Human papillomavirus infection and lung adenocarcinoma: special benefit is observed in patients treated with immune checkpoint inhibitors

Background: Human papilloma virus (HPV) has been associated with the development and modulation of response in a series of neoplasms. In the case of lung adenocarcinoma, its role in etiology and pathogenesis is still controversial. Considering that this infection brings foreign epitopes, it could be of prognostic significance in patients with lung adenocarcinoma treated with immunotherapy.

Methods: In a retrospective cohort study we evaluated the presence of HPV genomic material in lung adenocarcinoma primary lesions with the INNO-LiPA platform. Viral replication was also evaluated by detecting the presence of oncoprotein E6/E7 messenger RNA (mRNA) by quantitative RT-PCR. To confirm possible hypotheses regarding viral oncogenesis, vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1 (HIF1) were evaluated with immunohistochemistry and immunoscore.

Results: A total of 133 patients were included in the analysis, of whom 34 tested positive for HPV, reaching an estimated prevalence of 25.6% [95% confidence interval (CI) 18.2% to 32.9%]. E6/7 mRNA was identified in 28 out of the 34 previously positive cases (82.3%). In immune checkpoint inhibitor (ICI)-treated patients, the median overall survival reached 22.3 months [95% CI 19.4 months- not reached (NR)] for HPV-negative and was not reached in HPV-positive (HPV+) ones (95% CI 27.7-NR; \( P = 0.008 \)). With regard to progression-free survival, HPV– patients reached a median of 9.2 months (95% CI 7.9-11.2 months) compared to 14.3 months (95% CI 13.8-16.4 months) when HPV was positive (\( P = 0.001 \)). The overall response rate for HPV+ patients yielded 82.4% compared to 47.1% in negative ones. No differences regarding programmed death-ligand 1, VEGF, HIF1, stromal fibrosis, or immunoscore were identified.

Conclusions: In patients with HPV+ lung adenocarcinoma, a significant benefit in overall response and survival outcomes is observed.

Key words: immunotherapy, HPV infection, lung cancer
INTRODUCTION

In recent years, the incidence of lung cancer in never smokers has been increasing. This subgroup of individuals corresponds to 17 000-26 000 cancer-attributable deaths only in the United States. Data from developing nations, particularly from Asia and Latin America, further support this trend. Several risk factors have been associated with this phenomenon, including, but not limited to, secondhand smoke, indoor air pollution, occupational exposure, and genetic susceptibility, among others. In this population, adenocarcinoma represents the most common histology.

Interestingly, when compared with tumors from smokers, tumors from nonsmokers appear less histologically complex and have a higher prevalence of targetable driver mutations, specifically epidermal growth factor receptor mutations (EGFRm), human epidermal growth factor receptor-2 mutations (HER2m), as well as anaplastic lymphoma kinase (ALK) and ROS proto-oncogene 1 (ROS1) translocations. Several studies have pointed to a different hypothesis and found a relationship between human papilloma virus (HPV) infection and increased risk of developing lung adenocarcinoma [odds ratio (OR) 5.32, 95% confidence interval (CI) 1.75-16.17]. Furthermore, it is worth mentioning that this association has not been found in all latitudes. Several case-control studies limit these findings to Latin American and Asian countries, whereas it has not been described in Europe and North America.

Although a causal role for HPV and lung cancer is controversial, the effects of HPV infection on lung tissue have been documented. On the one hand, due to an increased expression of interleukin 8 (IL-8) and upregulation of proangiogenic MPP-2 and MPP-9, the malignant transformation potential of lung cells is increased in parallel to epithelial to mesenchymal transition induced by hypervascularization of STAT3. Moreover, by reduction of the expression of LKB1 mRNA, the overexpression of the E6 and E7 oncoproteins further promotes cell proliferation. The aforementioned mechanisms are also strengthened by increased HIF1 and VEGF gene expression levels, modulating inflammation and antitumoral immune responses. These results indicate possible different disease biology associated with HPV-related lung cancer. We previously reported a high HPV exposure in Hispanic lung adenocarcinoma patients and described that the presence of viral DNA results in a better prognosis in mutant EGFR and KRAS lung adenocarcinoma.

