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Classification of TP53 Mutations and HPV Predict Survival in Advanced Larynx Cancer

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Objectives/Hypothesis: Assess tumor suppressor p53 (*TP53*) functional mutations in the context of other biomarkers in advanced larynx cancer.

Study Design: Prospective analysis of pretreatment tumor *TP53*, human papillomavirus (HPV), Bcl-xL, and cyclin D1 status in stage III and IV larynx cancer patients in a clinical trial.

Methods: *TP53* exons 4 through 9 from 58 tumors were sequenced. Mutations were grouped using three classifications based on their expected function. Each functional group was analyzed for response to induction chemotherapy, time to surgery, survival, HPV status, p16INK4a, Bcl-xl, and cyclin D1 expression.

Results: *TP53* mutations were found in 22 of 58 (37.9%) patients with advanced larynx cancer, including missense mutations in 13 of 58 (22.4%) patients, nonsense mutations in four of 58 (6.9%), and deletions in five of 58 (8.6%). High-risk HPV was found in 20 of 52 (38.5%) tumors. A classification based on Evolutionary Action score of p53 (EAp53) distinguished missense mutations with high risk for decreased survival from low-risk mutations ($P = 0.0315$). A model including this *TP53* classification, HPV status, cyclin D1, and Bcl-xL staining significantly predicts survival ($P = 0.0017$).

Conclusion: EAp53 functional classification of *TP53* mutants and biomarkers predict survival in advanced larynx cancer.

Key Words: *TP53*, p53, larynx cancer, Bcl-xL, cyclin D1, HPV.

Level of Evidence: NA.

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INTRODUCTION

Larynx cancer is diagnosed in 12,260 patients in the United States each year, causing 3,630 deaths.[1] The high morbidity associated with the traditional surgical treatments led to the development of larynx-sparing chemoradiotherapy regimens.[2,3] Furthermore, many biomarkers have been studied in attempts to better classify patients for different treatment modalities and increase their survival.[4]

The tumor suppressor p53 (*TP53*) is mutated in up to 60% of head and neck cancers in some series.[5] It has a central role in determining cell fate in response to stressors.[6–10] The role of p53 as a prognostic biomarker in larynx cancer is controversial. Some studies show an association of p53 mutations with better sensitivity to chemotherapy,[11] whereas others associate it with poor response.[12,13] Almost 75% of the *TP53* mutations reside in the DNA-binding domain, and more than 30% are in one of six hotspot codons. These mutations can promote cancer development. Thus, analysis based primarily on protein function is appropriate in assessing clinical outcome linked to p53 mutants rather than only on its expression, as frequently was done in the past. Functional classifications of p53 can be based on Evolutionary Action score of *TP53* (EAp53),[14] yeast-based transcriptional activity,[15] and published gain of function (GOF) mutations.

In larynx cancer, B-cell lymphoma-extra large (Bcl-xl) and cyclin D1 are predictors of response to therapy and survival that are linked to p53 status.[4,16] Bcl-xl is an anti-apoptotic protein member of the Bcl-2 family[17–19] that inactivates proapoptotic proteins at the mitochondrial membrane.[19,20] Cyclin D1 phosphorylates and inactivates retinoblastoma protein (Rb), allowing cell cycle progression.

Human papillomavirus (HPV) studies in larynx cancer have been limited to recurrent laryngeal

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papillomatosis, which is caused by infection with low-risk HPV types 6 and 11.[21] High-risk HPV (hrHPV), especially HPV16, has become increasingly common in head and neck cancer.[22–27] In the current study, we assess the role of hrHPV, *TP53* functional classifications, Bcl-xl, and cyclin D1, each as a single variable factoring outcome as well as part of a multivariable model for advanced larynx cancer outcome.

