies), the expression of 55 immune-related genes was screened. Furthermore, we used immunohistochemistry to detect HPV infection in the samples, while focusing on the p16 expression in tumor cells.

4. Statistical analysis

Various statistical tests were used to evaluate the differences between groups. The categorical variables were analyzed using the Fisher's exact or Pearson's chi-square test. The continuous variables were analyzed using the Student's t test or Wilcoxon rank-sum test. PFS and OS were estimated using the Kaplan-Meier method, and they were compared between groups using the log-rank test. To analyze PFS and OS in relation to each genomic alteration, univariate and multivariate Cox proportional hazards regression models were used. The multivariate analysis was performed using a stepwise backward selection approach. The unadjusted and adjusted hazard ratios (HRs) and their 95% confidence intervals were calculated. All statistical analyses were performed using IBM SPSS ver. 20 (IBM Corp.). Statistical significance was set at $p < 0.05$.

Results

1. Characteristics of the patients

In the TRIUMPH trial, 179 patients met the eligibility criteria and were assigned to one of the five groups according to the genomic results. Groups 1, 2, 3, 4, and 5 comprise of 45, 17, 10, 34, and 73 patients, respectively (Fig. 1). The baseline characteristics of patients according to the presence of a *TP53* mutation are described in Table 1. Of the 179 patients, 116 (64.8%) had *TP53* mutations. The median age of the participants in the *TP53* MT group and *TP53* WT group was 64

Table 1. Characteristics of the patients in the *TP53* mutant and *TP53* wild-type groups

	Total (n=179)	*TP53* wild-type (n=63, 35.2%)	*TP53* mutant (n=116, 64.8%)	p-value
Age (yr)				
Median (range)	63 (32-85)	61 (32-80)	64 (37-85)	
< 75	158 (88.3)	60 (33.5)	99 (55.3)	0.045
≥ 75	21 (11.7)	3 (1.7)	17 (9.5)	
Sex				
Male	159 (88.8)	55 (30.7)	103 (57.5)	0.767
Female	20 (11.2)	8 (4.5)	13 (7.3)	
Smoking				
Never smoked	50 (27.9)	21 (11.7)	29 (16.2)	0.124
Ex-smoker	111 (62.0)	40 (22.3)	71 (39.7)	
Current smoker	16 (8.9)	2 (1.1)	14 (7.8)	
Unknown	2 (1.1)	0	1 (0.6)	
Primary tumor location				
Oral cavity	57 (31.8)	12 (6.7)	45 (25.1)	< 0.001
Oropharynx	37 (20.7)	28 (15.6)	9 (5.0)	
Hypopharynx	34 (19.0)	5 (2.8)	29 (16.2)	
Larynx	32 (17.9)	8 (4.5)	24 (13.4)	
Others	19 (10.6)	10 (5.6)	9 (5.0)	
Histologic differentiation				
Well differentiated	26 (14.5)	4 (2.2)	22 (12.3)	0.081
Moderately differentiated	79 (44.1)	30 (16.8)	49 (27.4)	
Poorly differentiated	32 (17.9)	15 (8.4)	17 (9.5)	
NA	42 (23.5)	14 (7.8)	28 (15.6)	
HPV status				
Negative	86 (48.0)	20 (11.2)	66 (36.9)	< 0.001
Positive	40 (22.3)	27 (15.1)	13 (7.3)	
Unknown	53 (29.6)	16 (8.9)	37 (20.7)	
No. of the previous lines of systemic therapy				
1	72 (40.2)	26 (14.5)	46 (25.7)	0.911
2	86 (48.0)	29 (16.2)	57 (31.8)	
3	21 (11.7)	8 (4.5)	13 (7.3)	
BMI (kg/m²)				
< 18.5	43 (24.0)	8 (4.5)	35 (19.6)	0.029
18.5-24.9	109 (60.9)	43 (24.0)	66 (36.9)	
≥ 25	27 (15.1)	12 (6.7)	15 (8.4)	
Allocated treatment group				
Group 1 (alpelisib)	45 (25.1)	23 (12.8)	22 (12.3)	< 0.001
Group 2 (poziotinib)	17 (9.5)	3 (1.7)	14 (7.8)	
Group 3 (nintedanib)	10 (5.6)	3 (1.7)	7 (3.9)	
Group 4 (abemaciclib)	34 (19.0)	1 (0.6)	33 (18.4)	
Group 5 (no actionable MT)	73 (40.8)	33 (18.4)	40 (22.3)	

Values are presented as number (%). BMI, body mass index; HPV, human papillomavirus; MT, mutation; NA, not applicable; *TP53*, tumor protein 53.

and 61 years, respectively. The *TP53* MT group consisted of a higher proportion of patients aged ≥ 75 years than the *TP53* WT group (9.5% vs. 1.7%, p=0.045). *TP53* MT was observed more commonly in the oral cavity (25.1%), hypopharynx (16.2%), and larynx (13.4%). However, the *TP53* WT was more prevalent in the oropharynx (15.6%, p < 0.001). Furthermore, the proportion of HPV-positive tumors was higher in the *TP53* WT group than in the *TP53* MT group (15.1% vs.