Mirroring the results from pivotal clinical trials of metastatic head and neck cancer treated with immunotherapy or its combination with chemotherapy, that suggested that HPV status could be associated with better clinical outcomes, we speculate that the previously depicted mechanisms could also correlate with treatment outcomes in lung cancer. Bearing this hypothesis in mind, the objective of the present study was to explore clinical outcomes in non-small-cell lung cancer (NSCLC) patients with tumors harboring HPV mRNA compared to negative ones and assess the different aspects of tumor biology such as HIF1 and VEGF gene expression, tumor-infiltrating lymphocytes (TILs), in the form of the immunoscore, and stromal fibrosis as indirect evidence of increased angiogenesis.

METHODS

Patients and study design

This retrospective cohort included patients diagnosed with advanced lung adenocarcinoma and treated in a reference center in Bogotá, Colombia. Clinical characteristics, treatment, and response variables were recovered from medical reports. Patients were stratified based on the presence or absence of oncogenic drivers, including EGFR mutations, ALK translocation, and also based on programmed death-ligand 1 (PD-L1) expression. Only patients whose tumor tissue sample was enough to perform tissue microdissection, nucleic acid extraction (DNA and RNA), and immunohistochemistry were included in the study. The local ethics and research committee approved the study (Cayre-34738-2019). Response evaluation was carried out according to RECIST 1.1 criteria.

Molecular markers determination

EGFR mutational status was determined with the Cobas®, VENTANA, Lightcycler (F. Hoffmann-La Roche AG Diagnostics, Basel, Switzerland) EGFR Mutation Test v2 kit, and the ALK status with the VENTANA ALK (DSF3) CDx Assay® kit. PD-L1 status was assessed with the VENTANA PD-L1 (SP263) Assay kit® and PD-L1 IHC 22C3 pharmDx® (Agilent Technologies, Santa Clara, CA). A positive expression was considered with staining of at least 1% of tumor cells. HPV status was determined directly on extracted DNA from formalin-fixed paraffin-embedded (FFPE) adenocarcinoma samples using the INNO-LiPA® HPV Genotyping Extra II on the TENDIGO® platform (Innogenetics NV, Ghent, Belgium), standardized for genotyping of 32 different HPV types, including high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68), probable high-risk HPV (26, 53, 66, 70, 73, 82) as well as several low-risk HPV genotypes (06, 11, 40, 42, 43, 44, 54, 61), according to manufacturer’s protocols. This assay consisted of an initial PCR conducted as follows: DNA samples were incubated at 37°C for 10 min; 40 cycles of 94°C for 9 min, 94°C for 30 s, 52°C for 45 s, 72°C for 4 s, and, a final hold, at 72°C for 45 min. The thermocycler speed rate was 2.3°C/s (Bio-Rad, Foster City, CA). After PCR amplification, the 65-base pair (bp), HPV L1, PCR products were denatured under alkaline conditions and joined with a previously soaked membrane in hybridization buffer. Then, a reverse line hybridization assay with a biotin—streptavidin system was carried out and conducted by automated baths on the TENDIGO® platform. HIF1 and VEGF gene expression was evaluated with quantitative RT-PCR (qRT-PCR). B-Actin gene was used as the control. After extraction, cDNA was retro-transcribed using the GoScript™ Reverse Transcriptase kit (Promega, Promega Corporation, Madison, WI). Subsequently, the
concentration and purity of the samples were evaluated by spectrophotometry in the NanoDrop 2000/2000c equipment and the integrity in the Qubit® 3.0 Fluorometer, Catalogue Number Q33216. Real-time PCR was conducted on the LightCycler® 480 using SYBR Green-I (Thermo Fisher Scientific, Waltham, MA) Master-I according to the manufacturer’s recommendations. Forward and reverse primer sequences, as well as reaction parameters, are presented in Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2022.100500. Positive expression was considered by detecting amplification before cycle number 38. Differences in expression levels between markers in each group and stratified by HPV status were evaluated using the ΔΔCt method. HPV oncoviral E6 and E7 mRNA was detected in a similar manner. qRT-PCR was conducted according to the aforementioned methodology. Primer sequences and reaction parameters are presented in Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2022.100500.

