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ORIGINAL ARTICLE

High-risk TP53 mutations predict poor primary treatment response of patients with head and neck squamous cell carcinoma

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Abstract

Objectives: Head and neck squamous cell carcinoma (HNSCC) poses a diagnostic and therapeutic challenge worldwide and is associated with a poor survival rate. Due to the variability in the efficacy of treatments for HNSCC, new predictive biomarkers of therapy outcomes are needed. Recently, we developed an algorithm that employs the mutational profile of TP53 as an independent prognostic factor in HNSCC. In this study, we investigated its role as a predictive biomarker of treatment outcomes in HNSCC patients. We also tested the usefulness of two classification systems for TP53 mutational landscapes.

Materials and Methods: Clinical and genomic data were retrieved from The Cancer Genome Atlas database. We built a multivariate stepwise backward binary regression model to assess the role of TP53 mutations in predicting therapeutic outcomes.

Results: Cases harbouring high-risk-of-death mutations reported an odds ratio of 3.301 for stable or progressive disease compared to wild-type cases, while no significant difference in treatment outcomes was found between cases with low-risk-of-death mutations and wild-type TP53. Our analysis found that older patients with a history of alcohol consumption had a higher risk of stable/progressive disease.

Conclusions: This study improves current evidence on the role of TP53 mutations in treatment response in HNSCC patients.

KEYWORDS
biomarkers, mutation, precision Medicine, squamous Cell Carcinoma of Head and Neck, TP53 genes

1 | BACKGROUND

Annually, over 600.000 people worldwide receive a diagnosis of head and neck squamous cell carcinoma (HNSCC), making this class of tumours the seventh most common malignancy globally (Sung et al., 2021). Although anatomical and molecular differences exist within HNSCCs, over 90% of cases are diagnosed as squamous cell carcinoma (Ali et al., 2017). Successful treatment of these patients is challenging and, despite the development of new treatment options (Perrotti et al., 2021), the 5-year survival rate has not changed for decades and remains at around 65% (Svahn et al., 2016). At present, human papillomavirus (HPV) status and staging are the main means to predict outcomes (Amin et al., 2017) and are also used to inform the choice of therapeutic options. Overall, treatment is guided by TNM stage, HPV status, anatomical localization, age and functional considerations while meeting patients' needs (Gottgens et al., 2019; Sabatini & Chiocca, 2020). Success rates may reach 80% for patients with small primary cancer and no lymph node involvement after resective surgery or radiation therapy (Lee et al., 2018). Surgical resection is mostly indicated in patients with oral cavity cancer, while patients with HPV-positive oropharyngeal or laryngeal cancer show better outcomes after radiation therapy (Johnson et al., 2020; Lyhne et al., 2015). Unfortunately, most patients receive a diagnosis of HNSCC with local or regional involvement, resulting in a decline of 5-year survival rate to 40% (Esaki et al., 2020); yet, first-line therapeutic protocols for such advanced tumours include surgical excision matched by chemotherapy and/or radiation therapy (Perrotti et al., 2022).

Despite the availability of novel treatment modalities for HNSCC, patients do not benefit significantly from these advancements in terms of improvement in overall survival, and approximately 50% of treated patients develop recurrent or metastatic disease in the following 2 years (Perrotti et al., 2021). Current evidence suggests that there is high variability in the clinical efficacy of different therapeutic modalities (Gavrielatou et al., 2020; Perrotti et al., 2021), however, clinicians lack predictive biomarkers to successfully correlate treatments with clinical response (Bouhaddou et al., 2021; Cohen et al., 2019; Gavrielatou et al., 2020; Ow et al., 2015). To date, smoking status (Ma et al., 2022), age (Stromberger et al., 2020) and HPV infection (Sabatini & Chiocca, 2020) have been shown to influence therapy outcomes in HNSCC. Additionally, mutations in the TP53 gene have been proposed as predictive biomarkers for therapy response, although inconsistent and controversial results have been reported (Basyuni et al., 2022; Ow et al., 2015). Studies have shown that HNSCC cells with disruptive mutations of TP53 have a higher resistance to radiotherapy (Skinner et al., 2012) and similar results were obtained in other studies using different radio/chemotherapy protocols, where patients with wild-type TP53 reported better therapy response (Hoffmann et al., 2008; Lindenbergh-van der Plas et al., 2011; Perrone et al., 2010; Temam et al., 2000). However, one study showed no difference between wild-type and mutated TP53 tumours in their response to radiation therapy (Koch et al., 1996). These inconsistencies could be explained by the fact that different mutations harbour variable biological and functional profiles in terms of loss or gain of function and dominant-negative activity, with consequent differential effects on therapy response (Poeta et al., 2007; Vikhanskaya et al., 2005; Zhou et al., 2016). Accounting for this heterogeneity in TP53 mutations, we recently developed a new classification system and highlighted the existence of specific high-risk of death mutations of the gene TP53 in patients with HNSCC (Caponio et al., 2020). Our model is an improvement of the TP53 mutation classification system originally proposed by Poeta et al. (Poeta et al., 2007), which was based on types of mutation, affected structural domain and changes in polarity. We improved Poeta's et al. criteria by including secondary structures, homozygous alteration, zing ligand and ability in forming hydrogen bonds and developed a new classification system (Caponio et al., 2020).