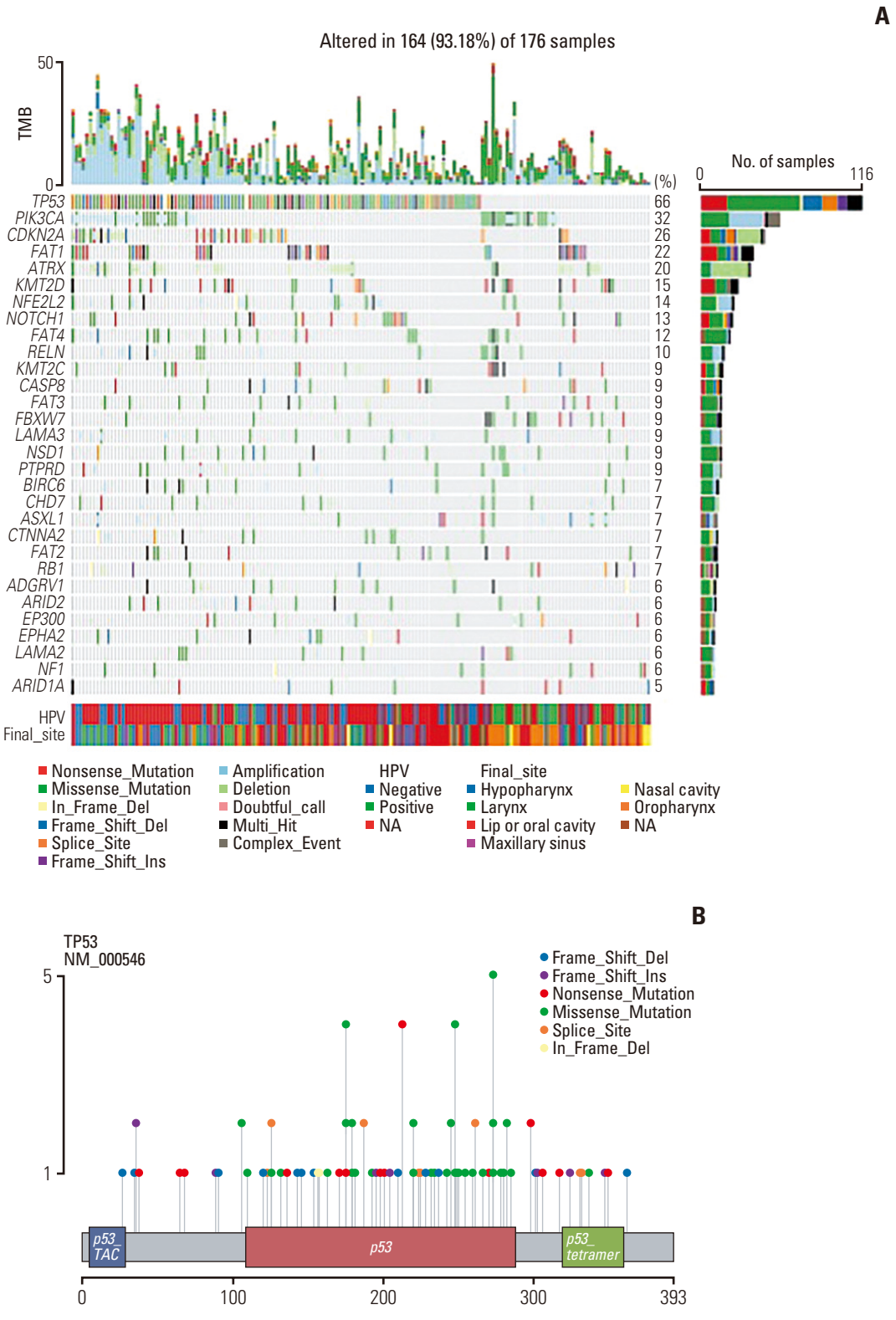


Fig. 2. Genomic landscape and profile of tumor suppressor p53 (*TP53*) mutations. (A) Oncoplot of total population. (B) Lollipop plot of *TP53* mutation. The percentage of each gene mutation in (A) was calculated based on 176 samples, after filtering out three samples due to the absence of variants. HPV, human papillomavirus; NA, not available; TMB, tumor mutational burden.

