CASE REPORT

Inflamed Tumor Phenotype as Predictor of Long-Term Response to Pembrolizumab in an *EGFR*-Mutated Non-Small Cell Lung Cancer (NSCLC) Patient with Acquired Resistance to Afatinib: a Case Report and Review of the Literature

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ABSTRACT

Treatment with immune checkpoint inhibitors (ICIs) that target the programmed cell death 1/programmed cell death ligand-1 (PD-1/PD-L1) axis is usually ineffective in patients with epidermal growth factor receptor (*EGFR*)-mutated advanced non-small cell lung cancer (NSCLC), either as first-line treatment or in later lines. By contrast, especially for patients with common *EGFR* mutations (exon 19 deletion/L858R point mutation), an orally bioavailable EGFR tyrosine kinase inhibitor (EGFR-TKI) is the best upfront therapy, being able to provide response rates well above 50% and a median progression-free survival ranging from 11 to 19 months, depending on whether a second-generation (e.g., afatinib) or a third-generation (i.e., osimertinib) EGFR-TKI is used. Unfortunately, treatment options for these patients at the time of acquired resistance are limited. As for afatinib-pretreated patients, those who develop a T790M mutation may benefit from osimertinib, whereas platinum-based chemotherapy is the preferable therapeutic strategy for T790M-negative patients as well as for patients who progress on osimertinib administered as first-line therapy. Here, we describe the case of an exon-19-deleted patient who experienced a complete response to the anti-PD-1 agent pembrolizumab upon the development of T790M-negative acquired resistance to afatinib. Furthermore, we discuss this case in the context of the existing literature, especially focusing on the importance of evaluating multiple markers of immune response post-EGFR-TKI and prior to ICI treatment in order to select the best treatment strategy in this clinical scenario.

Keywords: Afatinib; *EGFR* mutation; EGFR-TKI, immune checkpoint inhibitors; PD-L1; Tumor mutation burden

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Key Summary Points

Treatment with immune checkpoint inhibitors is generally poorly effective for epidermal growth factor receptor (EGFR)-mutated non-small cell lung cancer (NSCLC) patients who experience acquired resistance to an EGFR-tyrosine kinase inhibitor (TKI).

Here, we present the case of an EGFR-mutated NSCLC patient who underwent a complete response to the anti-programmed cell death 1 (PD-1) agent pembrolizumab upon disease progression on the EGFR-TKI afatinib.

Emphasis is placed on the serial measurement of multiple immune markers in tumor tissue as potential predictors of response to immunotherapy.

In our case, programmed cell death ligand-1 (PD-L1) expression, tumor mutation burden, as well as the presence/absence of CD8+ and FOXP3+ Tregs tumor-infiltrating lymphocytes were useful markers for the existence of an inflamed phenotype.

Evaluating the aforementioned markers can help guide treatment decisions on whether EGFR-mutated NSCLCs with acquired resistance to an EGFR-TKI could benefit from immunotherapy.

INTRODUCTION

Treatment with an epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) is highly effective for *EGFR*-mutated advanced non-small cell lung cancer [1]. However, limited treatment options exist at the time of acquired resistance to an EGFR-TKI, especially for T790M-negative patients who progress on afatinib and for those who receive osimertinib as a first-line therapy. Unfortunately, despite being

extensively studied in clinical trials, immunotherapy with immune checkpoint inhibitors (ICIs) that target the programmed cell death 1/programmed cell death ligand-1 (PD-1/PD-L1) axis has been shown to be poorly effective in the aforementioned post-EGFR-TKI clinical scenario [2]. Here we present the case of an *EGFR*-mutated (exon 19 deletion) advanced NSCLC patient who underwent a rapid complete response to the anti-PD-1 agent pembrolizumab used right after the development of T790M-negative acquired resistance to afatinib. The case is discussed in light of the existing literature on the basis of serial biopsies that were performed over time in order to measure potential markers of immune response, with the ultimate aim of predicting the clinical benefit of ICI treatment.