The aim of this study was to investigate the role of mutations in TP53 as a predictor of therapy response outcome in patients with HNSCC and to examine whether our classification system can be a useful tool to predict adverse therapy response.

2 | MATERIALS AND METHODS

2.1 | Data source and data collection

This study was conducted according to the Recommended Guidelines for Validation, Quality Control and Reporting of TP53 Variants Clinical Practice (Leroy et al., 2017). Data access and download were detailed in a previous publication from our group (Caponio et al., 2020) and included 207 HNSCC cases from The Cancer Genome Atlas database. Exclusion criteria were as follows: (a) cases with double mutation of the TP53 gene; (b) patients treated with neoadjuvant therapy; (c) patients with more than two relapsing events or a history of other malignancies; (d) patients with a histological diagnosis other than squamous cell carcinoma and postoperative death. Data were entered in an Excel file for the collection of all clinical-pathological variables and matched mutations. Data were entered into an Excel file for the collection of all clinical-pathological variables and matched mutations. Data were further processed in SPSS 21.0 for statistical analysis.TP53 mutations were assessed and classified as previously reported by Caponio et al., referred to as "Model C" (Caponio et al., 2020) (Table 1) and Poeta et al., referred to as "Model P" (Poeta et al., 2007) (Table 2).

2.2 | Statistical analysis

To assess the role of TP53 mutations in predicting therapeutic outcomes, a multivariate stepwise backward binary regression model was built. TP53 mutations were categorized according to the two different mutation-based models (Caponio et al., 2020; Poeta et al., 2007). Patients were grouped together in the case of progressive/stable disease or partial remission after the treatment regimen versus patients showing complete remission, defined as the disappearance of all signs of cancer in response to treatment. Stepwise backward multivariate analysis included only the clinical-pathological

TABLE 1 Model C: classification of TP53 mutations according to Caponio et al., 2020.

TP53 categorization	Characteristics
Low-risk	1. Missense mutations occurring outside the binding domains of L2-L3 (codons 163–195 or 236–251) 2. Missense mutations occurring in the binding domains of L2-L3, characterized by a replacement of an amino acid with another from the same polarity/charge category 3. Missense mutations involving any other secondary structure (excluding unknown), while maintaining the ability to donate or accept H^+ bond
High-risk	1. Stop, frameshift, splice and inframe mutations along the whole sequence of the gene 2. Missense mutations occurring in L2-L3 binding domains and replacing an amino acid from one polarity/charge category with an amino acid from another category 3. Mutation occurring in homozygous loci, such as DNA–VAF from 65% to 100% 4. In zinc-ligand amino acid 5. Missense mutations in which amino acid change lead to the destruction of H^+ bond 6. Missense mutations involving a non-assisgned secondary structure (defined as unknown) of the protein

TABLE 2 Model P: classification of TP53 mutations according to Poeta et al., 2007.