Table 2. Mutational profiles in the *TP53* mutant and *TP53* wild-type groups

	Total (n=179)	*TP53* wild-type (n=63, 35.2%)	*TP53* mutant (n=116, 64.8%)	p-value
Tumor mutation burden				
TMB low (< 10/mb)	73 (40.8)	37 (20.7)	36 (20.1)	< 0.001
TMB high (≥ 10/mb)	100 (55.9)	21 (11.7)	79 (44.1)	
Microsatellite instability				
Microsatellite stable	124 (69.3)	49 (27.4)	75 (41.9)	0.069
Microsatellite instable	55 (30.7)	14 (7.8)	41 (21.9)	
Co-mutation				
IK3CA	56 (31.3)	21 (11.7)	35 (19.6)	0.663
DKN2A	45 (25.1)	5 (2.8)	40 (22.3)	< 0.001
CCND1	41 (22.9)	2 (1.1)	39 (21.8)	< 0.001
FAT1	38 (21.2)	10 (5.6)	28 (15.6)	0.197
ARAF	28 (15.6)	3 (1.7)	25 (14.0)	0.003
NFE2L2	24 (13.4)	3 (1.7)	21 (11.7)	0.012
EGFR	25 (14.0)	4 (2.2)	21 (11.7)	0.03
NOTCH1	23 (12.8)	5 (2.8)	18 (10.1)	0.148
FBXW7	15 (8.4)	12 (6.7)	3 (1.7)	< 0.001
CDKN1B	12 (6.7)	2 (1.1)	10 (5.6)	0.164
RB1	12 (6.7)	5 (2.8)	7 (3.9)	0.627
FGFR1	11 (6.1)	2 (1.1)	9 (5.0)	0.223
PIK3CB	10 (5.6)	5 (2.8)	5 (2.8)	0.313
CCND2	10 (5.6)	1 (0.6)	9 (5.0)	0.113
KDM5A	10 (5.6)	1 (0.6)	9 (5.0)	0.086
EP300	10 (5.6)	4 (2.2)	6 (3.4)	0.743
ERBB2	6 (3.4)	1 (0.6)	5 (2.8)	0.334
PDGFRA	6 (3.4)	1 (0.6)	5 (2.8)	0.334
FGFR3	5 (2.8)	1 (0.6)	4 (2.2)	0.471

Values are presented as number (%). TMB, tumor mutation burden; *TP53*, tumor protein 53.

