Received: 20 June 2021 | Revised: 27 September 2021 | Accepted: 30 September 2021

DOI: 10.1111/tan.14441

ORIGINAL ARTICLE

HLA Immune Response Genetics WILEY

Immunohistochemical detection of "ex novo" HLA-DR in tumor cells determines clinical outcome in laryngeal cancer patients

Chiara Prampolini[1] | Giovanni Almadori[1,2] | Davide Bonvissuto[3] | Marta Barba[4,5] | Luca Giraldi[6] | Stefania Boccia[6,7] | Gaetano Paludetti[1,2] | Jacopo Galli[1,2] | Ornella Parolini[4,5] | Stefano Settimi[2] | Gabriella Cadoni[1,2]

[1]Department of Head-Neck and Sensory Organs, Università Cattolica del Sacro Cuore, Rome, Italy

[2]Department of Aging, Neurologic, Orthopedic and Head-Neck Sciences, Otorhinolaryngology Unit, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy

[3]Department of Neurosciences, Università Cattolica del Sacro Cuore, Rome, Italy

[4]Department of Life Science and Public Health, Università Cattolica del Sacro Cuore, Rome, Italy

[5]Biobank for Personalized Medicine, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy

[6]Department of Life Science and Public Health, Section of Hygiene and Public Health, Università Cattolica del Sacro Cuore, Rome, Italy

[7]Department of Woman and Child Health and Public Health, Public Health Area, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy

Correspondence
Stefano Settimi, Department of Aging, Neurologic, Orthopedic and Head-Neck Sciences, Otorhinolaryngology Unit, Fondazione Policlinico Universitario "A. Gemelli" – IRCCS, Largo A. Gemelli, 8 00168 Rome, Italy.
Email: settimi.stefano90@gmail.com

There are controversial results about the role of "ex novo" HLA-DR expression by tumor cells and its correlation with the oncological outcomes. Unfortunately, little is known about HLA-DR expression in laryngeal cancer tumor cells. The main purpose of this retrospective study is to strengthen the usefulness of studying "ex novo" HLA-DR expression on tumor cells from primary laryngeal squamous cell carcinoma (LSCC) patients and investigate its correlation with clinical outcome. We analyzed HLA-DR expression by immunohistochemical analysis in 56 patients with LSCC. The "ex novo" HLA-DR expression on laryngeal cancer tumor cells, assessing non-neoplastic LSCC – adjacent tissue, and the association of HLA-DR expression (HLA-DR+) with clinical outcomes were investigated. HLA-DR+ tumor cells were detected in 18/56 LSCC patients (32.1%). All specimens of non-neoplastic laryngeal carcinoma-adjacent tissue resulted HLA-DR negative (HLA-DR-). A statistically significant association was observed between HLA-DR + and well differentiated tumors (G1) ($p<0.001$). The Kaplan-Meier method showed how HLA-DR+ is significantly associated with both a better disease specific survival (HLA-DR+=100% vs. HLA-DR-=77.4%; $p=0.047$) and a better relapse free survival (HLA-DR+=100% vs. HLA-DR-=72.3%; $p=0.021$). Cox regression univariate analysis for death of disease confirmed a higher HR for HLA-DR absence on the surface of epithelial tumor cell [HR:37.489; 95% CI:0.750-18730.776; $p=0.253$] and for high-grade (G3) tumors [HR:18.601; 95% CI:3.613-95.764; $p<0.0001$]. Our results confirm that MHC class II HLA-DR expression is activated in a sub-set of LSCC patients. Evaluation of HLA-DR expression in LSCC could be useful for prognosis and future approaches towards personalized therapy.

KEYWORDS

HLA-DR, immunohistochemistry, laryngeal squamous cell carcinoma, MHC II, personalized medicine, tumor immunology

1 | INTRODUCTION

Laryngeal squamous cell carcinoma (LSCC) is a common head and neck cancer (HNC), accounting for about 5.7% to 7.6% of all HNC.[1] Despite recent therapeutic advancements, the survival of LSCC patients remains poor.[2] Various factors predict the outcome of laryngeal cancer. These can be grouped into host, tumor, and treatment. The development and application of molecular biology tools to analyze biopsy material may be predictive for the biological behavior of LSCC. Significant progress is being made and biomarkers may inform both prognosis and optimum treatment in the future.[3]

The host-tumor immune response has been explored as prognostic indicator for LSCC.[4] Nowadays immunological markers have laid the foundations for promising new immunomodulatory treatments to head and neck cancers. Recently, programmed death-1 immune-checkpoint inhibitors were approved as a treatment for platinum chemotherapy-treated patients with recurrent or metastatic HNC and have shown durable responses and survival improvements, albeit in a small number of patients.[5] Thus, there is a medical need to improve immunotherapies for HNC.