CASE

A 61-year-old woman who had never smoked was diagnosed with resectable stage III adenocarcinoma of the left lung. The patient was initially treated with three cycles of neoadjuvant cisplatin/gemcitabine followed by pneumonectomy. However, no pathologic response was documented at histologic examination, which confirmed the presence of stage IIIA (pN2) adenocarcinoma. Three months later, a CT scan documented progression in the liver for the presence of a single metastasis that was surgically resected. Unfortunately, the disease progressed again within 2 months following the hepatic metastasectomy, as documented by the development of multiple nodules in the right lung and a single asymptomatic brain lesion. Next-generation sequencing (NGS) (Ion Ampliseq Colon and Lung Research Panel v.2 and Ion GeneStudio S5 Systems, Thermo Fisher Scientific) showed the presence of an exon 19 deletion in the *EGFR* gene in both the primary lung tumor and hepatic metastasis (Fig. 1A, B), with a PD-L1 tumor proportion score (TPS) at immunohistochemistry of 60% (clone 22C3, pharmDx kit, Dako) in both samples (Fig. 2A, D). On this basis, the patient started treatment with the EGFR tyrosine kinase inhibitor (TKI) afatinib, which led to a partial response in the
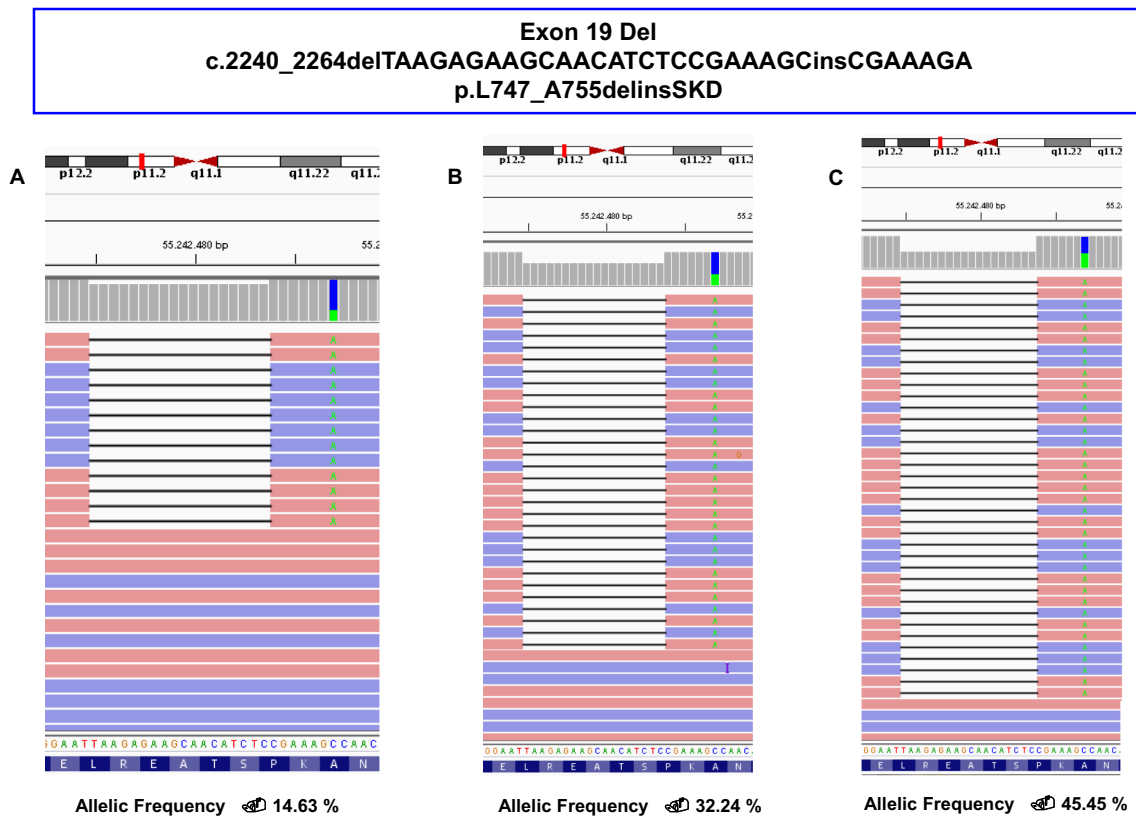
Fig. 1 Next-generation sequencing (NGS) performed on the primary lung tumor at the time of first diagnosis (**A**), on the hepatic metastasis prior to the start of treatment with afatinib (**B**), and on a mediastinal lymph node at the time of acquired resistance to afatinib before the start of immunotherapy (**C**). The presence of an *EGFR* exon 19 deletion was consistently shown in each tumor sample. No T790M mutation was documented in the mediastinal lymph-node sample collected at the time of acquired resistance to afatinib

lung and in the brain. Nevertheless, 8 months after the initiation of afatinib, the disease progressed further in the mediastinal lymph nodes, right lung, and brain (Fig. 3A, D, G). Re-biopsy was performed on the right lower paratracheal lymph-node station, which confirmed the presence of adenocarcinoma with an *EGFR* exon 19 deletion at NGS and without the presence of the secondary T790M resistance mutation (Fig. 1C). In this sample, a maintained PD-L1 TPS of 60% was found (Fig. 2G). At that time, all three tissue samples of the patient were further analyzed for tumor mutation burden (TMB) using the Oncomine Tumor Mutation Load (TML) Assay on the Ion GeneStudio S5 System (Thermo Fisher Scientific) (Fig. 4), as well as for tumor-infiltrating lymphocytes (TILs), particularly CD8+ T cell infiltrates and FOXP3+ regulatory T cells (Tregs) by immunohistochemistry