TP53 categorization	Characteristics
Conservative	1. Missense mutations occurring outside the binding domains of L2-L3 (codons 163–195 or 236–251) 2. Missense mutations occurring in the binding domains of L2-L3, characterized by a replacement of an amino acid with another from the same polarity/charge category
Disruptive	1. Stop, frameshift, splice and inframe mutations along the whole sequence of the gene 2. Missense mutations occurring in L2-L3 binding domains and replacing an amino acid from one polarity/charge category with an amino acid from another category

variables with evidence of significant contribution to the outcome at univariate analysis and an α-value of 0.157 was set for p-value in the stepwise backward model, as suggested by Sahoo et al. (Caponio et al., 2021; Heinze & Dunkler, 2017; Sahoo et al., 2020). Treatment outcomes were considered as dependent variable against sex, alcohol history and age.

The adequacy of the model was determined by the Hosmer-Lemeshow goodness-of-fit criterion, and the relative risk to different treatment outcomes was estimated by odds ratio (OR) with a 95% confidence interval (CI) (Temam et al., 2000). The power of logistic regression model was evaluated by the receiver operating characteristic (ROC) curve while the model fit was evaluated by AIC, in STATA v16 software.

The chi-square test, Fisher's exact test or Mann–Whitney test were used to explore differences between clinical-pathological variables, where applicable. The tests were performed using SPSS software v.21 and statistical significance was set as $p < 0.05$.

3 | RESULTS

3.1 | Patients' characteristics and treatment plan

A total of 207 cases were analysed from The Cancer Genome Atlas database (Table 3). Of these, 145 cases had a single mutation in the TP53 gene, while 62 cases had wild-type TP53 gene (WT). Specifically, 28 cases had a frameshift mutation, 3 had an in-frame mutation, 81 had a missense mutation, 11 had a splice-site mutation and 22 had a stop mutation. The average follow-up period for patients was 34.13 months (S.D. 30.79).

Based on the treatment plan, 73 cases underwent surgery alone (35.3%), 69 were treated with surgery and additional radiation therapy (33.3%), while in only 6 cases, surgery was followed by chemotherapy (2.9%). Finally, 59 cases were treated with surgery accompanied by both radio- and chemotherapy (28.5%). In total, 128 cases received radiation therapy, with the radiation total dose ranging between 600 and 8000 cGy and a mean of 6005.45 cGy (1250.57 S.D.). The radiation was delivered in a total number of fractions between 2 and 40, with a mean of 29.6 (6.65 S.D.). Sixty-five cases underwent chemotherapy alone or combined with radiation therapy. Chemotherapy protocols differed among patients and information about prescribed total dose and number of treatment cycles was not retrievable in most cases. Cisplatin was administered as first-line treatment in 35 cases, followed by Carboplatin in 8 cases. Erbitux was administered to 4 cases; Vectibix, Taxol and Paclitaxel were administered to 2 cases each, while Methotrexate, Docetaxel and Cetuximab were each given to one patient.

3.2 | Treatment outcomes and clinical-pathologic correlations

Out of the 207 cases included, 77.8% (161/207) achieved complete remission, while 22.2% (46/207) experienced progressive

TABLE 3 Clinical-pathological characteristics of included patients with HNSCC.

Clinical-pathological parameters	Groups	Number of patients n° of cases/total number and percentage									
		Oral cavity		Larynx		Oropharynx		Hypopharynx		Total	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%
Sex	Male	83	62.40	39	86.60	20	90.90	3	60.00	145	70.70
	Female	50	37.60	6	13.40	2	9.10	2	40.00	60	29.30
Age		*n* = 133		*n* = 45		*n* = 22		*n* = 5		*n* = 205	
	<60 years old	61	45.90	14	31.10	17	70.80	1	20.00	93	44.90
	≥60 years old	72	54.10	31	68.90	7	29.20	4	80.00	114	55.10
Grade		*n* = 133		*n* = 45		*n* = 24		*n* = 5		*n* = 207	
	1 and 2	113	84.90	31	70.40	9	52.90	3	60.00	156	78.30
	3	20	15.10	13	29.60	8	47.10	2	40.00	43	21.70
Stage		*n* = 133		*n* = 44		*n* = 17		*n* = 5		*n* = 199	
	I-II	43	32.80	6	13.60	7	29.10	0	0.00	56	27.40
	III-IV	88	67.20	38	86.40	17	70.90	5	100.00	148	72.60
		n = 131		*n* = 44		*n* = 17		*n* = 5		*n* = 204	
HPV status	Negative	–	–	–	–	3	12.50	–	–	3	12.50
	Positive	–	–	–	–	21	87.50	–	–	21	87.50
		–		–		*n* = 24		–		*n* = 24	
Mutational status for TP53 Model C	Wild type	30	22.50	10	22.20	20	83.30	2	40.00	62	29.90
	Low-risk	33	24.80	12	26.70	1	4.20	0	0.00	46	22.20
	High-risk	70	52.70	23	51.10	3	12.50	3	60.00	99	47.90
		n = 133		*n* = 45		*n* = 24		*n* = 5		*n* = 207	