HLA, also known as human major histocompatibility complex (MHC), is encoded by the HLA gene complex. The HLA gene is located on the short arm of chromosome 6 (6p21.31), covering an area of 7.6 Mb and containing more than 250 genes with different functions, and is currently known to have the highest gene density and the most polymorphisms among human chromosomal areas. HLA is divided into class I antigens, class II antigens, and class III antigens according to distribution and function. Classical HLA class I antigens include HLA-A, HLA-B, and HLA-C; HLA class II antigens include HLA-DP, HLA-DQ, and HLA-DR; nonclassical HLA class I and II molecules include HLA-F, E, H, X, DN, DO, and DM; and others, such as complement, are class III antigens.[6] Abnormalities in MHC participate in the host-tumor immune response of tumor cells, a mechanism that has been observed in various tumors. Researchers have detected the expression of MHC class I and class II proteins on the surface of tumor cells in 181 untreated melanoma patients, analyzed their transcription levels and genomes, and investigated the relationship between MHC and the clinical response to anti-CTLA-4 therapy, anti-PD-1 therapy or combined treatment. The results showed that the response to anti-CTLA-4 treatment requires melanoma cells to strongly express MHC class I molecules, while the response to anti-PD-1 treatment is related to IFN-gamma mediated immune activation, including the expression of HLA-DR.[7,8]

MHC class II HLA-DR antigen expression is typical of antigen-presenting cells (APC), such as dendritic cells, monocytes, macrophages, B cells and activated T cells, but its expression can be induced in cancer cells.[9,10] This mechanism represents one of the most effective evolutionary conquests by cancer cells. In fact, they are able to express the HLA-DR antigens "ex novo", after stimulation by IFN-gamma, which induces the activation of mediators including JAK/STAT and trans-activators such as CIITA, RFX5, RFXAP, and NF-Y16,19; in particular, the CIITA-PIV promoter isoform was involved in the inducible gene expression by IFN-gamma.[11–13]

There are controversial results about the role of "ex novo" HLA-DR expression by tumor cells and its correlation with the oncological outcomes.[13–20] Unfortunately, little is known about HLA-DR expression in laryngeal cancer tumor cells. The main purpose of this retrospective study is to strengthen the usefulness of studying "ex novo" HLA-DR expression on tumor cells from primary LSCC patients and investigate its correlation with clinical outcome.

2 | MATERIALS AND METHODS

2.1 | Design of the study

This study is a no-profit retrospective observational study, based on histological and immunohistochemical analysis of fragments of tumor tissue from patients with squamous cell carcinoma of the larynx, who underwent partial or total laryngectomy and who agreed to participate, by signing the informed consent; for those who were dead at the time of the study, approval was given to the treatment of biological samples by the Ethics Committee of the Fondazione Policlinico Universitario "A.Gemelli" - IRCSS of Rome, Italy. The research protocol was designed and implemented according to the European guidelines of good clinical practice (GCP) and was conducted in accordance with the ethical principles set out in the Helsinki Declaration for medical research involving human subjects. The Ethics Committee has granted full approval (protocols number 34474/18; ID 2222).

2.2 | Population and sample collection

The present study included primary laryngeal squamous cell carcinoma (LSCC) patients undergoing surgery in a period between 2007 and 2014, at the Otorhinolaryngology Institute of the Fondazione Policlinico Universitario "A. Gemelli", IRCSS of Rome, Italy. Hematoxylin & eosin-stained full slides were examined to reevaluate the

histopathologic diagnosis and grade. One hundred-ten patients were initially included in our cohort. Selection was based on the possibility to obtain both neoplastic and non-neoplastic laryngeal carcinoma-adjacent tissue specimens for the same patient. After surgery, the surgical tissue specimens were excised aseptically immediately after operation from the tumor center and normal non-cancerous laryngeal epithelium as far as possible away from the tumor of the same tumor patients. Out of 110 patients, 54 could not be included in the present study because of the limitation of the material. The tumor staging and grading was determined according to the UICC classification system (2016).[21] Clinical data were collected retrospectively by reviewing patients' medical files. Relapse-free survival (RFS) was calculated as time from date of diagnosis and time to local or regional recurrence, distant metastasis. Disease specific survival (DSS) was calculated as time from date of diagnosis and time of death for laryngeal cancer. Overall survival (OS) was calculated as time from date of diagnosis and time of death. The median follow-up time was 69 months (range 12-144 months). Patients clinicopathological features were showed in Table 1.