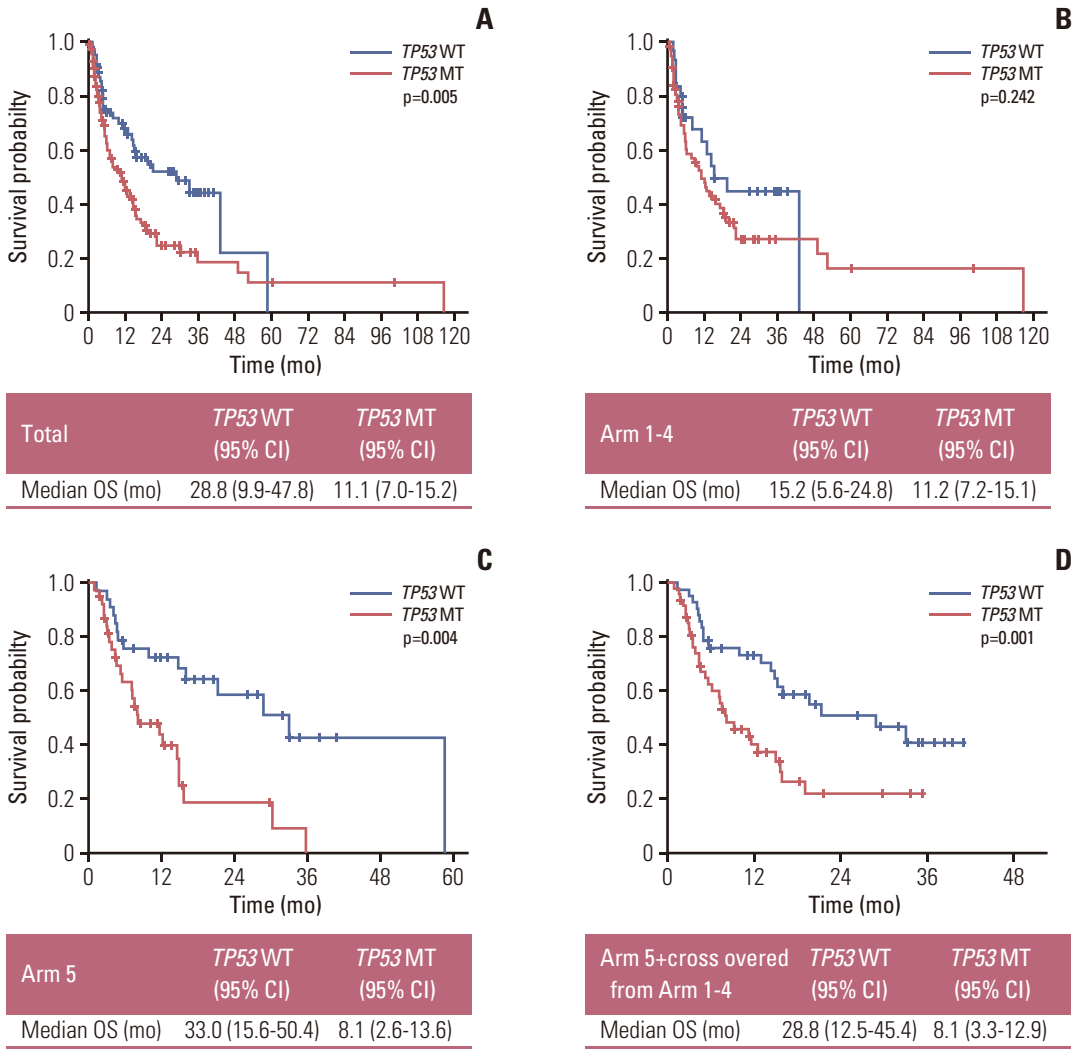
7.3%, p < 0.001). The *TP53* MT group had more underweight patients with a body mass index (BMI) of < 18.5 than the *TP53* WT group (19.6% vs. 4.5%, p=0.029). In the treatment groups 2, 3, and 4, more patients exhibited *TP53* MT than *TP53* WT.

2. *TP53* mutations in HNSCC

Three patients were filtered out because of the lack of a variant in the oncoplot of the total population (Fig. 2A). The *TP53* gene harbored the highest number of mutations in the HNSCC samples. Moreover, missense mutations were the most common *TP53* mutation in HNSCC (50.8%). These mutations were mainly located in the DNA binding domain of p53 (Fig. 2B). The other mutations observed were nonsense mutations (16.4%), frameshift deletions (12.5%), frameshift insertions (8.6%), and splice site mutations (8.6%) (Fig. 2A and B).

3. Mutational profiles of the *TP53* MT and *TP53* WT groups

The *TP53* MT group had significantly more patients with a high tumor mutational burden (TMB; ≥ 10 mutations/mb) than the *TP53* WT group (44.1% vs. 11.7%, p < 0.001). Although the microsatellite instability distribution was not significantly different between the two groups (p=0.069), more unstable cases were observed in the *TP53* MT group than in the *TP53* WT group (21.9% vs. 7.8%). Several comutations, including mutations of the *EGFR* (11.2% vs. 2.2%, p=0.03), *CDKN2A* (22.3% vs. 2.8%, p=0.001), *CCND1* (21.8% vs. 1.1%, p < 0.001), *NFE2L2* (11.7% vs. 1.7%, p=0.012), and *ARAF* (14.0% vs. 1.7%, p=0.003) genes, were more prevalent in the *TP53* MT group than in the *TP53* WT group. However, the *FBXW7* mutation was more common in the *TP53* WT group than in the *TP53* MT group (6.7% vs. 1.7%, p < 0.001) (Table 2).



Total	*TP53* WT (95% CI)	*TP53* MT (95% CI)
Median OS (mo)	28.8 (9.9-47.8)	11.1 (7.0-15.2)

Arm 1-4	*TP53* WT (95% CI)	*TP53* MT (95% CI)
Median OS (mo)	15.2 (5.6-24.8)	11.2 (7.2-15.1)

Arm 5	*TP53* WT (95% CI)	*TP53* MT (95% CI)
Median OS (mo)	33.0 (15.6-50.4)	8.1 (2.6-13.6)

Arm 5+cross overed from Arm 1-4	*TP53* WT (95% CI)	*TP53* MT (95% CI)
Median OS (mo)	28.8 (12.5-45.4)	8.1 (3.3-12.9)

Fig. 3. Overall survival (OS) of patients according to the tumor suppressor p53 (*TP53*) mutations. (A) All participants (groups 1-5). (B) Targeted therapy group (groups 1-4). (C) Immunotherapy group (group 5). (D) Immunotherapy group 2 (group 5+participants who crossed over to group 5 from groups 1-4). CI, confidence interval.