Evaluation of TILs and other histopathological characteristics

Cytoplasmic immunoreactivity for CD3 and CD8 in lymphocytes was determined by the immunohistochemical reaction on the BenchMark® ULTRA SystemTissue platform (Roche Diagnostics) using CONFIRM anti-CD3 (2GV6) rabbit monoclonal primary antibody and CONFIRM anti-CD8 (SP57) rabbit monoclonal primary antibody (Roche—Ventana) on selected FFPE slides. Immunoscore, which has been validated in several other tumors and surpassed the American Joint Committee on Cancer/International Union Against Cancer TNM (tumor—node—metastasis) classification as a prognostic factor in colon cancer, was evaluated using the immunostained TILs.23 Stromal fibrosis, defined as the proportion of connective tissue fibers evident by hematoxylin-eosin staining in the neoplastic stroma in a high-power field of view, was used as an indirect measure of stromagenesis and angiogenesis.24 Stromal fibrosis was stratified into low or absent (<10% of fibrosis), moderate (10%-75%), and high (75%-100%).

Statistical analysis

All variables were collected and curated in a centralized anonymized database. The analysis plan consisted of an initial description of all variables using central tendency and dispersion measures. To determine the relationship between HPV infection status and clinical outcomes, inferential statistics were used. First of all, the determination of confounder variables that could potentially lead to differences in relevant outcomes was conducted using logistic regression. If no relevant confounders were determined, subsequent analyses to determine the association between molecular markers and response rate were carried out with the construction of tables and a chi-square test. With regard to PD-L1 levels, due to the non-normality of the variable, the Wilcoxon rank-sum test was chosen.

Survival analysis was conducted using the Kaplan—Meier method, and differences between survival curves were evaluated with the log-rank test. Results were validated with Cox’s regression method after confirming hazard proportionality. In order to determine cut-off values that could properly categorize two survival groups, a support vector machine model was constructed for both immunoscore and stromal fibrosis. Graphical representation of the resulting vector, indicative of the categorizing value, is presented in Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2022.100500. All analyses were conducted on R 4.0.2 (The R Foundation, Vienna, Austria).

RESULTS

A total of 133 patients with stage IV disease were included in the analysis, with a median follow-up time of 29.9 months (95% CI 26.6-34.7 months). 51.1% were male, the majority had a Karnofsky performance score (KPS) >80, and only 19.6% (n = 26) were exposed to cigarette smoke. Patient characteristics are presented in Table 1. All the patients in the cohort except for one had adenocarcinoma, whereas the remaining cases had an adenosquamous carcinoma. Seven patients (5%) had locally advanced unresectable disease, 61 patients (45.9%) had only one extrapulmonary organ involvement, while 47 (35%) and 18 (13.5%) had two or three or more metastatic sites, respectively. Considering molecular profiles, EGFR mutations were identified in 29 patients (21.8% [95% CI 14.8% to 28.8%]), ALK rearrangements in 8 [6% (95% CI 1.9% to 10.1%)], and PD-L1 expression in 28 [21.1% (95% CI 14.1% to 28%)]. Treatment information for the first line was obtained from 121 patients, and their characteristics were as follows: among 15 patients, EGFR-positive tumors (51.7%) were treated with afatinib, 8 with erlotinib (27.6%), 4 with gefitinib (13.8%), and 2 with osimertinib (6.9%). All ALK-positive patients were treated with crizotinib. Chemotherapy alone or in combination with

| Variable                      | n (%)          |
|-------------------------------|----------------|
| Age (years), median (range)   | 67 (25-92)     |
| Female sex                    | 65 (48.9)      |
| Karnofsky performance score   |                |
| 100                           | 33 (24.8)      |
| 90                            | 11 (8.3)       |
| 80                            | 51 (38.3)      |
| 70                            | 30 (22.6)      |
| 50                            | 8 (6)          |
| Smoking status                |                |
| Never smoker                  | 84 (63.2)      |
| Former smoker                 | 18 (13.5)      |
| Active smoker                 | 8 (7.3)        |
| Unknown                       | 23 (17.2)      |
| Metastatic involvement        |                |
| Liver                         | 45 (33.8)      |
| Pleura                        | 44 (33.1)      |
| Brain                         | 38 (28.6)      |
| Bone                          | 55 (41.3)      |