TABLE 1 Demographic and clinical characteristics of the 56 LSCC patients

	No. patients (%)
Sex	
Males	47 (83.9 %)
Females	9 (16.1 %)
Age at diagnosis	
Mean ± SD	63 ± 2.3
>50	56 (100%)
<50	0 (0%)
cT status	
T1-T2	22 (39.3%)
T3-T4	34 (60.7%)
cN status	
N0	42 (75%)
N+	14 (25%)
Stage	
I-II	21 (37.5 %)
III-IV	35 (62.5 %)
Grading	
Well differentiated (G1)	18 (32.1%)
Moderately differentiated (G2)	27 (48.2%)
Poorly differentiated (G3)	11 (19.7%)

2.3 | Histological analysis

Pathological samples by every patient were fixed in formalin 4% and included and conserved in paraffin. 3-4 μm-thick sections were cut on a microtome (Leica SM2000) and directly mounted on slides. The slides were used both for hematoxylin and eosin staining to perform morphological analysis and for immunohistochemistry analysis. In particular, the sections used for histological analysis were firstly deparaffinated in xylene (two passages of 5 min each one), dehydrated with alcohol passages (100%-95%-70%) and finally, after a washing in spring water, incubated with Mayer's Hematoxylin for 5 min. After another washing in spring water, slides were stained with eosin for 1 min. Finally, the sections were rehydrated through a series of graded alcohols (70%-95%-100%), clarified in xylene again, and cover-slipped with Eukitt mounting medium (03989 Fluka). The slides were then examined using a Zeiss Axiophot optic microscope by our pathologists.

2.4 | Immunohistochemistry

After deparaffinization and passage in decreasing alcohol (100%-95%-70%), antigen retrieval was carried out using citrate buffer Ph 6 in the microwave at high-temperature (750w) for two 5-min cycles. "Mouse and Rabbit Specific HRP/DAB IHC Detection Kit" (ab236466, Abcam) was used for all immunohistochemical staining. Sections were stained using a 1:10000 dilution of HLA-DR primary antibody (clone TAL 1B5, Abcam, Cambridge, UK), and a Goat anti-rabbit HRP Conjugate secondary antibody-DAB system. Sections were rinsed and counterstained in Mayer's hematoxylin. Finally, the sections were dehydrated through a series of graded alcohols (70%-95%-100%), clarified in xylene, and cover-slipped with Eukitt mounting medium (03989 Fluka). The slides were then examined using a Zeiss Axiophot optic microscope: immunohistochemical staining was assessed at different magnifications (10×, 20×, 40×, 63×). Negative controls were performed by omitting the primary antibody, while positive controls were performed on formalin-fixed, paraffin-embedded biopsy sections of human skin melanoma (Figure 1)

2.5 | HLA-DR positivity system

HLA-DR expression was evaluated using immunostaining and a positive reaction will be indicated as a brown color at the antigen site. The slides were examined by two independent pathologists (Institute of Pathological Anatomy), who had no prior knowledge of the

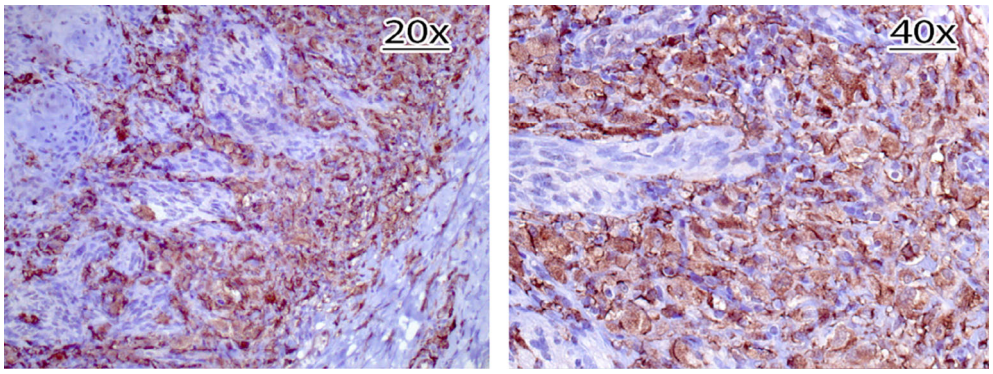

FIGURE 1 Photos at 10× and 20× magnification obtained at the optical microscope of IHC reaction with Abcam Ab HLA-DR [TAL B5] on melanoma sections of human skin from formalin-fixed, paraffin-embedded biopsy