4. Survival in the *TP53* MT and *TP53* WT groups following targeted treatments and immunotherapy

The median OS was significantly shorter in the *TP53* MT group than in the *TP53* WT group (11.1 vs. 28.8 months, p=0.005) (Fig. 3A). The median PFS was also shorter in the *TP53* MT group than in the *TP53* WT group (1.7 vs. 3.8 months, p=0.002). In addition, we compared the survival between patients who were treated with targeted therapies (groups 1-4) and those who were treated immunotherapy (group 5). In 105 patients who were treated with targeted therapies, the OS was not statistically significantly different between those with *TP53* MT or *TP53* WT (11.2 vs. 15.2 months, p=0.242) (Fig. 3B). However, among the patients treated with targeted therapies, the PFS was shorter in patients with *TP53* MT than in those with *TP53* WT (2.5 vs. 7.3 months, p=0.009). Among the 73 patients in group 5, the median OS was shorter in patients with *TP53* MT than in those with *TP53* WT (8.1 vs. 33.0 months, p=0.001) (Fig. 3C). However, there was no significant difference in PFS between the 73 patients in group 5 who exhibited *TP53* MT or *TP53* WT. Thereafter, we analyzed the survival of patients in the immunotherapy group 2, which consisted of patients in group 5 and patients who crossed over to group 5 from groups 1-4 after failure of these treatments. Among the 109 patients in the immunotherapy group 2, the median OS was shorter in patients with *TP53* MT than in those with *TP53* WT (3.3 vs. 11.6 months, p=0.002) (Fig. 3D). However, the median PFS of this population was not statistically different between those with *TP53* MT and

Table 3. Multivariate analysis of prognostic genetic alterations

	Overall survival		p-value
	Hazard ratio	95% CI	
EGFR mutation or amplification	1.40	0.87-2.26	0.170
CDK4/6 mutations, *CCND1* amplification, *CDKN2A* mutations	1.16	0.76-1.79	0.488
TP53 mutations	1.61	1.02-2.58	0.049

CDKN2A, cyclin dependent kinase inhibitor 2A; CI, confidence interval; *EGFR*, epidermal growth factor receptor; *TP53*, tumor protein 53.

those with *TP53* WT (1.6 vs. 3.6 months, p=0.057).

The univariate analysis revealed that the presence of *EGFR* mutations or amplifications, which were allocated to the group 2, and cell cycle pathway-related gene alterations, including *CDK4/6* mutation, *CCND1* amplification, or *CDKN2A* mutations, which were allocated to group 4, were significant predictors of a poor prognosis. In addition, we conducted univariate analyses for various clinical factors including HPV status, smoking, BMI, Charlson Comorbidity Index, different age criteria, sex, site of primary tumor location, and the number of prior lines of systemic treatment. However, none of these clinical factors showed significant differences in OS. Therefore, we included genomic factors as covariates for the multivariate analysis, which showed statistical significance in univariate analysis. The multivariate analysis revealed that a *TP53* mutation was an independent poor prognostic factor (HR, 1.61; p=0.049) (Table 3).

Discussion

TP53 gene mutation is frequently reported in most human cancers. Since its initial discovery in 1979, numerous studies have been conducted to identify the role of *TP53* mutation in cancer biology [11]. Both germline and somatic *TP53* mutations affect cancer development. Germline *TP53* mutations contribute to the development of Li-Fraumeni syndrome, which is characterized by the early onset of multiple cancers [12]. According to the National Cancer Institute–sponsored *TP53* database, which was originally established in 1994 by the International Agency for Research on Cancer and the World Health Organization, the frequency of somatic mutations of the *TP53* gene ranges from 20% to 90% in various sporadic cancers [13,14]. The incidence of *TP53* mutations in HNSCC has been primarily reported in Western populations, and it varies widely across different studies. In The Cancer Genome Atlas (TCGA) analysis of 279 patients with HNSCC, *TP53* mutations were reported in 41% of the tumors tested [15]. In larger cohorts of HNSCC from publicly available datasets such as cBioPortal [16], American Association for Cancer Research Project GENIE [17], and Caris Life Sciences (Phoenix), the incidence of *TP53* mutations is 68% [18]. Among the Asian countries, the incidence of *TP53* mutations was reportedly 38.8% in a Japanese cohort (n=283) and 47% in a Chinese cohort (n=89) [19,20]. In our study, *TP53* mutations were reported more frequently (64.8%). Considering these results, the frequency of *TP53* mutations in Korean HNSCC appears to be relatively higher than that in other Asian countries and similar to that in Western populations.