The definition for never smoker/smoker/nonsmoker follows the CDC standardized terminology (https://www.cdc.gov/nchs/nhis/tobacco/tobacco_glossary.htm#:text=Former%20smoker%3A%20An%20adult%20who%20has%20not%20smoked%20at%20all%20after%20age%2018%20years%20of%20life.).
bevacizumab was given to 18 and 5 patients, respectively, for a total of 23 patients [17.3% (95% CI 10.8% to 23.7%)]. Immunotherapy alone or combined with chemotherapy [immune checkpoint inhibitor (ICI) treatment group] was used in 63 individuals, of which 7 (11.1%) received pembrolizumab as monotherapy. The remainder, 56 (88.9%), received pembrolizumab in combination with platinum and pemetrexed. Median overall survival (OS) for the whole cohort was 31.4 months (95% CI 25.8-41.7 months), and median progression-free survival (PFS) was 11 months (95% CI 10.3-13.4 months).

Of 133 patients, 34 tested positive for HPV, reaching an estimated prevalence of 25.6% (95% CI 18.2% to 32.9%). Oncoprotein E6/E7 mRNA was identified by qPCR in 28 out of the 34 previously positive cases (82.3%). Positivity rates among distinctive molecular subtypes are presented in Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2022.100500. When comparing prevalence among subgroups, EGFR-mutated samples, and PD-L1 expression was statistically significantly associated with an increased HPV positivity ($P = 0.012$ and $P = 0.0234$, respectively). ALK rearrangements were not associated ($P = 0.111$). HPV and its association with other clinical variables are presented in the Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2022.100500. Logistic regression indicates that the only factor related to increased HPV positivity is the molecular subgroup. Among patients treated with ICIs (pembrolizumab ± chemotherapy), mean PD-L1 expression reached 14.7% in the HPV-negative (HPV−) group compared to 21.9% in the positive group ($P = 0.08$). Overall PD-L1 positivity was estimated at 24.4% in HPV− patients, as opposed to 40.9% in the HPV-positive (HPV+) subgroup ($P = 0.17$).

Regarding survival by molecular subgroups and treatment protocols, EGFR-mutated patients achieved a median OS of 54.3 months (95% CI 44.5 months-NR), and ALK-rearranged, a median of 41.7 months, whereas patients treated with chemotherapy only reached 23.3 months (95% CI 18.8-25.4 months), compared to 27.1 months (95% CI 22.1 months-NR) in the immunotherapy ± chemotherapy group (hazard ratio [HR] 0.26 [95% CI 0.08-0.757]; $P = 0.0136$). Median PFS for individuals with EGFR-mutated and ALK-rearranged tumors was 17.4 months (95% CI 15.1-25.6 months) and 14.1 months (95% CI 13.3 months-NR), respectively. The combination of chemotherapy and immunotherapy or immunotherapy alone prolonged PFS from 8.3 months (95% CI 6.97-9.63 months) to 11 months (95% CI 8.9-13.2 months) [HR 0.39 (0.22-0.69); $P = 0.0014$] when compared to chemotherapy only. Survival outcomes stratified based on treatment received and HPV status are presented in Table 2. Survival curves of ICI-treated patients depending on HPV status are presented in Figure 1. Furthermore, overall response rates for this cohort were 82.3% for HPV+ cases compared to 47.1% in negative individuals ($P < 0.001$). Survival curves based on the type of response are presented in Figure 2. No complete responses were observed for ICI-treated HPV− patients, and no progressive diseases were observed in HPV+ ICI-treated patients. Survival curves and response types are presented in Supplementary Figures S3 for HPV+ and S4 for HPV−, available at https://doi.org/10.1016/j.esmoop.2022.100500.