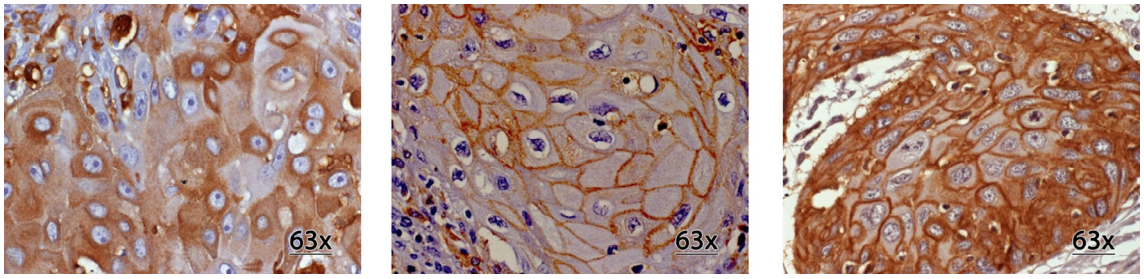

FIGURE 2 Photos at 63× magnification obtained at the optical microscope of laryngeal squamous carcinoma tissue samples analyzed for HLA-DR antigen expression by immunohistochemistry. The positive reaction is indicated as a brown color at the antigen site

clinical and pathological parameters. Epithelial tissue compartments were assessed for HLA-DR staining. The intensity of staining was classified into two grades: positive (HLA-DR positive cells ≥10%) and negative (<10% of positive cells) as previously reported in literature.[22]

2.6 | Statistical analyses

Statistical analyses were performed using SPSS (IBM SPSS Statistics for Macintosh, Version 26.0. Armonk, NY: IBM Corp). The Chi-square test was used to analyze the association between HLA-DR expression and clinicopathological characteristics and p-value were corrected with Bonferroni method. $p<0.05$ was considered to indicate a statistically significant difference. Analysis was conducted for the overall survival (OS), disease specific survival (DSS), and relapse-free survival (RFS). Survival analyses were performed by the Kaplan Meier method, and the log-rank test was used to compare survival curves. Cox proportional hazards regression model was conducted to calculate the hazard ratio (HR) and 95% confidence interval (CI) of possible predictors of death. Univariate Cox proportional hazards models were performed to assess each parameter's power in predicting the death of disease. Then factors with $p < 0.05$ in univariate analyses were further assessed in multivariate Cox proportional hazards models to determine significant prognostic factors.

3 | RESULTS

3.1 | Immunohistochemical analysis

HLA-DR+ tumor cells were detected in eighteen out of 56 LSCC patients (32.1%). From the observation under the optic microscope, it was possible to see HLA-DR + both at the cytoplasmic level and at the surface membrane (Figure 2). All non-neoplastic laryngeal carcinoma-adjacent tissue samples resulted HLA-DR - on epithelial cells. Neoplastic laryngeal tissue samples positive and negative for HLA-DR expression on their epithelial cells, together with negative controls, are shown in Figure 3. The difference of antigen expression between an HLA-DR + tumor tissue and normal tissue, from the same patient, is shown in Figure 4.

3.2 | HLA-DR and clinicopathological features

The association between HLA-DR + and the clinicopathological characteristics are showed in Table 2. A statistically significant association was observed between

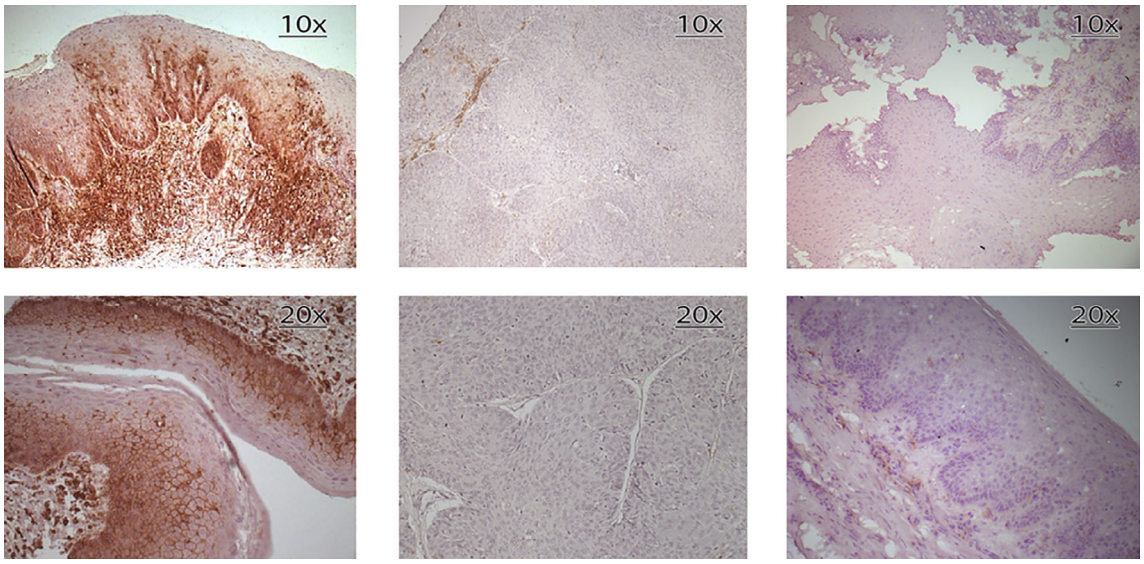

FIGURE 3 Photos at 10× and 20× magnification obtained at the optical microscope of HLA-DR + tumor tissue samples (left column), HLA-DR – tumor tissue samples (middle column), and negative controls (right column) analyzed for HLA-DR antigen expression by immunohistochemistry. The positive reaction is indicated as a brown color at the antigen site