or stable disease after completing their treatments. There was no statistically significant difference in treatment outcomes among anatomical groups (Fisher exact test *p*-value = 0.276—complete remissions 102/133 in oral cavity; 22/24 in oropharynx; 3/5 in hypopharynx; 34/45 in larynx) or clinical stage (Fisher exact test p-value = 0.582—complete remissions 8/10 in Stage I; 33/46 in Stage II; 41/49 in Stage III; 76/99 in Stage IV). Treatment outcomes also did not differ significantly among different treatment protocols (Fisher exact test *p*-value = 0.070—complete remissions 57/73 in cases undergoing surgery alone; 57/69 in cases with additional radiation therapy; 2/6 in cases with additional chemotherapy and 45/59 in cases with additional radio- and chemotherapy). When smoking status was examined, the cases were categorized as smokers and never-smokers and, further classified as ex-smokers who had quit for more or less than 15 years and current smokers. No differences in treatment outcomes were observed (chi-square *p*-value = 0.938 and 0.314). Additionally, the number of cigarettes smoked was not associated with different treatment outcomes (Mann–Whitney *p*-value = 0.362). Treatment outcomes did not differ significantly among different tumour grading statuses (chi-square *p*-value = 0.960). For patients receiving radiotherapy, no significant differences were found in treatment outcomes based on radiation therapy total dose and number of cycles (Mann–Whitney *p*-value = 0.232 and 0.946, respectively). Similarly, limited data on the total dose administered drugs showed no significant differences in treatment outcomes (Mann–Whitney *p*-value = 0.769). HPV status was available for 201 cases, of which only 28 were positive. Complete remission was achieved in 27/28 cases and for this reason, HPV status was excluded from the multivariate regression logistic model. These findings suggest that, except for HPV status, anatomic site and clinical staging of the tumour do not correlate with therapy outcome in this cohort. Likewise, smoking status and treatment regimens did not predict therapy response outcomes in HNSCC patients.

3.3 | Multivariate stepwise backward binary regression

Since treatment response could not be predicted using the above clinical-pathologic variables mentioned earlier, a multivariate logistic regression model was developed to assess the impact of TP53 mutations in predicting therapeutic outcomes. In this model, the dependent variable was treatment outcomes, while patient-related characteristics that were found to predict therapy response at univariate analysis (such as sex, alcohol history and age with p-values of 0.039, 0.095 and 0.006, respectively) were considered as independent variables. When mutations were classified according to either Model C (Caponio et al., 2020) or Model P (Poeta et al., 2007), the multivariate logistic regression model was equally completed in one step. Based on Model P, the multivariate analysis found that patients with a documented history of alcohol consumption were at higher risk of stable or progressive disease (OR = 2.545, 95% CI: 1.142–5.670,

p-value = 0.022) (Table 4). Similarly, older patients were slightly at higher risk of stable or progressive disease (OR = 1.038, 95% CI: 1.005–1.071, *p*-value = 0.022). Female patients were more likely to achieve a complete remission, although this result was close to the significance level (OR = 0.511, 95% CI: 0.238–1.096, *p*-value = 0.085). Nearly identical results were obtained when mutations were input based on Model C (alcohol consumption history: OR = 2.402, 95% CI: 1.080–5.340, *p*-value = 0.032; age: OR = 1.040, 95% CI: 1.007–1.074, *p*-value = 0.016; female patients: OR = 0.526, 95% CI: 0.245–1.130, *p*-value = 0.100) (Table 5). In general, mutations of TP53 could predict patients at high risk of stable or progressive disease. In detail, when applying Model P classification, both conservative and disruptive mutations reported worse treatment outcomes compared to wild-type patients, without difference between conservative and disruptive mutations (Conservative mutations compared to wild-type: OR = 2.839, 95% CI: 1.034–7.796, *p*-value = 0.043; Disruptive mutations compared to wild-type: OR = 2.779, 95% CI: 1.069–7.225, *p*-value = 0.036). After applying Model C, a better predictive performance of TP53 mutational status was obtained. Specifically, the area under the curve generated in the multivariate model including mutations classified according to Model C returned a value of 0.72 and an Akaike Information Criterion (AIC) of 206.7, compared to the model including mutations based on Model P, for which the area under the curve was 0.70 and AIC was 0.208 (Figure 1).