VEGF and HIF1 mRNA expression levels were similar among the HPV+ and HPV− groups (Figure 3A and B) ($P = 0.093$ and $P = 0.082$, respectively). Furthermore, stromal fibrosis, absent or low, was associated with worse OS [25.1 months (95% CI 21.5-40.5 months)] compared with patients whose tumors had >10% fibrosis [38.7 months (95% CI 27.7 months-NR); HR 0.51 (95% CI 0.29-0.89); $P = 0.01$]. A 2-month prolongation in PFS was observed in the absent/low stromal fibrosis group [10.5 months (95% CI 8.8-13.2 months)] versus 12.5 months [95% CI 10.3-15.3 months; $P = 0.02$; HR 0.61 (95% CI 0.41-0.89)] (Supplementary Figure S5, available at https://doi.org/10.1016/j.esmoop.2022.100500). This benefit was neither associated with HPV positivity ($P = 0.78$) nor ICI exposure ($P = 0.151$), indicating that this difference does not explain clinical outcomes. On the other hand, as evaluated by the immunoscore, infiltrating lymphocytes was also associated with improved survival. OS improved from 22.1 months (95% CI 19.8-42.3 months) in the high-score (immunoscore ≥40%) group to 38.7 months [95% CI 31.1-58.6 months, HR 2.49 (95% CI 1.41-4.03); $P < 0.001$] for low (immunoscore < 40%) scoring individuals. Median PFS, in a similar manner, was estimated at 10.5 months (95% CI 7.97-12.7 months) compared to 13.1 months (95% CI 10.27-14.7 months), [P = 0.005, HR 1.77 (95% CI 1.19-2.63)], respectively (Supplementary Figure S6, available at https://doi.org/10.1016/j.esmoop.2022.100500). Similar to stromal fibrosis,
immunoscore did not differ between HPV+ and HPV− groups (P = 0.152), or ICI-treated patients (P = 0.369).

**DISCUSSION**

To the authors’ knowledge, this is the first study to evaluate the relationship of HPV infection by detecting transcriptionally active virus through the determination of the E6 and E7 mRNA oncoproteins and clinical outcomes, especially considering the effect on patients exposed to immunotherapy. Considering the high positivity rates in the cohort, it is worth considering that prevalence could vary extensively across latitudes, explaining no association with lung cancer risk observed in western populations.3–12 As mentioned previously, evidence from other tumors such as head and neck cancers provided reasons to consider that active HPV infection has strong modulatory properties that may increase the effectiveness of ICI.
From the mechanisms explored in this manuscript, no statistically significant difference between the expression of VEGF and HIF1 in HPV+, compared to HPV−, disease was found. It is worth noting that the original manuscript that identified enriched VEGF, HIF1, and interleukin (IL)-8 expression in HPV-infected NSCLC cells was conducted in vitro on commercially available cell lines (A549 and NCI-H460). One possible explanation for our results compared to the published literature could lay in the different methodology employed. Since samples represented tumors removed from a patient, malignant cells were in contact with a neoplastic stroma. This is relevant since tumor stroma has been amply reported to have a central role in disease progression and modulation. Tumor-associated fibroblasts have been associated with the induction of epithelial to mesenchymal transition (EMT) and of a stem cell-like phenotype in different NSCLC models.

Contrary to this phenomenon, VEGF and HIF1 have been linked with EMT inhibition. Considering the pro-and anti-EMT signaling that occurs due to HPV infection, it is possible to hypothesize that tumor stroma reverses the increased VEGF and HIF1 expression detected in cultured cell lines.
and therefore was not detected in the present study. Since IL-8 RT-PCR could not be standardized, this marker could not be evaluated in this study.

Further explanations regarding differences between HPV+ and HPV− patients were also explored with stromal fibrosis. This easy-to-evaluate parameter, requiring only light microscopy and a trained pathologist, was chosen as an indirect measure of angiogenesis and treatment sensitivity. Although there is controversy on whether increased fibrosis leads to tumor progression and metastatic potential, several studies have described the inhibitory effect of fibrosis on cancer growth.28 The present study indicates that in NSCLC, stromal fibrosis has a negative effect on disease aggressiveness as indicated by a longer OS and PFS in patients with moderate to high fibrosis, compared to patients harboring a low to absent interstitial collagen content. One theoretical mechanism that could explain this phenomenon is activating the hedgehog (Hh) signaling pathway. Several disease models like pancreatic, colon, and bladder cancer models indicate that tumor cells secrete Hh in a paracrine fashion leading to increased fibrosis. In models where a knockout of this gene was induced, an increased vascularized and low fibrotic stroma was observed, with the caveat that the neoplastic disease was less differentiated and more aggressive. Similar studies indicated that premalignant pancreatic lesions progressed more rapidly when HH was silenced, lacking desmoplasia and eliminating stromal to tumor restraints.29,30 Although stromal fibrosis was associated with better clinical outcomes in this study, HPV status was not associated with an increased or decreased stromal fibrosis-enriched phenotype. This, in turn, indicated that the benefit observed in HPV+ patients treated with ICI could not be explained by increased fibrosis.