MATERIALS AND METHODS

Study Population

The UMCC9520 larynx-sparring cancer trial enrolled 97 patients with stage III and IV squamous cell carcinoma of the larynx. Adequate pretreatment biopsy tissue was available for DNA isolation from 58 of 97. A detailed description of the clinical trial was previously reported.[3]

Tissue Handling and DNA Extraction

Tumor tissue was obtained from formalin-fixed paraffin-embedded pretreatment biopsies. A head and neck pathologist marked representative areas of tumor. Two cores were taken from the marked areas using a 20-gauge needle. DNA was extracted using the QiaAmp DNA Mini Kit (Qiagen, Valencia, CA). DNA quantity and quality were corroborated with ultraviolet-visible spectrophotometer using a NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE).

TP53 *Sequencing*

TP53 mutations were assessed by polymerase chain reaction (PCR) amplification of exons 4 to 9 and subsequent DNA sequencing. Seven pairs of primers were used (Supp. Table SI) with AmpliTaq DNA Polymerase (Invitrogen, Life Technologies, Grand Island, NY), as indicated by the manufacturer. Amplification was performed at 95°C for 10 minutes, 36 cycles of 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 45 seconds, and a final extension at 72°C for 10 minutes. The amplicons were examined with electrophoresis in a 1.5% agarose gel, purified using the Wizard SV Gel (Promega Corporation, Madison, WI) and PCR Clean-Up System (Promega Corporation), and submitted to the University of Michigan DNA Sequencing Core for bidirectional sequencing. Mutations were confirmed with a second independent PCR reaction and sequencing.

TP53 *Mutant Classifications*

Three classifications designed to classify *TP53* single-base pair mutations (point mutations) were studied. The EAp53 classification that was designed and validated for head and neck cancers[14] scores *TP53* mutations as high-risk mutations (H) and low-risk mutations (L) obtained through a computational algorithm based on evolutionary sensitivity and amino acid conservation. Tumors without mutations were designated as wild type (W) *TP53*. Insertion/deletions were not scored.

The p53 transactivation activities (TAs) classification is based on reporter gene activity placed under the control of eight p53 response elements in yeast models.[15] Transactivation activities were measured as a percentage of W p53 TA and classified as *supertrans* (median TA above 140%), *functional* (median TA of 76%–140%), *partially functional* (median TA of 21%–75%), and *nonfunctional* (median TA below or equal 20%).[15,28]

The p53 mutant GOF classification is compared to mutants with no evidence of GOF and W *TP53*. The p53 GOF mutations have been defined as those with activities independent and unrelated to W protein.[15] These activities were demonstrated by RNA interference silencing or direct transfection of the mutant *TP53*.[29] To identify GOF mutants, a literature search of PubMed without language limitation was performed using the search terms "mutant p53" and "gain of function." Additionally, every missense mutation in this study was used as a search term to find reports.

HPV Testing

DNA samples from the pretreatment biopsies were analyzed for the presence of high-risk HPV using the multiplex HPV PCR-MassArray assay as described.[25]

Bcl-xl, Cyclin D1, and p16INK4a Immunohistochemistry

Staining intensity (scale: 1: none, 2: weak, 3: moderate, and 4: strong) and proportion (scale 1: < 5% staining, 2: 5%–20% staining, 3: 21%–50% staining, and 4: 51%–100% staining) were assessed by immunohistochemistry using a tissue microarray and scored by a head and neck pathologist (J.B.MCH.) blinded to patient outcome, as previously described. [4,25]

Statistical Analysis

Associations between *TP53* groups and clinical characteristics were assessed using χ^2 statistics. Correlations with induction chemotherapy response, overall survival (OS), disease-specific survival (DSS), and time to indication for surgery (TIS) were tested by log-rank tests and logistic and Cox proportional hazards regression models. Time-to-event outcomes (OS, DSS, TIS) were measured from date of diagnosis. For illustration in figures, Bcl-xl and Cyclin D1 intensity scores were grouped as no (1), low (2), and high (3 or 4). Multivariable analyses controlling for stage, smoking, and hrHPV were performed using logistic and Cox proportional hazards models. Variables tested included *TP53* mutational status and presence of hrHPV DNA, Bcl-Xl, and CyclinD1 expression.