Only disruptive mutations, based on Model C categorization as high-risk mutations, predicted higher risk of stable or progressive disease after treatments compared to wild-type (OR = 3.301, 95% CI: 1.308–8.336, *p*-value = 0.011), while no statistically significant difference emerged between conservative mutations (low-risk mutations according to Model C) and TP53 wild-type tumours (OR = 1.829, 95% CI: 0.599–5.583, *p*-value = 0.289).

TABLE 4 Stepwise backward multivariate analysis included clinical-pathological variables and Model P.; 2007.

Parameters	Groups	Odds ratio	Confidence interval 95%	*p*-Value
Age		1.038	1.005–1.071	**0.022**
Sex	Male	1	1	0.085
	Female	0.511	0.238–1.096	
Alcohol history	No	1	1	**0.022**
	Yes	2.545	1.142–5.670	
Model P (2007)	Wild-type	1	1	–
	Conservative	2.839	1.034–7.796	**0.043**
	Disruptive	2.779	1.069–7.225	**0.036**

Note: Bold values indicate statistical significance ($p < 0.05$).

TABLE 5 Stepwise backward multivariate analysis included clinical-pathological variables and Model C.; 2020.

Parameters	Groups	Odds ratio	Confidence interval 95%	*p*-Value
Age		1.040	1.007–1.074	**0.016**
Sex	Male	1	1	0.100
	Female	0.526	0.245–1.130	
Alcohol history	No	1	1	**0.032**
	Yes	2.402	1.180–5.340	
Model C (2020)	Wild-type	1	1	–
	Low-risk	1.829	0.599–5.583	0.289
	High-risk	3.301	1.308–8.336	**0.011**

Note: Bold values indicate statistical significance ($p < 0.05$).

4 | DISCUSSION

In the present study, we demonstrate that high-risk TP53 mutations, along with a positive history of alcohol consumption and older age, are useful in predicting poorer treatment outcomes, such as stable or progressive disease, in HNSCC patients.

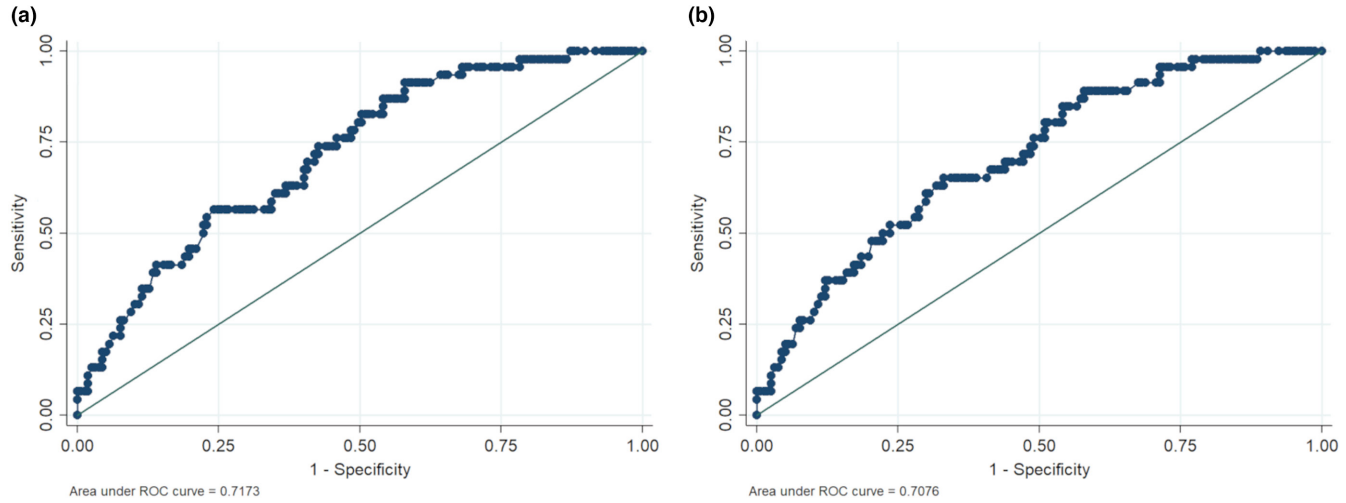

FIGURE 1 ROC analysis shows an area under the curve for the multivariate logistic regression for the treatment outcome, including as variables age, sex, documented alcohol history and mutations based on (a) Model C and (b) Model P.