In a similar fashion, immunoscore, a validated methodology to assess prognosis, especially in colon cancer, takes into account the immunophenotype of TILs. This score has also been associated with clinical outcome and prognosis in lung cancer, especially in early-stage disease.31 Taking into consideration the immunogenic potential of oncogenic viruses, this score was also included as a possible reflection of better responses in HPV+ patients. Since no differences in

![Figure 3. Comparative VEGF(A) and HIF1(B) mRNA expression levels between the HPV+ and HPV− groups.](image)

HIF1, hypoxia-inducible factor 1; HPV, human papilloma virus; mRNA, messenger RNA; VEGF, vascular endothelial growth factor.
this score were observed between HPV− or HPV+ patients, it could be assumed that tumor immunogenicity, as reflected by TILs amount and phenotype, was not influenced by HPV infection.

After a comprehensive analysis of several factors related to hypoxia, angiogenesis, inflammation, and antitumor immune response in NSCLC patients with and without HPV infection, no difference attributable to these parameters was observed. Considering the benefit of ICI in individuals with HPV+ tumors, without variation of PD-L1 levels, among both groups, it could be hypothesized that this oncogenic virus functions as a prognostic/predictive feature in these patients. The previously mentioned mechanisms cannot give a clear explanation for this finding. STK11-inactivating mutations are associated with an inadequate response to ICI, mainly when associated with other mutations such as KRAS, shortening survival.13 HPV has also been associated with decreased STK11 mRNA levels,15 suggesting that HPV-infected tumors might have a poorer prognosis and response to ICI, but this is not the case. Possible mechanisms that could increase immune response include decreased damage response and genomic repair mechanisms that result in incremental mutational loads. This, in turn, could induce the production of more cancer-related epitopes and increase immune responses.13

This study is not free of limitations. Due to its retrospective nature and the relatively small number of cases, especially HPV+ in the different molecular subgroups, these results should be validated in more extensive prospective clinical trials. The cohort, due to its retrospective nature, included samples from patients that, due to local availability of medications, were not tested for European Society for Medical Oncology- and National Comprehensive Cancer Network-recommended genomic drivers. Further genes are infrequent compared to EGFR and ALK, especially ones that could negatively influence immunotherapy-related outcomes such as LKB1 or KEAP1. Taking this into consideration, it would be highly improbable that further genomic testing would modify our results. Moreover, other hypotheses regarding the specific mechanism, such as modulation of inflammatory markers, regulatory lymphocytes, or oncolytic cytotoxicity, should be considered.

In conclusion, a 26% prevalence of HPV infection was observed in this population. Our data suggest that the detection of this virus could serve as a prognostic factor, especially in patients treated with ICI.

FUNDING

This work was supported by a grant from the Conquer Cancer Foundation—ASCO [grant number 13350_GOYIA (2018)].

DISCLOSURE

AFC discloses financial research support from Merck Sharp & Dohme, Boehringer Ingelheim, Roche, Bristol-Myers Squibb, Foundation Medicine, Roche Diagnostics, Thermo Fisher, Broad Institute, Amgen, Flatiron Health, Teva Pharma, Rochem Biocare, Bayer, INQBox and The Foundation for Clinical and Applied Cancer Research — FICMAC. Additionally, he was linked and has received honoraria as an advisor, participated in speakers’ bureau. He gave expert testimony to EISAI, Merck Serono, Janssen Pharmaceutical, Merck Sharp & Dohme, Boehringer Ingelheim, Roche, Bristol-Myers Squibb, Pfizer, Novartis, Cellidex Therapeutics, Foundation Medicine, Eli Lilly, Guardant Health, Illumina, and Foundation for Clinical and Applied Cancer Research — FICMAC. OA reports personal fees from Pfizer, grants and individual fees from AstraZeneca, grants and individual fees from Boehringer Ingelheim, Lilly, Merck, Bristol-Myers Squibb, and Roche, outside the submitted work. CR reports relation with Mylan, Archer Biosciences, Oncopass, Inviva, Merck Serono Novartis, MSD, Boehringer Ingelheim, Guardant Health, and AstraZeneca as part of speakers’ bureau. Also, he received research funding from Pfizer and had uncompensated relationships with OncoDNA, Biomark, and Guardant Health. GR discloses travel, accommodations, and expenses from AstraZeneca and Pfizer, and consulting advisory role with Roche, Amgen, and Pfizer. UM reports personal fees from Boehringer Ingelheim, AstraZeneca, Roche, MSD, Amgen, Merck, and BMS for participation in a speaker bureau; personal fees from Boehringer Ingelheim, MSD, Amgen, Merck, and BMS for acting in an advisory role; financial support from Boehringer Ingelheim and Amgen that was paid directly to his institution outside the submitted work. All other authors have declared no conflicts of interest.