RESULTS

Sample Characteristics

TP53 sequence and clinical data were obtained from a previously published study.[3] Basic clinical and tumor characteristics are shown in Table 1.

TP53 *Mutants*

Of the 97 eligible patients, *TP53* gene was sequenced in 58. Of these, 22 tumors contained *TP53* mutations. Five (8.6%) had deletions that were not classifiable. Fifty-three patients classified had either W *TP53* (36 of 53 [68%]) or single-base changes (17 of 53 [32%]) corresponding to missense in 13 of 53 (24.5%) or non-sense mutations in four (7.5%). No insertions were found. A *TP53* missense mutational spectrum is presented in Supporting Table SII. Bivariate associations were tested for each p53 classification with age, T stage, N stage, smoking, and HPV. The EAp53 classification[14] revealed a significant association of H mutations and node-positive tumors ($P = 0.03$) (Table 2). Seven (53.8%) of the 13 missense mutations were classified as GOF. No significant associations

TABLE 1.
Patient and Tumor Characteristics

Characteristic	N		No. of Patients	%[a]
Gender	58	Male	48	83%
		Female	10	17%
T stage	58	T2	5	9%
		T3	30	52%
		T4	23	40%
N stage	58	N0	29	50%
		N1	12	21%
		N2	15	26%
		N3	2	3%
AJCC Stage	58	Stage III	24	41%
		Stage IV	34	59%
Smoking Status	58	Never	2	4%
		Former	12	21%
		Current	42	75%
HPV	52[b]	Positive	20	39%
		Negative	32	62%
EAp53 Risk Classification	58	W TP53	36	62%
		Deletion	5	9%
		High (H)	10	17%
		Low (L)	7	12%
Yeast Assay Based Classification	58	Functional	38	66%
		Deletion	5	9%
		Partially functional	1	2%
		Nonfunctional	14	24%
Gain of Function (GOF) Classification	58	W TP53	37	64%
		Deletion	5	9%
		GOF Mutation	7	12%
		No GOF Mutation	9	16%
			Mean	**(std)**
Age (years)			57.2	(11.2)

a). % may not add to 100 due to rounding.
b). n=52 for HPV due to tissue availability.

with clinical parameters were found for GOF or transactivation classifications, L, and W.

HPV and p16INK4a

Tumor DNA from 52 patients was analyzed with the multiplex HPV PCR-MassArray assay.[25] High-risk HPV was identified in 20 of 52 (38.5 %) tumors; 19 of 20 had HPV16; and one had HPV 18. Of the HPV-positive tumors, 15 of 20 (75%) had W *TP53* (consistent with HPV-driven carcinogenesis); three of 20 (15%) had high-risk *TP53* mutations; and two of 20 (10%) had *TP53* deletion (questionable for HPV carcinogenesis). HPV PCR-MassArray–negative tumors presented a W *TP53* in only 53% (16 of 30) cases. Sixteen HPV PCR-MassArray–positive samples were assessed for p16INK4a status. Of those, 31% (5 of 16) were p16INK4a-positive. Whereas all HPV+/p16INK4a+ tumors (5 of 5) had a W *TP53*, only 64% (7 of 11) of the HPV+/p16INK4a- presented a W *TP53*.

A total of 46 tumors were stained for p16INK4a. Of those, 26% (12 of 46) were positive. From the 12 p16INK4a-positive samples, only 42% (5 of 12) were HPV PCR-MassArray–positive. The p16INK4a and HPV PCR-MassArray results were not concordant in larynx cancer (Pearson correlation coefficient Rho = 0.09).

TP53 *Mutants and Induction Chemotherapy*

Thirty-eight of the 58 patients (66%) responded to induction chemotherapy (tumor volume decrease > 50%). Multivariable logistic regression models with different models, including T stage and N stage separately, overall stage, HPV, and smoking, were run. No significant predictors of response to induction chemotherapy were found in any of the *TP53* classifications.