No statistically significant difference emerged between low-risk mutations and wild-type tumours.

Despite the administration of chemo-radiotherapy in patients with HNSCC, a certain number of patients show treatment failure due to resistance to such treatment protocols (Li et al., 2023; Ortiz-Cuaran et al., 2021; Perrotti et al., 2021). To our knowledge, no biomarker has been approved to date for clinical use to predict treatment outcomes (Li et al., 2023) and only HPV infection is considered a strong predictor of positive therapy response and patients' survival (Perri et al., 2020). Some studies have elucidated a possible association between smoking status (Smith et al., 2019; von Kroge et al., 2020) and older age with treatment failure (Bentzen & Overgaard, 1994; Chen et al., 2020; Perera et al., 2020). In our cohort, only age slightly contributed to predict a worse treatment outcome, while smoking status, smoking history or the total number of cigarettes smoked per year did not influence treatment response. On the contrary, documented alcohol history significantly contributed to treatment failure. While there is substantial evidence of alcohol's role as a prognostic factor of survival in cancer patients (Inoue et al., 2012; Kawakita et al., 2016; Leoncini et al., 2015; Li et al., 2014; Sawabe et al., 2017), to the best of our knowledge, our study is the first to highlight the negative role of alcohol consumption in treatment outcomes. The reasons for the negative influence of alcohol consumption in patients' prognosis are still unclear (Sawabe et al., 2017). However, some mechanisms might involve cell proliferation, decrease DNA-repair machinery and impaired antioxidant defence (Papalouka et al., 2023; Ratna & Mandrekar, 2017). In particular, alcohol consumption can lead to a decrease in patients' nutrient status, which can weaken the activity of the immune system (Meadows & Zhang, 2015).

The response of cancer cells to treatment may also be influenced by genomic alterations. TP53 is the most commonly mutated gene in HNSCC, and these mutations have a negative impact on patient's prognosis (Basyuni et al., 2022). Although the prognostic significance of TP53 mutations is increasingly recognized, their impact on therapeutic outcomes remains controversial and only a few studies have investigated this topic thus far (Ow et al., 2015). Most research has focused on cell-line models, in which mutated TP53 confers higher chemo- and radioresistance (de Bakker et al., 2021; Wang et al., 2023). Although these results have been poorly investigated in clinical studies, emerging evidence suggests a higher risk of treatment failure in patients bearing mutated TP53 gene (Temam et al., 2000; Zhou et al., 2016), although contrasting results have also been reported (Basyuni et al., 2022; Koch et al., 1996). These discrepancies may be due to the different kinds of mutational events that can affect TP53 (Monti et al., 2020). For example, in our previous study on 286 patients with HNSCC, there were 129 different types of mutations, with the most frequent being R273 in 13 patients (Caponio et al., 2020). Moreover, previous studies only evaluated mutations in the main transcribed segments of the whole TP53 gene, thus ignoring mutations that may occur throughout the entire gene locus (Koch et al., 1996; Temam et al., 2000). In this scenario, growing evidence suggests that different mutations result in variable biological profiles in terms of loss or gain of function and dominant-negative activity, with major consequences on pathway activation, therapy response and prognosis of patients (Wang et al., 2023). For these reasons, future studies should not only compare wild-type and mutated patients but should also apply additional classifications to translate these biologically different functions to clinical data. Poeta et al. attempted to overcome this issue by classifying patients based on conservative or disruptive mutations of TP53, with interesting prognostic results (Poeta et al., 2007). In a previous study, we developed a new classification system, that outperformed Poeta et al.'s model in terms of survival prognosis (Caponio et al., 2020). Specifically, our algorithm successfully stratified cancer patients with mutated TP53 into low-high-risk of death categories, while Poeta et al.'s method failed to identify this difference. In this present study, patients harbouring high-risk of death mutations (Model C) reported an OR of 3.301 for stable or progressive disease compared to wild-type patients, while no statistically significant difference was found between low-risk of death mutations and wild-type patients in treatment outcomes.