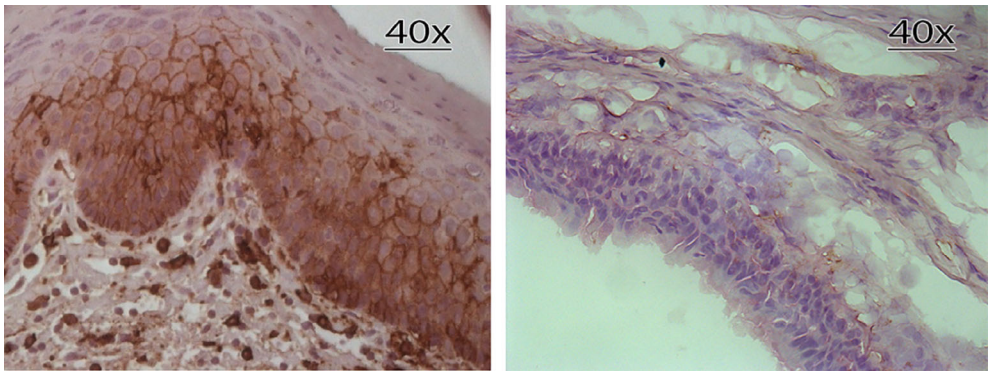

FIGURE 4 Photos at 40× magnification obtained at the optical microscope of HLA-DR + laryngeal squamous carcinoma tissue samples (left) and normal tissue sampled at least 3 cm distant from tumor (right), from the same patient, analyzed for HLA-DR antigen expression by immunohistochemistry. The positive reaction is indicated as a brown color at the antigen site

TABLE 2 Clinical characteristics of the 56 LSCC patients according to HLA-DR expression on tumor cells

	HLA-DR +	HLA-DR –	TOT.	*p*
cT1-T2	7 (31.8%)	15 (68.2%)	22	0.967
cT3-T4	11 (32.4%)	23 (67.6%)	34	
cN0	13 (30.9%)	29 (69.1%)	42	0.751
cN+	5 (35.7%)	9 (64.3%)	14	
Stage I-II	7 (33.3%)	14 (66.7%)	21	0.883
Stage III-IV	11 (31.4%)	24 (68.6%)	35	
Well differentiated (G1)	15 (83.3%)	3 (16.7%)	18	<0.01[a]
Moderately differentiated (G2)	2 (7.4%)	25 (92.6%)	27	
Poorly differentiated (G3)	1 (9%)	10 (91%)	11	

[a]The statistic used was Chi-square test with Bonferroni test correction. G1 versus G2-G3.

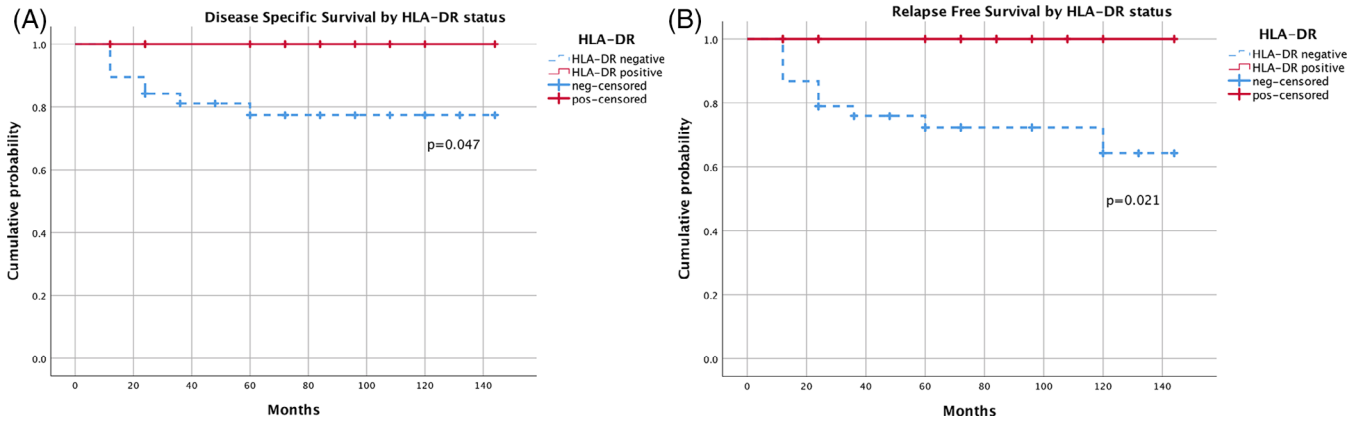

FIGURE 5 Kaplan-Meier curves of the disease specific survival (A) and relapse free survival (RFS) (B) based on HLA-DR expression