REFERENCES

1. Corrales L, Rosell R, Cardona AF, Martín C, Zatarain-Barrón ZL, Arrieta O. Lung cancer in never smokers: the role of different risk factors other than tobacco smoking. Crit Rev Oncol Hematol. 2020;148:102895.
2. Subramanian J, Govindan R. Lung cancer in never smokers: a review. J Clin Oncol Off J Am Soc Clin Oncol. 2007;25(5):561-570.
3. Gou LY , Niu FY, Wu YL, Zhong WZ. Differences in driver genes between smoking-related and non-smoking-related lung cancer in the Chinese population. Cancer. 2015;121(suppl 17):3069-3079.
4. Corrales-Rodriguez L, Arrieta O, Mas L, et al. An international epidemiological analysis of young patients with non-small cell lung cancer (AdJuV-CLIcap). Lung Cancer Amst Neth. 2017;113:30-36.
5. Okazaki I, Ishikawa S, Sohara Y. Genes associated with susceptibility to lung adenocarcinoma among never smokers suggest the mechanism of disease. Anticancer Res. 2014;34(10):5229-5240.
6. Arrieta O, Ramírez-Tirado LA, Báez-Saldaña R, Peña-Curiel O, Soca-Chafre G, Macedo-Perez EO. Different mutation profiles and clinical characteristics among Hispanic patients with non-small cell lung cancer could explain the “Hispanic paradox.” Lung Cancer Amst Neth. 2015;90(2):161-166.
7. Bae JM, Kim EH. Human papillomavirus infection and risk of lung cancer in never-smokers and women, an “adaptive” meta-analysis. Epidemiol Health. Published online 2015;1-5. https://dx.doi.org/10.4178/eph/e2015052.
8. Zhai K, Ding J, Shi HZ. HPV and lung cancer risk: a meta-analysis. J Clin Virol. 2015;63:84-90.
9. Koshiol J, Rotunno M, Gillison ML, et al. Assessment of human papillomavirus in lung tumor tissue. J Natl Cancer Inst. 2011;103(6):501-507.
10. Anantharaman D, Gheit T, Waterboer T, et al. No causal association identified for human papillomavirus infections in lung cancer. Cancer Res. 2014;74(13):3525-3534.
11. Sagerup CM, Nymoen DA, Halvorsen AR, Lund-Iversen M, Helland A, Brustugun OT. Human papilloma virus detection and typing in 334 lung cancer patients. Acta Oncol Stockh Swed. 2014;53(7):952-957.
12. Ramqvist T, Ortiz-Villalon C, Brandén E, et al. Analysis of human papillomaviruses and human polyomaviruses in lung cancer from Swedish never-smokers. *Acta Oncol* Stockh Swed. 2020;59(1):28-32.

13. Shiau MY, Fan LC, Yang SC, et al. Human papillomavirus up-regulates MMP-2 and MMP-9 expression and activity by inducing interleukin-8 in lung adenocarcinomas. *PLoS One*. 2013;8(1):e54423.

14. Zhang W, Wu X, Hu L, et al. Overexpression of human papillomavirus type 16 oncoproteins enhances epithelial-mesenchymal transition via STAT3 signaling pathway in non-small cell lung cancer cells. *Oncol Res*. 2017;25(5):843-852.

15. Zeng Q, Chen J, Li Y, et al. LKB1 inhibits HPV-associated cancer progression by targeting cellular metabolism. *Oncogene*. 2017;36(9):1245-1255.

