A detailed smoking history and determination of MYC status predict response to checkpoint inhibitors in advanced non-small cell lung cancer

Michelle Chiu, Mary Beth Lipka, Priyanka Bhateja, Pingfu Fu, Afshin Dowlati

Division of Hematology and Oncology, Case Western Reserve University and University Hospitals Seidman Cancer Center, Cleveland, Ohio, USA

Contributions: (I) Conception and design: A Dowlati, M Chiu, P Fu; (II) Administrative support: A Dowlati, MB Lipka, M Chiu; (III) Provision of study materials or patients: A Dowlati, M Chiu, P Bhateja; (IV) Collection and assembly of data: A Dowlati, M Chiu, MB Lipka, P Bhateja; (V) Data analysis and interpretation: A Dowlati, M Chiu, P Bhateja, P Fu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Afshin Dowlati, MD. Division of Hematology and Oncology, University Hospitals, 11100 Euclid Avenue, Cleveland, Ohio 44106, USA. Email: afshin.dowlati@case.edu.

Background: Although many studies have determined that PD-L1 expression by immunohistochemistry can be somewhat predictive of a response to checkpoint inhibitor the impact of specific genomic changes and smoking history in the context of PD-L1 expression is limited. This single-center study examined clinical and genomic factors beyond STK11 and EGFR in patients with advanced non-small cell lung cancer (NSCLC) to determine which patients benefit from therapy with immune checkpoint inhibitors (ICIs).

Methods: Clinical and genomic features of patients with NSCLC treated with immunotherapy were compiled into a database. Genomic information collected included gene mutations via next generation sequencing, tumor mutation burden (TMB), and PD-L1 tumor proportional scores.

Results: A total of 131 patients with advanced NSCLC treated with ICIs were examined. Race was not associated with response. A positive response to immunotherapy was associated with smoke year increase (P=0.042). KRAS mutation and MYC amplification were associated with a positive response to immunotherapy while EGFR, RB1, and NF1 mutations were associated with a lack of response. KRAS mutation (P=0.007) and high TMB (P=0.070) were positively associated with smoking history. EGFR mutation was negatively associated with smoking history (P=0.002). In multivariate analysis controlling for age and smoking history, MYC amplification continued to be the only predictive genomic marker with a trend toward response to therapy (P=0.092) beyond the smoking history.

Conclusions: Among the clinical and genomic factors examined in this study, smoking status is the most predictive of response to ICIs. Only MYC amplification continued to predict a trend toward response to immunotherapy when controlling for smoking history. Other genomic predictors such as EGFR and KRAS simply reflect their association with smoking. Detailed smoking history and MYC amplification alone can predict response to ICI.

Keywords: Immunotherapy; lung cancer; tobacco

Submitted Jun 25, 2019. Accepted for publication Dec 10, 2019.
doi: 10.21037/tlcr.2020.01.03
View this article at: http://dx.doi.org/10.21037/tlcr.2020.01.03
Introduction

Immune checkpoint inhibitors (ICIs) are emerging as an alternative to traditional chemotherapy for several cancers including non-small cell lung cancer (NSCLC) (1-6). Programmed death receptor (PD-1) inhibitors such as nivolumab and pembrolizumab in particular demonstrate remarkable results in certain patients with increased survival and a favorable safety profile (7,8). Despite these promising results, the overall response rate for second-line treatment with ICIs is about 20%, highlighting the need for clinically practical predictors of response (1-3).

Most trials demonstrated improved response rates in tumors with increased expression of programmed death-ligand (PD-L1), specifically in treatment with pembrolizumab for tumors with PD-L1 expression ≥50% (1,3,9). Still, PD-L1 expression has several limitations as a biomarker, such as assay variability (10,11). Recent studies appear to show that higher tumor mutation burden (TMB) correlates with response to therapy as well, independent of PD-L1 expression (12). These biomarkers may predict response for some patients, but it appears that certain subgroups are less apt to benefit from immunotherapy, such as NSCLC with EGFR mutations or ALK rearrangements despite high PD-L1 expression in some of these tumors (1,13). Thus, the need for a clinically available predictor of response to ICIs remains extremely important.