TABLE 3 Hazard ratios from the Cox univariate regression analysis for death of disease

	HR	CI (95%)		*p*
Sex (M)	1.543	0.189	12.588	0.685
Age (1-y increase)	0.974	0.896	1.059	0.542
T stage				
cT1-T2	1.00			
cT3-T4	2.080	0.418	10.347	0.371
N stage				
cN0	1.00			
cN+	1.823	0.435	7.640	0.411
HLA-DR expression				
HLA-DR +	1.00			
HLA-DR −	37.489	0.750	18730.776	0.253
Grading				
G1-G2	1.00			
G3	18.601	3.613	95.764	**<0.0001**

Abbreviations: CI, confidence interval; HR, hazard ratio.
Bold values denote statistical significance at the $p < 0.05$ level.

HLA-DR + and well differentiated tumors (G1): 15/18 (83.3%) well differentiated LSCCs resulted HLA-DR+, in comparison with 2/27 (7.4%) and 1/11 (9%) of moderately (G2) and poorly differentiated (G3) carcinomas, respectively ($p<0.001$).

3.3 | Survival analyses

The Kaplan-Meier curves (Figure 5) show that patients affected by squamous cell carcinoma expressing HLA-DR on epithelial cancer cells, compared with those not expressing HLA-DR, have a higher disease specific survival (DSS=100% vs. 77.4%) and relapse free survival (RFS=100% vs. 72.3%) at 5-years follow-up; this difference was significative at log-rank test ($p=0.047$ for DSS and $p=0.021$ for RFS). A better overall survival (OS) at 5-years follow-up (OS= 83.3% vs. 58.5%) was also observed but did not result statistically significative at log-rank test ($p=0.505$). Cox regression univariate analysis for death of disease (Table 3) confirmed a higher HR for HLA-DR absence on the surface of epithelial tumor cell [HR:37.489; 95% CI:0.750-18730.776; $p=0.253$], for poorly differentiated (G3) tumors [HR:18.601; 95% CI:3.613-95.764; $p<0.0001$], for tumor staged as T≥3 [HR:2.080; 95% CI:0.418-10.347; $p=0.371$], for a clinic involvement of lymph nodes at the diagnosis [HR:1.823; 95% CI:0.435-7.640; $p=0.411$] and for male sex [HR: 1.543; 95% CI: 0.189-12.588; $p=0.685$]. Therefore, considering the presence of only one significative variable, multivariate Cox proportional hazard model was not carried out.

4 | DISCUSSION

In the literature, we found few publications concerning the analysis of immunohistochemical "ex novo" HLA-DR expression in laryngeal cancer tumor cells. Firstly, Esteban et al.[14] with the monoclonal antibody GRB1 against DR antigen and then Sikorska et al.[23] using monoclonal antibody CR3/43 showed immunohistochemical HLA-DR + tumor cells in eight out of 69 and in 20 out of 68 LSCC patients, respectively. In 2007, Gomatos et al.[4] detected immunohistochemical HLA-DR+ in eighteen out of 37 LSCC patients using the monoclonal antibody MO746. Similarly, we studied a homogenous group of head and neck cancer patients with laryngeal squamous cell carcinoma and observed immunohistochemical HLA-DR+ tumor cells in 18 out of 56 LSCC, using the monoclonal antibody TAL 1B5. It should be emphasized that HLA-DR

expression on tumor cells has been investigated mostly in heterogeneous groups of cancers of the head and neck region, which are characterized by a different biology.[17,19,23–25] This does not allow direct comparisons of results obtained in patients with laryngeal cancer.

The role of HLA-DR expression in laryngeal squamous cell carcinoma cells is not fully known. Our results confirm that immunohistochemical HLA-DR+ tumor cells could identify a sub-set of LSCC patients with an "immune signature". It is well known that HLA-DR is expressed constitutively on stromal APCs such as macrophages, dendritic cells and activated B and T cells, being part of the molecular mechanism of extracellular antigen presentation to CD4+ T lymphocytes, which are activated to generate an immune response against it. On the other hand, the induction of HLA-DR expression on tumor cells is a not clarified phenomenon, at yet. Whether HLA-DR expression on tumor cells results in effective APC is unclear, but studies have shown that HLA-DR + epithelial cells are capable of antigen presentation and cross-presentation of tumor-associated antigens by HLA-DR has been hypothesized. HLA class II expression on epithelial cells has been shown to enhance tumor-specific immunity by bypassing the classical antigen-presenting cell-mediated pathway.[26–28] So, the effective antigen presentation by HLA-DR + tumor cells could represent an important immune key-step in the cancer-immune control of carcinoma progression and be considered an important and currently underutilized immunotherapeutic target.