TABLE 2.
Nodal status, HPV and EAp53 Classification

Nodal Status	Deletion	H	L	W	Total
N0	3	2	6	18	29
N+	2	8	1	18	29
Total	5	10	7	36	58
HPV Status	Deletion	H	L	W	Total
HPV-	2	6	6	18	32
HPV+	2	3	0	15	20
Total	4	9	6	33	52[a]

a). n=52 for HPV due to tissue availability.
H=High risk mutation; L=Low risk mutation; W=Wild type TP53

TP53 *Mutants and Survival*

Of the 58 patients with a median follow-up of 95 months, the Kaplan-Meier estimates for overall survival at 1, 2, and 5 years were 90%, 83% and 67%, respectively. Disease-specific survival estimates were 91%, 86%, and 80%, respectively.

Using the EAp53 classification and univariate analysis (log-rank tests), we find a significant DSS difference between strata ($P = 0.026$) (Fig. 1). Pairwise, the difference between the H versus L groups was significant ($P = 0.027$). The differences between H versus W ($P = 0.188$) and L versus W ($P = 0.988$) groups were not statistically significant. Using a Cox model after controlling for T stage, N stage, and HPV status, the effect of p53 mutation was no longer significant ($P = 0.114$). No significant differences were observed for DSS using the yeast assay-based classification ($P = 0.603$, log-rank; $P = 0.887$ Cox) or GOF classification ($P = 0.096$ log-rank; $P = 0.130$ Cox). Interestingly there was a trend for the GOF mutations to do better.

TP53 *Mutants and Time to Surgery*

Time to indication for surgery (TIS) was evaluated for the *TP53* classifications. No significant differences in TIS were found between groups using the EAp53, yeast transcriptional assay-based, or GOF classifications ($P = 0.654$, $P = 0.394$, and $P = 0.835$, respectively). After controlling for stage, smoking, and HPV status, the differences were still not significant ($P = 0.667$, $P = 0.245$, $P = 0.874$, respectively).

HPV, p16INK4a, Bcl-xL, and Cyclin D1: Effects on the EAp53 Classification and Survival

The role of HPV, p16INK4a, Bcl-xL, and cyclin D1 in the EAp53 classification was assessed. As a single variable, the presence of HPV did not reach significance (log-rank $P = 0.38$) When separating the HPV-positive tumors by p16INK4a status, we found that the patients with HPV+/p16INK4a+ tumors tended to have a better DSS compared to the HPV+/p16INK4a- and HPV-negative. This difference did not reach statistical significance (log-rank $P = 0.4037$ and $P = 0.3506$, respectively) (Fig. 2A). No patients with HPV+/p16INK4a+ tumors die due to cancer. By including only the HPV-positive cases with W *TP53*, we observed that HPV-positive W *TP53* patients tended to have better DSS than the HPV-negative patients. This difference did not reach statistical significance ($P = 0.195$) (Fig. 2B).

We found no evidence of an association between Bcl-xL intensity in different *TP53* groups. In multivariable analysis, Bcl-xL expression displayed a trend for poorer DSS ($P = 0.075$) (Table 3). By plotting the DSS of the *TP53* groups and sorting out the W *TP53* based on their biomarker status, we found no significant difference across Bcl-xL staining intensities. However, the group with W *TP53* and no expression of Bcl-xL had no deaths due to cancer (Fig. 2C.).

Alone, cyclin D1 was associated with poor DSS (HR 2.4, $P = 0.010$ Cox). When sorting the W *TP53* by cyclin D1 staining intensity, we found that high-cyclin D1 staining was a predictor of poor survival compared with low- or no cyclin D1 staining ($P = .051$ and $P = 0.044$, respectively) (Fig. 2D.) Cyclin D1 showed a trend ($P = 0.096$) for higher intensity in the high-risk group of p53 mutations.