As most patients with advanced lung cancer undergo genomic testing, in particular next-generation sequencing (NGS), and clinical data is readily obtainable (such as smoking history) we set to examine which genomic and clinical characteristics are predictive of response to immunotherapy in advanced NSCLC. We examined clinical characteristics including sex, age, and detailed smoking status and extensive NGS of targeted exomes in addition to PD-L1 expression, and TMB to determine what factors are correlated with response.

Methods

Patient population

Patients with NSCLC at UH Cleveland Medical Center are compiled into an IRB approved institutional database (N=3,169) that is continuously maintained and updated. From this database patients with advanced stage IV disease were identified to yield a total of 987 patients. Other inclusion criteria included patients treated with either pembrolizumab or nivolumab and age greater than 18. We collected data on age, sex, race, smoking status, histological subtype, and somatic genomic information.

Smoking status

Smoking status is defined as current smoker for patients smoking at the time of diagnosis or a quit date within 12 months of diagnosis. Former smoker are those who quit at 12 months or greater prior to diagnosis. Never smoker is defined as less than 100 cigarettes over an individual’s lifetime. Smoking index (SI) is defined as pack years multiplied by years smoked to yield smoke-years.

PD-L1 expression and genomic testing

Clarient Diagnostic Services are used to determine PD-L1 expression at our institution. Genomic information was gathered from the Foundation One sequencing platform, which utilizes next generation sequencing to interrogate 315 genes as well as introns of 28 genes involved in rearrangements (as previously described).

Statistical analysis

Chi-square tests were used to determine associations between response to immunotherapy and variables such as gene mutations and smoking status. The association between response and continuous variables (smoking quit time, smoke years, pack years, PD-L1 expression, and TMB) was estimated using logistic regression. The response rate and 95% confidence internals were estimated using Wilson’s method. All statistical tests were two-sided and P≤0.05 was considered statistically significant. However P values of ≤0.1 were considered as a trend.

Results

Patient characteristics

A total of 131 patients met the inclusion criteria. In regards to the specific immunotherapy agent used, 108 were treated with single agent nivolumab while 23 were treated with single agent pembrolizumab. Thirty-three patients underwent PD-L1 testing, which was determined using Calrient Diagnostic Services. Eighty-three patients underwent genomic testing with Foundation One next generation sequencing. Baseline characteristics including sex, race, smoking status, and tumor pathology are described in Table 1.
The overall response rate to immunotherapy is 22.1% (29 of 131). There is no significant difference between response rates for nivolumab vs. pembrolizumab (20.4% vs. 30.4%; P=0.192). Sex and race are not associated with response (P=0.853 and 0.722, respectively). Increasing patient age is associated with positive response to immunotherapy [odds ratio (OR) 1.05; 95% confidence interval (CI), 1.01–1.09; P=0.019].

Only 9 patients in the cohort were never smokers while 39 were current smokers and 83 former smokers. 0 of the 9 never smokers responded to immunotherapy. In comparison, a current or former smoking status showed a trend with response to immunotherapy (0% vs. 23.8%; P=0.097). Univariate logistic regression demonstrates a positive association to immunotherapy response with smoke year increase (OR 1.03; 95% CI, 1–1.06; P=0.042). Quit-time (OR 1; 95% CI, 0.97–1.03; P=0.091) showed a trend with response by univariate logistic regression.

The majority of pathology was either adenocarcinoma or squamous cell carcinoma (123 of 131; 94%); neither of which is associated with response to immunotherapy. PD-L1 expression by univariate logistic regression (per percent increase) is not associated with response to immunotherapy (OR 1.01; 95% CI, 0.99–1.03; P=0.423).