In our study, non-neoplastic laryngeal carcinoma-adjacent epithelial cells resulted HLA-DR-, according to Esteban results.[14] These findings suggest a minor role of HLA-DR in initial stages of laryngeal carcinogenesis. In the investigated LSCC cases, we noted a tendency towards HLA-DR expression in well differentiated tumors that appears to be gradually lost with the lack of cell differentiation, indicating a possible interplay of HLA-DR expression with the differentiation of tumor cells in LSCC. Given that in other malignant neoplasms, such as colorectal carcinomas[17] the expression of this molecule is not related to the histological grade of the tumor, further investigation is needed to elucidate the mechanisms that regulate HLA-DR expression regarding cell differentiation in laryngeal cancer.

The clinical significance of HLA-DR expression in malignant tumors remains unclear. In the present study HLA-DR + tumor cells are significantly associated with both better disease specific survival and better relapse free survival. The link between HLA-DR expression in LSCC and improved specific survival and relapse free survival could reflect effective immunosurveillance and anti-carcinoma immune involvement, that is, activation of a robust adaptive T cell response. Since HLA-DR mediated antigen presentation is critical for priming CD4+T cell effector functions, HLA-DR can also be considered to be an integral, upstream part of this anti-carcinoma, Th1 axis, suggesting that future studies may benefit from inclusion of HLA-DR into prognostic scoring panels.

Up to now, few adoptive immunotherapies using MHC class II-restricted helper T lymphocytes (HTL) have been tested clinically but have clearly shown long-term tumor regression. Interestingly, recent studies in melanoma suggested that high expression of HLA-DR in tumor cells is associated with improved response rates and clinical benefit of PD-1/PD-L1 targeted therapy.[19] Quite recently, Hayashi et al.[29] hypothesized the use of a therapeutic vaccine to effectively stimulate antigen-specific HTLs against a restricted sub-set of laryngeal HLA-DR+ cancers.

The limitations of the present study include a small sample size. It appears clear the importance to further extensive studies involving large population with laryngeal cancer to verify the opportunity to add HLA-DR detection in LSCC samples to better select sensible immunotherapy patients. Further research is warranted to elucidate the functional aspects of ex novo HLA-DR expression on laryngeal cancer cells. In conclusion, we confirm that MHC class II HLA-DR expression is activated in a sub-set of LSCC patients. Such knowledge may ultimately help guide immuno-therapy in laryngeal cancer.

CONFLICT OF INTEREST

The authors declare that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

AUTHOR CONTRIBUTION

All authors substantially contributed to conception and design of the study, acquisition of the data, or analysis and interpretation of the data; drafted the article or revised it for important intellectual content; gave final approval of the version to be submitted; agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