Multivariable Model for Prediction of Survival

A model with Bcl-xL, cyclin D1, EAp53 classification, and hrHPV status was highly significant as a prognostic model for DSS ($P = .002$, likelihood ratio test) (Table 3). A full model including EAp53 classification of *TP53*, Bcl-xL intensity, cyclin D1 intensity, stage, smoking status, and HPV status was also performed (Supp. Table SIII). This model was significant for modeling DSS ($P = 0.011$), although a backward selection algorithm suggested dropping stage and smoking for a better model. We found no significant models for induction chemotherapy response or to time to indication for surgery.

DISCUSSION

Three classifications for *TP53* mutations were analyzed. Only the EAp53 classification[14] based on mutant

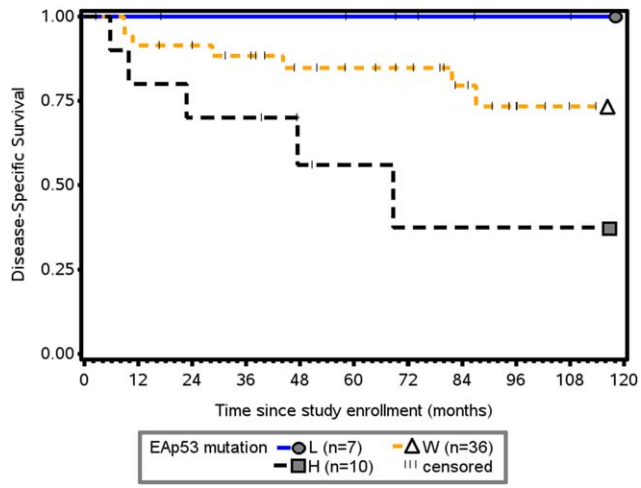

Fig. 1. Disease-specific survival for patients with advanced larynx cancer classified by *TP53* mutation type based on EAp53 as low-risk mutations, high-risk mutations, and wild-type mutations.
EAp53 = Evolutionary Action score of p53; H = high-risk mutations; L = low-risk mutations; *TP53* = tumor suppressor p53; W = wild-type mutations.