16. Zhang E, Feng X, Liu F, Zhang P, Liang J, Tang X. Roles of PI3K/Akt and c-Jun signaling pathways in human papillomavirus type 16 oncoprotein-induced HIF-1α, VEGF, and IL-8 expression and in vitro angiogenesis in non-small cell lung cancer cells. *PLoS One*. 2014;9(7):e103440.

17. Cardona AF, Rosell R, Vargas C, et al. P1.01-005. EGFR and KRAS mutations in patients having lung adenocarcinoma associated with human papilloma virus infection. *J Thorac Oncol*. 2013;8(suppl 2):S428.

18. Outh-Gauer S, Alt M, Le Tourneau C, et al. Immunotherapy in head and neck cancers: a new challenge for immunologists, pathologists and clinicians. *Cancer Treat Rev*. 2018;65:54-64.

19. Mehra R, Seiwert TY, Gupta S, et al. Efficacy of pembrolizumab vs investigator’s choice in recurrent/metastatic head and neck squamous cell carcinoma: pooled analyses after long-term follow-up in KEYNOTE-012. *Br J Cancer*. 2018;119(2):153-159.

20. Ferris RL, Blumenschein G, Fayette J, et al. Nivolumab vs investigator’s choice in recurrent or metastatic squamous cell carcinoma of the head and neck: 2-year long-term survival update of CheckMate 141 with analyses by tumor PD-L1 expression. *Oral Oncol*. 2018;81:45-51.

21. Cohen EEW, Soulières D, Le Tourneau C, et al. Pembrolizumab versus methotrexate, docetaxel, or cetuximab for recurrent or metastatic head-and-neck squamous cell carcinoma (KEYNOTE-040): a randomised, open-label, phase 3 study. *Lancet*. 2019;393(10167):156-167.

22. Burtness B, Harrington KJ, Greil R, et al. Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): a randomised, open-label, phase 3 study. *Lancet*. 2019;394(10121):1915-1928.

23. Bruni D, Angell HK, Galon J. The immune contexture and immuno-score in cancer prognosis and therapeutic efficacy. *Not Rev Cancer*. 2020;20(11):662-680.

24. Yamauchi M, Barker TH, Gibbons DL, Kurie JM. The fibrotic tumor stroma. *J Clin Invest*. 2018;128(1):16-25.

25. Bremnes RM, Dannem T, Al-Saad S, et al. The role of tumor stroma in cancer progression and prognosis: emphasis on carcinoma-associated fibroblasts and non-small cell lung cancer. *J Thorac Oncol Off Publ Int Assoc Study Lung Cancer*. 2011;6(1):209-217.

26. Shintani Y, Abulaiti A, Kimura T, et al. Pulmonary fibroblasts induce epithelial mesenchymal transition and some characteristics of stem cells in non-small cell lung cancer. *Ann Thorac Surg*. 2013;96(2):425-433.

27. Hong JP, Li XM, Li MX, Zheng FL. VEGF suppresses epithelial-mesenchymal transition by inhibiting the expression of Smad3 and miR-192, a Smad3-dependent microRNA. *Int J Mol Med*. 2013;31(6):1436-1442.

28. Chandler C, Liu T, Buckanovich R, Coffman LG. The double edge sword of fibrosis in cancer. *Transl Res J Lab Clin Med*. 2019;209:55-67.

29. Rhim AD, Oberstein PE, Thomas DH, et al. Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. *Cancer Cell*. 2014;25(6):735-747.

30. Lee JJ, Perera RM, Wang H, et al. Stromal response to Hedgehog signaling restrains pancreatic cancer progression. *Proc Natl Acad Sci U S A*. 2014;111(30):E3091-E3100.

31. Zhao Z, Zhao D, Xia J, Wang Y, Wang B. Immunoscore predicts survival in early-stage lung adenocarcinoma patients. *Front Oncol*. 2020;10:691.

32. Skoulidis F, Goldberg ME, Greenawalt DM, et al. STK11/LKB1 mutations and PD-1 inhibitor resistance in KRAS-mutant lung adenocarcinoma. *Cancer Discov*. 2018;8(7):822-835.

33. Gao P, Lazare C, Cao C, et al. Immune checkpoint inhibitors in the treatment of virus-associated cancers. *J Hematol Oncol*. 2019;12(1):58.