### Association of tumor mutation and response to immunotherapy

Of 131 patients, 83 had genomic data available. Gene mutations represented in less than 4 patients were eliminated yielding 29 gene mutations. Only those with a P≤0.2 are reported here. Via this analysis, 5 gene mutations were identified: *EGFR*, *KRAS*, *MYC* amplification, *NF1*, and *RB1*. *MYC* amplification and *KRAS* mutation are associated with a positive response to immunotherapy while *EGFR*, *NF1*, and *RB1* mutations are associated with a lack of response as summarized in Figure 1. Examination of TMB by univariate logistic regression does not demonstrate an association with response (OR 0.99; 95% CI, 0.91–1.07; P=0.717).

### Incorporation of clinical and genomic factors for predicting response

The relationship between smoking history and genomic factors was further examined by dividing smoking history into 2 groups, <20 vs. ≥20 pack-years. Those with *KRAS* mutations compared to those without a mutation were more likely to have a high smoking history (84.9% vs. 56.6%; P=0.007). Patients with *EGFR* mutations were more likely to have a low smoking history compared to those without a mutation (100% vs. 27.2%; P=0.002). Additionally those with a TMB ≥10 were more likely to have a high smoking

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**Table 1** Demographic characteristics of the patient cohort and their association with response to immunotherapy

| Factor                  | No. of patients (%) or median (range) | P value |
|-------------------------|--------------------------------------|---------|
| Sex                     |                                      |         |
| Women                   | 73 (55.7)                            | 0.858   |
| Men                     | 58 (44.3)                            |         |
| Age, y                  | 62 (29, 88)                          |         |
| Race (40 missing)       |                                      |         |
| White                   | 49 (53.9)                            | 0.722   |
| Black                   | 41 (45.1)                            |         |
| Other                   | 1 (1.1)                              |         |
| Smoking                 |                                      |         |
| Never                   | 9 (6.9)                              | 0.097   |
| Former/current          | 122 (93.1)                           |         |
| Pathology (2 missing)   |                                      |         |
| Adenocarcinoma          | 91 (70.5)                            | 0.442   |
| Squamous                | 32 (24.8)                            |         |
| Other                   | 6 (4.7)                              |         |

**Figure 1** Response rates shown by gene mutation/wild-type (WT) with the corresponding P values.
history when comparing to those with a TMB <10 (85.7% vs. 50%; P=0.070) (Figure 2). Using logistical regression TMB did not increase with pack/year history of smoking (P=0.157) (Figure 3).

Multivariable logistic regression using clinical, PD-L1 expression, and genomic data demonstrated that only SI and MYC amplification have an independent effect on response. It also demonstrates that MYC amplification continues to predict response to immunotherapy after controlling for the effects of age and smoking history (OR 1.04; 95% CI, 0.8–18.4; P=0.092). In this analysis, age, per year increase, is no longer significant at predicting response (OR 1.04; 95% CI, 0.98–1.1; P=0.17). Conversely, SI is predictive of response to immunotherapy per 1000 SI increase (OR 1.04; 95% CI, 1–1.08; P=0.048).

**Discussion**

This study demonstrates that increased smoking history and certain genomic characteristics are important factors in predicting response to ICIs in patients with advanced NSCLC, particularly when considered in combination.

Our single institution program captures a very detailed smoking history not available in the context of clinical trials and highlights the importance of this work. Clinical trial details include capturing never smokers/former smokers and current smokers but do not provide details on the amount and the duration the patients smoked. For example in a systematic review of the effects of tobacco smoking and PD-L1 inhibitors no mention is made of pack/years smoked (14). Both KRAS mutation and MYC amplification are associated with a response to immunotherapy in our study. In contrast to EGFR-positive tumors, KRAS mutation did not predict response in multivariate analysis when accounting for smoking history and age. TMB is not a predictive biomarker in this study, likely due to lack of power, but more importantly high TMB is associated with a high smoking history and the use of a detailed smoking history may predict the TMB status of a patient with lung cancer. Examined together, these results suggest that smoking history is the most important factor predicting response to immunotherapy. As smoking history is the only independent factor (outside of MYC amplification as described below) to predict response, it is likely that TMB and PD-L1 changes are simply a reflection...
of patients smoking habits and provide no additional information beyond smoking details.