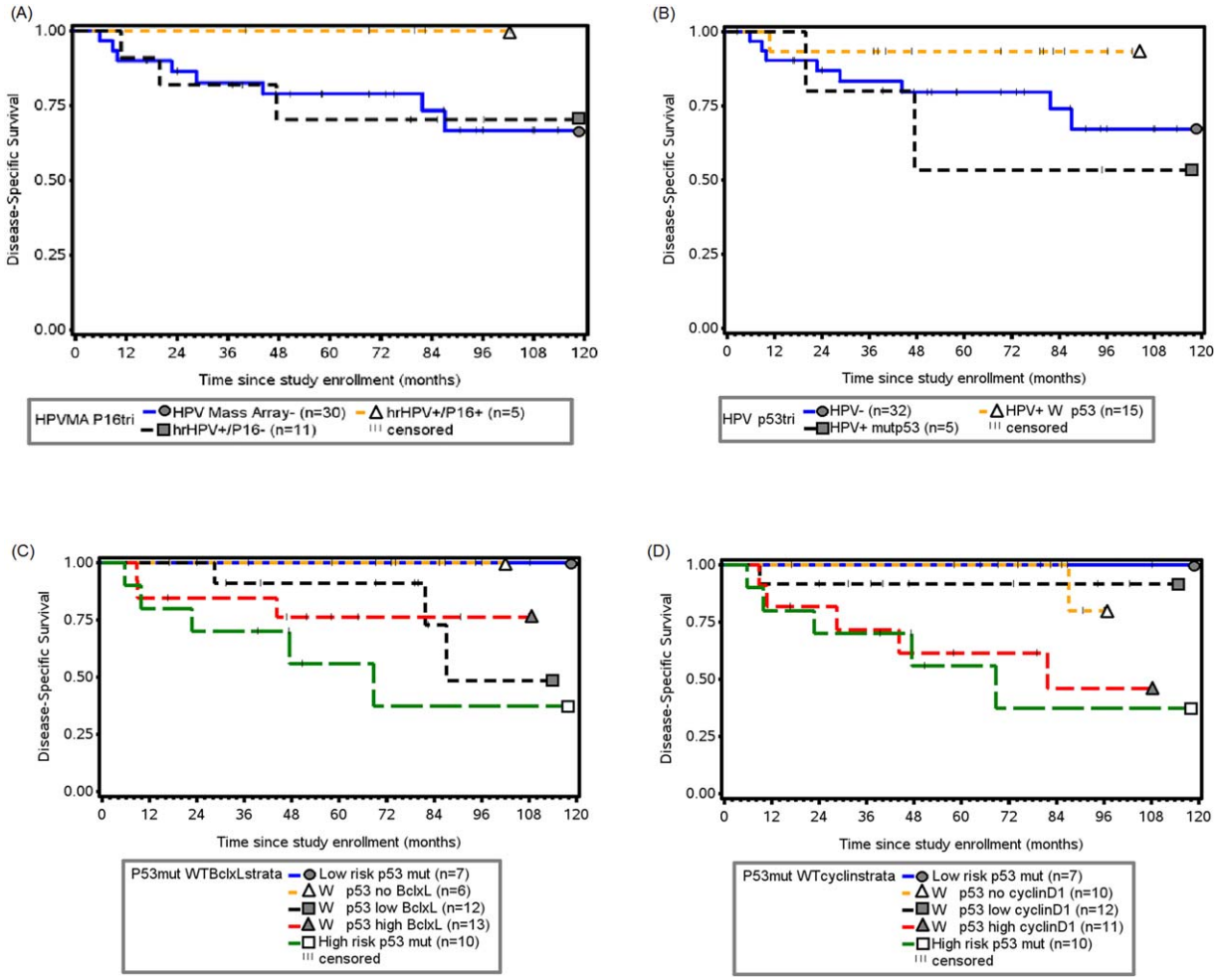

Fig. 2. Disease-specific survival by (A) HPV status with HPV-positives sorted by p16INK4a status; (B) EAp53 classification with *TP53* wild types sorted by HPV status; (C) EAp53 classification with *TP53* wild types sorted by Bcl-xL intensity staining; and (D) EAp53 classification with *TP53* wild types sorted by cyclin D1 intensity staining and W mut.
Intensity staining scores: 1 = no, 2 = low, 3 or 4 = high.
EAp53 = Evolutionary Action score of p53; H = high-risk mutations; HPV = human papillomavirus; L = low-risk mutations; mut = mutation; *TP53* = tumor suppressor p53; W = wild-type mutations Bcl-xl = B-cell lymphoma-extra large.

TP53 transcriptional activity showed clinical significance. This classification predicted DSS ($P = 0.026$). In this clinical trial, patient selection through induction chemotherapy resulted in larynx preservation in 70% of patients and DSS of 87%.[3] Although patient survival was improved, there was still a subset of patients with poor outcome. The EAp53 classification has potential to identify this subset of patients.

Bivariate associations were tested for EAp53 classification with age, T stage, N stage, smoking status, hrHPV, Bcl-xL, and cyclin D1 staining. Of interest, high-risk mutations found by EAp53 classification were associated

TABLE 3.
Cox Proportional Hazards Model Results from Selected Model for Prediction of Disease-Specific Survival

Parameter	Value	p-value	Hazard Ratio	95% Hazard Ratio Confidence Limits	
Cyclin D1		0.0325	3.782	1.117	12.803
Bcl-xL		0.0751	0.307	0.084	1.127
EAp53 Classification	H vs. W	0.0186	7.591	1.404	41.043
EAp53 Classification	L vs. W	ie.	ie	ie	ie
HPV	Neg vs. Pos	0.0262	58.123	1.619	2086.187

H=High risk mutation; L=Low risk mutation; W=Wild type TP53; Bcl-xl=B-cell lymphoma-extra large; ie=inestimable because zero events in the L group.