Unfortunately, clinicians lack strong biomarker predictors of treatment outcomes in patients with HNSCC, and the TNM Staging system has failed to serve this purpose (Saada-Bouzid et al., 2019). In this study, we have provided the first evidence that TP53 mutations, classified according to our model, represent a useful tool in predicting the therapy response of patients with HNSCC. The protein p53 plays a key role in cellular physiology by regulating cell cycle and apoptosis in response to DNA stress, damage and hypoxia. Numerous mechanisms have been proposed to explain the role of mutant p53 in radiochemoresistance. For instance, some p53 mutants can enhance the expression of MDR1 by binding to its promoter, whereas wild-type p53 inhibits MDR1 expression. MDR1 encodes an ATP-dependent drug efflux pump that contributes to chemoresistance (de Bakker et al., 2021; Wang et al., 2023). Apoptosis is the primary process responsible for inducing cancer cell death, and mutations in the TP53 gene can disrupt its transcriptional activity and its ability to bind and activate BCL2-family proteins, resulting in the inhibition of apoptosis (Chen et al., 2022; Zhou et al., 2019). Our findings may indicate biological differences that extend beyond variations in the TP53 gene mutations (El Baroudi et al., 2017). While Model P (Poeta et al., 2007) did not identify any significant difference in treatment outcomes between cases with conservative and disruptive mutations, our classification system demonstrated that patients with high-risk mutations were the only group at a higher risk of treatment failure. This might be explained by dysregulated pathways, influenced by specific changes affecting high-risk mutations. For example, p53 mutants have been found to sequestrate p53 homologs, TAp63 and TAp73, thus impairing the apoptotic process further (Chen et al., 2022). In colorectal cancer, mutant p53 has been shown to promote chemoresistance by activating JNK–c-Jun and Src–ERK pathways (Alam et al., 2016). Mutated TP53 products, such as p53-R175H, induce miRNA-128-2 expression which, in turn, targets the transcription factor E2F5, thus leading to Cisplatin, Doxorubicin or 5-Fluorouracil-induced apoptosis resistance (Donzelli et al., 2012). Another mechanism contributing to chemoresistance involves the downregulation of miR-233

(Masciarelli et al., 2014). Chemo- and radiotherapy are also able to induce cell death by autophagy. Moreover, mutated p53 proteins can impair such mechanisms by differential activation of mTOR and AMPK pathways (Cordani et al., 2016; Zhang et al., 2015).

Although our study provides promising results for predicting therapy response in HNSCC patients, its clinical application may face certain limitations. Firstly, our classification system is based on sequencing characterization of the tumour, which may not be easily accessible in many hospital settings and hence could not be included in routine tumour characterization. Future studies may need to establish a correlation between this mutation classification system and immunohistochemical expression of surrogate markers to improve applicability. However, previous attempts to correlate TP53 mutations and surrogate immunohistochemical expression, have produced controversial results (Basyuni et al., 2022; Murnyak & Hortobagyi, 2016).

5 | CONCLUSIONS

The current study provides further insight into the prognostic and predictive value of TP53 mutations in HNSCC patients. While our results are encouraging, this investigation is limited to a retrospective cohort of patients, without the possibility of testing in other patient cohorts. Well-designed prospective studies are needed to confirm the role of TP53 mutations in HNSCC before this biomarker can be used to inform clinical decision making.

AUTHOR CONTRIBUTIONS

Marco Mascitti: Data curation; writing – original draft; writing – review and editing. **Vito Carlo Alberto Caponio:** Conceptualization; data curation; formal analysis; writing – original draft. **Khrystyna Zhurakivska:** Methodology; writing – original draft. **Lucrezia Togni:** Methodology; writing – review and editing. **Francesca Spirito:** Writing – original draft. **Nicola Cirillo:** Writing – original draft. **Lorenzo Lo Muzio:** Methodology. **Giuseppe Troiano:** Conceptualization; formal analysis; writing – original draft.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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