In our study, the *TP53* mutations were associated with aggressive clinical characteristics such as HPV-negativity, advanced age, and oral cavity origin. *TP53* mutations were also significantly associated with poor survival in the Korean population. Moreover, the multivariate analysis revealed that patients harboring *TP53* mutations demonstrated a 1.61-fold increase in risk of death.

The inverse relationship between the presence of HPV DNA and *TP53* mutations has been documented in several reports. In 2015, the report by TCGA presented the difference in carcinogenesis between HPV-related HNSCCs and non-HPV–related HNSCCs via genomic sequencing [15]. Furthermore, it stated that accumulation of tumor mutations and chromosomal gains and losses were more in non-HPV–related HNSCCs than HPV-related HNSCCs. Moreover, the most prevalent mutation in the non-HPV–related HNSCCs was *TP53* mutation. Therefore, the prevalence of *TP53* mutations might be lower in larynx or hypopharynx cancers, which are weakly related to HPV, than oropharynx cancers. Additionally, the prevalence of *TP53* mutations might be higher in older adults, who have a higher incidence of larynx or hypopharynx cancers, than younger adults. Our findings on the differences in characteristics between the *TP53* MT an *TP53* WT groups are consistent with those of previous studies.

In our study, the prevalence of *TP53* mutations differed according to the BMI. *TP53* mutations were more common in patients with a lower BMI, particularly underweight patients with a BMI of < 18.5, than in those with a higher BMI. A lower BMI in patients with HNSCCs may be attributed to the weight loss associated with the destruction of structures

involved in chewing and swallowing in the hypopharynx or oral cavity. The higher incidence of *TP53* mutations observed in these patients may be related to the fact that patients with hypopharyngeal or oral cavity cancer are more likely to have a low BMI. Furthermore, BMI is regarded as a surrogate marker of the nutritional status of patients with cancer, which affects the OS [21,22]. Previous studies have demonstrated that a higher BMI in HNSCC is associated with better survival outcomes, while being underweight is associated with a higher risk of death [23,24]. Comprehensively, these clinical characteristics may explain the poor prognosis of the *TP53* MT group.

Our study findings demonstrated that the PFS associated with the treatment option significantly impacts the OS. The median PFS of the entire cohort was shorter in the *TP53* MT group than in the *TP53* WT group, and this difference was also observed among patients who were administered targeted agents. However, the median PFS did not differ between the *TP53* MT and *TP53* WT groups among patients who were allocated to the group without any druggable genomic targets. This finding indicates that *TP53* mutation is not a predictor of treatment response of immunotherapy, at least in patients without druggable genetic alterations. However, *TP53* MT was associated with a significantly poor OS, especially in patients in the immunotherapy group. Thus, *TP53* mutations is a powerful prognostic marker in patients without druggable genomic alterations. Our study findings also demonstrated that *EGFR* gene aberrations and cell cycle pathway gene alterations, including *CDK4/6* mutation, *CCND1* amplification, or *CDKN2A* mutations, were associated with a poor survival. However, the multivariate analysis revealed that only *TP53* was independently associated with poor survival. Therefore, a *TP53* mutation, which plays a major role in the various steps and multiple pathways of carcinogenesis, is the most powerful prognostic factor in HNSCC.

In our study, a high TMB or microsatellite instability was seen more frequently in the *TP53* MT group than in the *TP53* WT group. In a report by Klinakis and Rampias, *TP53* mutations were associated with a high TMB, particularly in metastatic HNSCC [25]. Furthermore, data from the Caris Life Sciences cohort demonstrate that tumors harboring *TP53* mutations exhibit a significantly higher average TMB than tumors lacking *TP53* mutations [18]. Because *TP53* has multiple functions that attribute to its interaction with numerous target genes involved in DNA damage, cell cycle, cell metabolism, and apoptosis, *TP53* mutations are naturally correlated with genetic instability or higher TMB [26]. In this study, we also found that comutations in *CDKN2A* or *CCND1* were higher in the *TP53* MT group than in the *TP53* WT group. The normal functioning of the *TP53* gene is negatively regulated by the *MDM2* gene, which encodes an E3 ubiquitin ligase, the *MDM2* protein [27,28]. *CDKN2A* is also involved in stabilizing *TP53*. The p14ARF protein, which is encoded by *CDKN2A*, reportedly binds to *MDM2* and stabilizes *TP53* by suppressing its E3 ubiquitin ligase function [29]. Consistent with our study findings, previous studies have demonstrated the low frequency of tumors with *CDKN2A* alterations in the absence of an accompanying *TP53* mutation [18]. However, a high incidence of *FBWX7* co-mutation was found in our study's *TP53* WT group. *FBWX7* is a well-established tumor suppressor gene that is inactivated by mutations in numerous cancers [30]. *FBWX7* acts as a ubiquitin E3 ligase and regulates the p53 protein level. In response to DNA damage, p53 accumulates and induces *FBXW7* expression. The *FBXW7* expression subsequently promotes p53 degradation, keeping the p53 level in check. Gong et al. [31] reported that *FBXW7* inactivation induces significant cellular senescence in *TP53* WT cells, but not in *TP53* MT or normal cells. Furthermore, they determined that the simultaneous p53 inactivation abrogated the induced cellular senescence [31]. This finding is similar to our study finding that *FBXW7* inactivation triggers tumorigenesis predominantly in the *TP53* WT cells.