Unlike the other mutations, multivariate analysis shows that MYC amplification remains marginally significant in predicting response when controlling for patient age and smoking status with an OR of 3.85. Furthermore unlike KRAS mutation and TMB that follow the smoking pattern, MYC amplification does not correlate with smoking status and may explain why MYC amplification is an independent predictive marker of response. MYC is known to influence immune effector cells as well as regulatory cytokines, and its overexpression leads to tumor evasion of the immune response (16). This may explain why NSCLC with MYC amplification is particularly responsive to ICIs.

To our knowledge, this is the first study to show that Caucasian vs. African American race is not predictive of response to immunotherapy. Likewise, pathology of the tumor, predominantly adenocarcinoma and squamous carcinoma, is not associated with response to treatment.

It has been shown in subgroup analysis of previous studies and other investigations that EGFR-mutant tumors are less likely to respond to immunotherapy (1,13,17). This finding is supported in our study with 0 of 6 EGFR-mutants responding to immunotherapy. Similarly, 6 patients had RB1-mutant tumors and 6 had NF1-mutant tumors, of which 0 responded to immunotherapy for both groups. It has been shown that RB1 mutation correlates with a shorter disease-free survival in early stage adenocarcinoma (18).

Our group also recently demonstrated that RB1 mutation is associated with worse prognosis in advanced NSCLC (19). To our knowledge, NF1 mutation has been suggested as a potential target in NSCLC, but has not been associated with prognosis (20). EGFR-positive tumors are associated with a negative or low smoking history in NSCLC, which could account for the lower response rates seen in these tumors. One prevailing theory is that these tumors represent a lower neoantigen and TMB, and thus do not respond well to immunotherapy (21). This hypothesis is supported by our study, as the EGFR-positive tumors were strongly associated with a lower smoking history. Although RB1 and NF1 mutations are not associated with smoking history, they may also confer decreased immunogenicity through a separate mechanism.

A limitation of this study is lack of power. The cohort examined in this study contains 131 patients, of which a subset lacked TMB and PD-L1 data. Another weakness in our trial is the risk of multiple testing and risk of false positive results. Nevertheless the strength and uniqueness of our dataset is the detailed smoking history not available in the setting of clinical trials.

This single-institution study demonstrates that smoking history is perhaps the most important factor at predicting response to ICIs in advanced NSCLC. The other commonly use predictive factors including TMB, PD-L1 expression and KRAS mutations are simply a reflection of smoking history. Obtaining a detailed smoking history is a much more cost effective strategy. Furthermore, MYC amplification is a potential predictive biomarker independent of smoking history.

Acknowledgments

Funding: None.

Footnote

Conflicts of Interest: A Dowlati—consulting or advisory role: Takeda, Abbvie, Seattle Genetics, Astra Zeneca, Bristol Myers Squibb; research funding: Loxo, Bayer, Incuron, Takeda, Regeneron, Tesaro, Amgen, Seattle Genetics, Symphogen, Abbvie, Ipsen. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are 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. The study was approved by institutional review board of University Hospitals Cleveland Medical Center (Protocol DBR0014).

Data Sharing Statement: No additional data available.

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Cite this article as: Chiu M, Lipka MB, Bhateja P, Fu P, Dowlati A. A detailed smoking history and determination of MYC status predict response to checkpoint inhibitors in advanced non-small cell lung cancer. Transl Lung Cancer Res 2020;9(1):55-60. doi: 10.21037/tlcr.2020.01.03