with lymphatic metastasis at presentation ($P = 0.03$) and a trend for high expression of cyclin D1 ($P = 0.096$). It is known that node-positive patients have poorer survival.[30] Therefore, EAp53 classification could potentially guide therapeutic approaches such as neck dissection, neck irradiation, or the addition of chemotherapy to radiation treatment. More aggressive treatment to the neck of patients with high-risk *TP53* mutations could improve patient outcome. We also found a trend for high-risk *TP53* mutants to have high expression of cyclin D1. Cyclin D1 was previously found to be associated with overall and disease-specific survival.[4] We propose a mechanism in which cyclin D1 overexpression is unchecked by defective p53. High-risk *TP53* mutations have a lower expression of BTG2 (B-cell translocation gene 2) protein compared to their W and low-risk counterparts.[14] BTG2 inhibits cyclin D1 transcription.[31,32] Thus, its low expression is a likely mechanism for cyclin D1 overexpression. Furthermore, cyclin D1 itself could be constitutively overexpressed by dysregulation in the β-catenin,[33,34] RAS/MAPK, or IP3K pathways.[35] The EAp53 classification and cyclin D1 have proven to be powerful predictors of survival in advanced larynx cancer patients, but further studies are needed to characterize the cellular mechanisms by which these markers interact and affect survival in this type of cancer.

Gain of function mutations affect important cellular activities such as proliferation, apoptosis, chemoresistance, metabolism, cell-to-cell interactions, or cytoskeleton conformation.[29] Only seven of 58 (12%) of the sequenced patients had a *TP53* GOF mutation. These showed no impact on response to induction chemotherapy, time to surgery, or survival. We grouped all GOF mutations together. More specific classification of GOF mutations based on the cellular functions altered could offer a better understanding of how these mutations affect patient outcome. Larger studies and a more comprehensive GOF classification are needed for assessment of their impact in larynx cancer.

The proportion of tumors with mutant *TP53* (22 of 58 [37.9%]), was lower than expected [36] *TP53* mutations are uncommon in HPV-positive tumors.[37] The low rate of *TP53* mutations in this study is consistent with the high proportion of hrHPV-positive larynx tumors, (20 of 52 [38.5%]), that we found. This is also consistent with previous reports of HPV in up to 25% of larynx carcinomas,[38,39] and with the increasing frequency of HPV-related head and neck cancers in general.[40,41] A recent report from the Tissue Cancer Genome Atlas (TCGA) head and neck cancer group included 72 larynx cancer cases. From these, 66 of 72 (91.6%) have mutated TP53, and only five of 72 (7%) are HPV PCR-MassArray–positive.[42] Patient selection could be causative of the discrepancy found between the studies. Although samples in the TCGA study were collected without regard to disease stage or patient functional status, in our study patients were part of a clinical trial and were selected with a good functional status and an advanced stage of disease (stage III and IV).[3,42] Differences in geographical distribution of the patients could be another factor affecting the HPV detection rates; geographic variability of HPV-related HNSCC have been reported.[43,44]

The biology of the HPV-positive tumors was assessed using p16INK4a immunohistochemistry. P16INK4a status did not correlate with HPV status (Rho = 0.09), showing a dichotomy of HPV-related larynx cancer. All HPV-positive tumors with p16INK4a-positive staining presented a W *TP53* and showed a better DSS with zero deaths caused by cancer. These HPV-related tumors show characteristics of the typical HPV E6- and E7-driven cancers. A different subset of HPV-positive tumors with p16INK4a-negative staining did not show a clear correlation with *TP53* mutational status, and patients presented a DSS similar to the HPV-negative patients (Fig. 2A). Further studies addressing other mechanisms of HPV carcinogenesis, such as viral DNA integration, methylation profiling, miRNA profiling, and characterization of other HPV-related cellular pathways, will determine the role of HPV in this subset of HPV-positive larynx tumors.