Our study results validate *TP53* as a prognostic factor and provide clinically relevant insights for the application of emerging targeted therapies in the treatment of patients with recurrent or metastatic head and neck cancer. Currently, various targeted therapies are under development and clinical trials. These targeted therapies are likely to be utilized as NGS-guided treatments in the near future. In such cases, the presence of *TP53* mutations may influence the choice of targeted therapy in patients with *TP53* MT may be associated with a poor PFS. Moreover, current clinical trial data suggest minimal efficacy of immunotherapies following failure of platinum-based therapies [32]. Thus, targeted therapies rather than immunotherapy can be prioritized in patients with targetable genetic alterations and *TP53* WT after failure of platinum-based therapies. However, in patients with *TP53* mutations and no other actionable genetic alterations, treatment with the immune checkpoint inhibitor is unlikely to increase survival, potentially guiding the avoidance of overtreatments.

The limitations of this study include the small sample sizes within each targeted therapy group, which may have restricted the analysis and interpretation of PFS differences according to the *TP53* mutational status. Further studies on its use as a predictive or prognostic marker of *TP53* mutation is required with larger sample sizes in each targetable gene alteration group. Furthermore, immune checkpoint inhibitors were only administered to patients without targetable driver mutations in the TRIUMPH study. Therefore,

the prognostic role of *TP53* mutations in an entire patient population receiving immune checkpoint inhibitor remains unclear. However, our study has some notable strengths. The analyses were performed by utilizing a large-scale genomic dataset obtained from the nation-wide Korean patients. Future studies should analyze a larger patient cohort and explore other potential markers or therapeutic strategies to improve the poor prognosis associated with *TP53* mutations.

In conclusion, *TP53* mutation is an independent poor prognostic factor in patients with advanced HNSCC. Aggressive clinical characteristics, which are more common in patients with *TP53* mutations and poor response to targeted therapies, might be the cause for the shorter OS. Furthermore, patients with *TP53* MTs exhibited poor prognosis even with immunotherapy. Considering the high frequency of *TP53* mutations in patients with HNSCC, additional investigations should be performed to personalize strategies targeted at *TP53* MTs in this population.

Ethical Statement

This study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. This study was reviewed and approved by the Institutional Review Board and Ethics Committee of the Chungnam National University Hospital (IRB approval number: Asan Medical Center; 2017-1295, Yonsei Cancer Center; 4-2017-0695) and the local committees of all other participating centers. Written informed consent was obtained from all the patients prior to being enrolled in the TRIUMPH trial.

Author Contributions

Conceived and designed the analysis: Kang EJ, Lee YG, Choi JK, Shin SH, Choi YH, Lee KW, Lee HW, Kim MK, Lim ST, Yun HJ, Park SG, Kim SB.
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Conflicts of Interest

Conflict of interest relevant to this article was not reported.