DATA AVAILABILITY STATEMENT

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

ORCID

Stefano Settimi https://orcid.org/0000-0003-0104-1501

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68:394-424.
2. Steuer CE, El-Deiry M, Parks JR, Higgins KA, Saba NF. An update on larynx cancer. *Ca Cancer J Clin*. 2017;67:32-50.
3. Bradford CR, Ferlito A, Devaney KO, Mäkitie AA, Rinaldo A. Prognostic factors in laryngeal squamous cell carcinoma. *Laryngoscope Investig Otolaryngol*. 2020;5:74-81.
4. Gomatos IP, Georgiou A, Giotakis J, et al. The role of host immune response and apoptosis in patients with laryngeal squamous cell carcinoma. *ORL*. 2007;69:159-166.
5. Szturz P, Vermorken JB. Immunotherapy in head and neck cancer: aiming at EXTREME precision. *BMC Med*. 2017;15:110.
6. El-Awar N, Jucaud V, Nguyen A. HLA Epitopes: the targets of monoclonal and alloantibodies defined. *J Immunol Res*. 2017; 2017:3406230.
7. Liu B, Shao Y, Fu R. Current research status of HLA in immune-related diseases. *Immun Inflamm Dis*. 2021;9:340-350.
8. Najjar YG, McCurry D, Lin H, et al. Neoadjuvant pembrolizumab and high dose interferon Alfa-2b in resectable regionally advanced melanoma. *Clin Cancer Res*. 2021;27(15):4195-4204. doi:10.1158/1078-0432
9. Cabrera T, Ruiz-Cabello F, Garrido F. Biological implications of HLA-DR expression in tumours. *Scand J Immunol*. 1995;41: 398-406.
10. Mulder DJ, Pooni A, Mak N, Hurlbut DJ, Basta S, Justinich CJ. Antigen presentation and MHC class II expression by human esophageal epithelial cells. *Am J Pathol*. 2011;178:744-753.
11. Satoh A, Toyota M, Ikeda H, et al. Epigenetic inactivation of class II transactivator (CIITA) is associated with the absence of interferon-gamma-induced HLA-DR expression in colorectal and gastric cancer cells. *Oncogene*. 2004;23:8876-8886.
12. Ting JP-Y, Trowsdale J. Genetic control of MHC class II expression. *Cell*. 2002;109:S21-S33.
13. Muhlethaler-Mottet A, Otten L, Steimle V, Mach B. Expression of MHC Class II molecules in different cellular and functional compartments is controlled by differential usage of multiple promoters of the transactivator CIITA. *EMBO J*. 1997;16:2851-2860.
14. Esteban F, Ruiz-Cabello F, Concha A, Pérez-Ayala M, Sánchez-Rozas JA, Garrido F. HLA-DR expression is associated with excellent prognosis in squamous cell carcinoma of the larynx. *Clin Exp Metastasis*. 1990;8:319-328.
15. Ma X, Hattori T, Kushima R, Terata N, Kodama M. Expression of HLA-class II antigen in gastric carcinomas. Its relationship to histopathological grade, lymphocyte infiltration and five-year survival rate. *Acta Oncol*. 1994;33:187-190.
16. Matsushita K, Takenouchi T, Shimada H, et al. Strong HLA-DR antigen expression on cancer cells relates to better prognosis of colorectal cancer patients: Possible involvement of c-myc suppression by interferon-gamma in situ. *Cancer Sci*. 2006;97: 57-63.
17. Sconocchia G, Eppenberger-Castori S, Zlobec I, et al. HLA Class II antigen expression in colorectal carcinoma tumors as a favorable prognostic marker. *Neoplasia*. 2014;16:31-42.
18. Diao J, Xia T, Zhao H, Liu J, Li B, Zhang Z. Overexpression of HLA-DR is associated with prognosis of glioma patients. *Int J Clin Exp Pathol*. 2015;8:5485-5490.
19. Johnson DB, Estrada MV, Salgado R, et al. Melanoma-specific MHC-II expression represents a tumour-autonomous phenotype and predicts response to anti-PD-1/PD-L1 therapy. *Nat Commun*. 2016;7:10582.
20. Dunne MR, Michielsen AJ, O'Sullivan KE, et al. HLA-DR expression in tumor epithelium is an independent prognostic indicator in esophageal adenocarcinoma patients. *Cancer Immunol Immunother*. 2017;66:841.
21. James DB, Mary KG, Christian W. *TNM Classification of Malignant Tumours*. 8th ed. Wiley-Blackwell; 2016.
22. Sikorska B, Danilewicz M, Wagrowska-Danilewicz M. HLA-DR expression is a significant prognostic factor in laryngeal cancer. A morphometric study. *APMIS*. 1999;107:383-388.
23. Kambayashi T, Laufer TM. Atypical MHC class II-expressing antigen-presenting cells: can anything replace a dendritic cell? *Nat Rev Immunol*. 2014;14:719-730.
24. Rangel LB, Agarwal R, Sherman-Baust CA, et al. Anomalous expression of the HLA-DR alpha and beta chains in ovarian and other cancers. *Cancer Biol Ther*. 2004;3:1021-1027.
25. Samuels S, Spaans VM, Osse M, et al. Human leukocyte antigen-DR expression is significantly related to an increased disease-free and disease-specific survival in patients with cervical adenocarcinoma. *Int J Gynecol Cancer*. 2016;26:1503-1509.
26. Armstrong TD, Clements VK, Martin BK, Ting JP, Ostrand-Rosenberg S. Major histocompatibility complex class II-transfected tumor cells present endogenous antigen and are potent inducers of tumor-specific immunity. *Proc Natl Acad Sci USA*. 1997;94: 6886-6891.
27. Armstrong TD, Clements VK, Ostrand-Rosenberg S. MHC class II-transfected tumor cells directly present antigen to tumor-specific CD4+ T lymphocytes. *J Immunol*. 1998;160: 661-666.
28. Axelrod ML, Cook RS, Johnson DB, Balko JM. Biological consequences of MHC-II expression by tumor cells in Cancer. *Clin Cancer Res*. 2019;25:2392-2402.
29. Hayashi R, Nagato T, Kumai T, et al. Expression of placenta-speci c 1 and its potential for eliciting anti-tumor helper T-cell responses in head and neck squamous cell carcinoma. *Oncoimmunology*. 2020;10:1856545.

How to cite this article: Prampolini C, Almadori G, Bonvissuto D, et al. Immunohistochemical detection of "ex novo" HLA-DR in tumor cells determines clinical outcome in laryngeal cancer patients. *HLA*. 2021; 1-8. doi:10.1111/tan.14441