Kumar et al.,[16] in their analysis of the Department of Veterans Affairs (VA) Laryngeal Cancer Trial,[2] found that low Bcl-xL and high p53 staining were predictors of larynx preservation in patients treated with induction chemotherapy followed by definitive radiation. Here, we found no significant effect of Bcl-xL on clinical outcome, although in multivariable analysis a trend was noted for poorer disease-specific survival to be associated with Bcl-xL expression. The association with organ preservation was not explored in this analysis. It is interesting to note that among WTp53 patients with no Bcl-xL expression, no tumor failures were seen. We observed a similar trend to improved survival with Bcl-xL expression in the VA Study. Both studies gave induction chemotherapy (cisplatin 100 mg/m2 on day 1 and fluorouracil 1,000 mg/m2/day for 5 days). In the VA study, tumor response was assessed after two cycles of induction, and responders went on to an additional cycle of chemotherapy followed by radiotherapy alone.[2] In contrast, in UMCC9520 a single round of the same induction therapy was followed by response assessment, and either immediate surgery/radiotherapy (nonresponders) or radiotherapy with concurrent cisplatin (100 mg/m2) given on day 1, 22, 43.[3] The addition of concurrent cisplatin to radiation in UMCC9520 could change the association of Bcl-xL expression as a biomarker of outcome. Recent studies suggest that cisplatin can activate extrinsic apoptotic pathways or induce necrosis[45] or cell senescence.[46] In these scenarios, cell death is no longer completely dependent on the intrinsic mitochondrial apoptotic machinery,[47] which could explain the loss of power for Bcl-xL as a predictive biomarker. Nonetheless, the importance of a functional intrinsic apoptotic pathway in the response to chemoradiotherapy is evidenced by the fact that, in our study patients, whose tumor did not stain for the apoptotic inhibitor Bcl-xL and had W *TP53* did not die due to cancer (Fig. 2C).

We constructed a model for the prediction of disease-specific survival based on a combination of biomarkers and clinical characteristics. The model including classification of *TP53,* Bcl-xL intensity, cyclin D1 intensity, stage, smoking status, and HPV status was able to predict survival ($P = 0.011$). By including only variables with a strong predictive value (i.e., Bcl-xL, cyclin D1, p53 mutation classification, and HPV status), we obtained a selected model that

was highly significant for the prediction of disease-specific survival ($P = 0.0017$) (Likelihood Ratio Test Table III). In this model, we can distinguish two types of patients: 1) high-risk *TP53* mutation was predictive of poor DSS; and 2) low-risk *TP53* mutation was predictive of good DSS ($P = 0.026$) (Fig. 1.). In patients with W *TP53*, high staining of cyclin D1 was predictive of poor DSS, whereas no/low staining for cyclin D1, no staining for Bcl-xL, and HPV positivity were predictors of good DSS (Fig. 2) (Supp. Table SIV). Thus, treatment modalities targeting p53 and cyclin D1 could increase survival in advanced larynx cancer patients that fail to respond to current treatment. Novel targeted therapies such as mutant p53 reactivating small molecules; mdm2 inhibitors; cyclin-dependent kinase inhibitors; or inhibitors of β-catenin, RAS/MAPK, or IP3K pathways could potentially counteract these markers and offer better outcome. The model should be tested in larger cohorts.

CONCLUSION

A functional classification of *TP53* mutants based on EAp53 predicts DSS in advanced larynx cancer patients with mutant *TP53*. A high proportion of hrHPV-related larynx cancers in this cohort reflects the epidemic of HPV-related head and neck cancers. A dichotomy in the biology of hrHPV is observed in larynx cancer. A model including Bcl-xL staining, cyclin D1 staining, p53 mutation classification, and HPV status was highly significant for predicting disease-specific survival. High-cyclin D1 expression and high-risk *TP53* mutations were associated with poor DSS. New therapies targeting these pathways could benefit patients that fail to respond to current treatment modalities.

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