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References

1. Feroz W, Sheikh AM. Exploring the multiple roles of guardian of the genome: P53. Egypt J Med Hum Genet. 2020;21:49.
2. Kastenhuber ER, Lowe SW. Putting p53 in context. Cell. 2017;170:1062-78.
3. 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;117:2682-92.
4. 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.
5. 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.
6. Chen S, Zhou Y, Chen Y, Gu J. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics. 2018;34:i884-90.
7. 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.
8. Kandoth C, Gao J, Qwangmsk, Mattioni M, Struck A, Coursin Y, et al. mskcc/vcf2maf: vcf2maf v1.6.19 [Internet]. Zenodo; 2020 [cited 2024 Jun 28]. Available from: 10.5281/zenodo.593251.
9. Amemiya HM, Kundaje A, Boyle AP. The ENCODE Blacklist: identification of problematic regions of the genome. Sci Rep. 2019;9:9354.
10. 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:e1004873.
11. Lane DP, Crawford LV. T antigen is bound to a host protein in SV40-transformed cells. Nature. 1979;278:261-3.
12. Malkin D, Li FP, Strong LC, Fraumeni JF Jr, Nelson CE, Kim DH, et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science. 1990;250:1233-8.
13. Olivier M, Hollstein M, Hainaut P. TP53 mutations in human cancers: origins, consequences, and clinical use. Cold Spring Harb Perspect Biol. 2010;2:a001008.
14. Chen X, Zhang T, Su W, Dou Z, Zhao D, Jin X, et al. Mutant p53 in cancer: from molecular mechanism to therapeutic modulation. Cell Death Dis. 2022;13:974.
15. Cancer Genome Atlas Network. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature. 2015;517:576-82.
16. cBioPortal for Cancer Fenomics [Internet]. cBioPortal for Cancer Fenomics; 2024 [cited 2024 Jun 28]. Available from: http://www.cbioportal.org/index.do.
17. AACR PROJECT GENIE: powering precision medicine [Internet]. American Association for Cancer Research; 2024 [cited 2024 Jun 28]. Available from: https://www.aacr.org/professionals/research/aacr-project-genie/
18. Deneka AY, Baca Y, Serebriiskii IG, Nicolas E, Parker MI, Nguyen TT, et al. Association of TP53 and CDKN2A mutation profile with tumor mutation burden in head and neck cancer. Clin Cancer Res. 2022;28:1925-37.
19. Maruyama H, Yasui T, Ishikawa-Fujiwara T, Morii E, Yamamoto Y, Yoshii T, et al. Human papillomavirus and p53 mutations in head and neck squamous cell carcinoma among Japanese population. Cancer Sci. 2014;105:409-17.
20. Li Q, Zhu G, Zhang L, Zeng B, Cai T, Wu J, et al. Mutational landscape of head and neck cancer and cervical cancer in Chinese and Western population. Head Neck. 2024;46:528-40.
21. Campbell PT, Newton CC, Dehal AN, Jacobs EJ, Patel AV, Gapstur SM. Impact of body mass index on survival after colorectal cancer diagnosis: the Cancer Prevention Study-II Nutrition Cohort. J Clin Oncol. 2012;30:42-52.
22. Kampman E, Vrieling A, van Duijnhoven FJ, Winkels RM. Impact of diet, body mass index, and physical activity on cancer survival. Curr Nutr Rep. 2012;1:30-6.
23. den Hollander D, Kampman E, van Herpen CM. Pretreatment body mass index and head and neck cancer outcome: a review of the literature. Crit Rev Oncol Hematol. 2015;96:328-38.
24. Hobday S, Armache M, Paquin R, Nurimba M, Baddour K, Linder D, et al. The body mass index paradox in head and neck cancer: a systematic review and meta-analysis. Nutr Cancer. 2023;75:48-60.
25. Klinakis A, Rampias T. TP53 mutational landscape of metastatic head and neck cancer reveals patterns of mutation selection. EBioMedicine. 2020;58:102905.
26. Riley T, Sontag E, Chen P, Levine A. Transcriptional control of human p53-regulated genes. Nat Rev Mol Cell Biol. 2008;9:402-12.
27. Kubbutat MH, Jones SN, Vousden KH. Regulation of p53 stability by Mdm2. Nature. 1997;387:299-303.
28. Buschmann T, Minamoto T, Wagle N, Fuchs SY, Adler V, Mai M, et al. Analysis of JNK, Mdm2 and p14(ARF) contribution to the regulation of mutant p53 stability. J Mol Biol. 2000;295:1009-21.
29. Sherr CJ, Weber JD. The ARF/p53 pathway. Curr Opin Genet Dev. 2000;10:94-9.
30. Yeh CH, Bellon M, Nicot C. FBXW7: a critical tumor suppressor of human cancers. Mol Cancer. 2018;17:115.
31. Gong L, Cui D, Liu D, Shen X, Pan H, Xiong X, et al. FBXW7 inactivation induces cellular senescence via accumulation of p53. Cell Death Dis. 2022;13:788.
32. He Y, Zeng J, Wei Z, Huang Y, Yang L, Hu X, et al. Comparison of second-line treatments for patients with platinum-resistant recurrent or metastatic head and neck squamous cell carcinoma: a systematic review and Bayesian network meta-analysis. Cancers (Basel). 2022;14:4472.