(Fig. 2B, E, H, and C, F, I, respectively). On the one hand, although low, TMB levels (defined as the total number of nonsynonymous mutations within a tumor genome/per megabase (Mb)) were found to increase over time (Fig. 4). On the other, the TILs presented both a peripheral and, although with lower density, an intratumoral localization (not evaluable in the re-biopsy mediastinal lymph-nodal specimen), showing high levels of CD8+ T cell infiltrates with scant presence of FOXP3+ Tregs across all tumor samples, which suggested the presence of an inflamed tumor phenotype. On this basis, the patient started pembrolizumab 200 mg i.v. every three weeks, with a first CT scan performed after 2 months of treatment showing the achievement of a partial response at all sites, brain included (Fig. 3B, E, H). Subsequent CT scans showed that the disease continued to
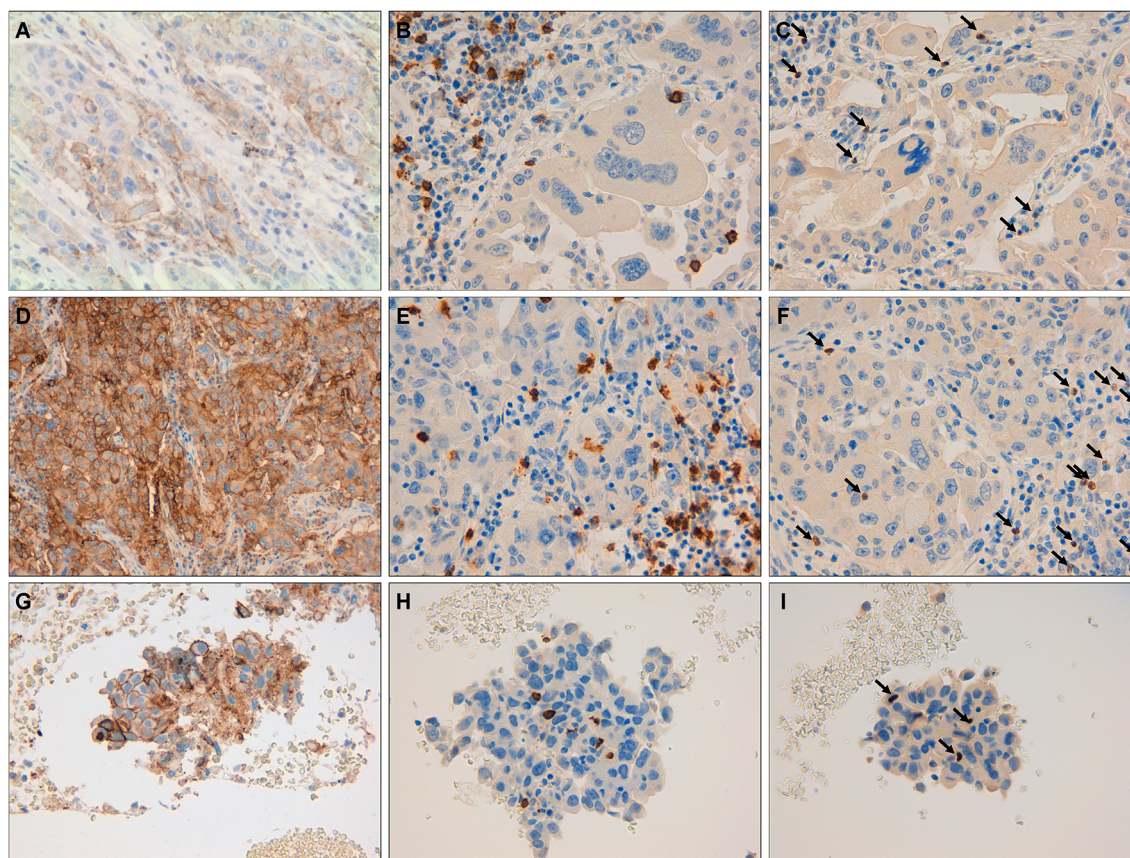
Fig. 2 PD-L1 membranous immunostaining of 60% of tumor cells, showing high intensity of immunolabeling in the primary lung tumor (**A**), hepatic metastasis (**D**), and mediastinal lymph node (**G**). CD8+ T cell infiltrates highlighted by membranous immunostaining (*brownish label*): localized at the edge and between the tumor cells for both primary lung carcinoma (**B**) and hepatic metastasis (**E**) and within the tumor cluster for the nodal relapse (**H**). scant FOXP3 nuclear immunostaining of Treg cells (*arrows*) in the primary lung carcinoma (**C**), hepatic metastasis (**F**), and nodal relapse (**I**). Original magnification **A–I**: ×400

shrink until it underwent a complete response after 9 months of treatment (Fig. 3C, F, I). Maintenance of the complete response was observed at the most recent CT scan performed 24 months since the initiation of pembrolizumab.

Written informed consent was obtained from the patient before publishing her case in an anonymous form.

DISCUSSION

ICI therapy is generally poorly effective for *EGFR*-mutated NSCLC. Two pooled meta-analyses evaluating a few trials of treatment with PD-1/PD-L1 inhibitors as a second-line therapy for advanced NSCLC found that immunotherapy does not improve survival as compared to chemotherapy [3, 4]. Similarly, poor activity of ICI therapy in *EGFR*-mutated NSCLC has also been reported in a large retrospective analysis by Mazeries et al., in which a response rate of 12.2% was observed in the subset of patients harboring an *EGFR* mutation [5]. On the other hand, several phase 1 trials exploring the association of ICI with an EGFR-TKI found that this combination resulted into a high percentage of treatment-related adverse events, which discouraged further clinical development of such a strategy [6–8]. For instance, the phase 1b
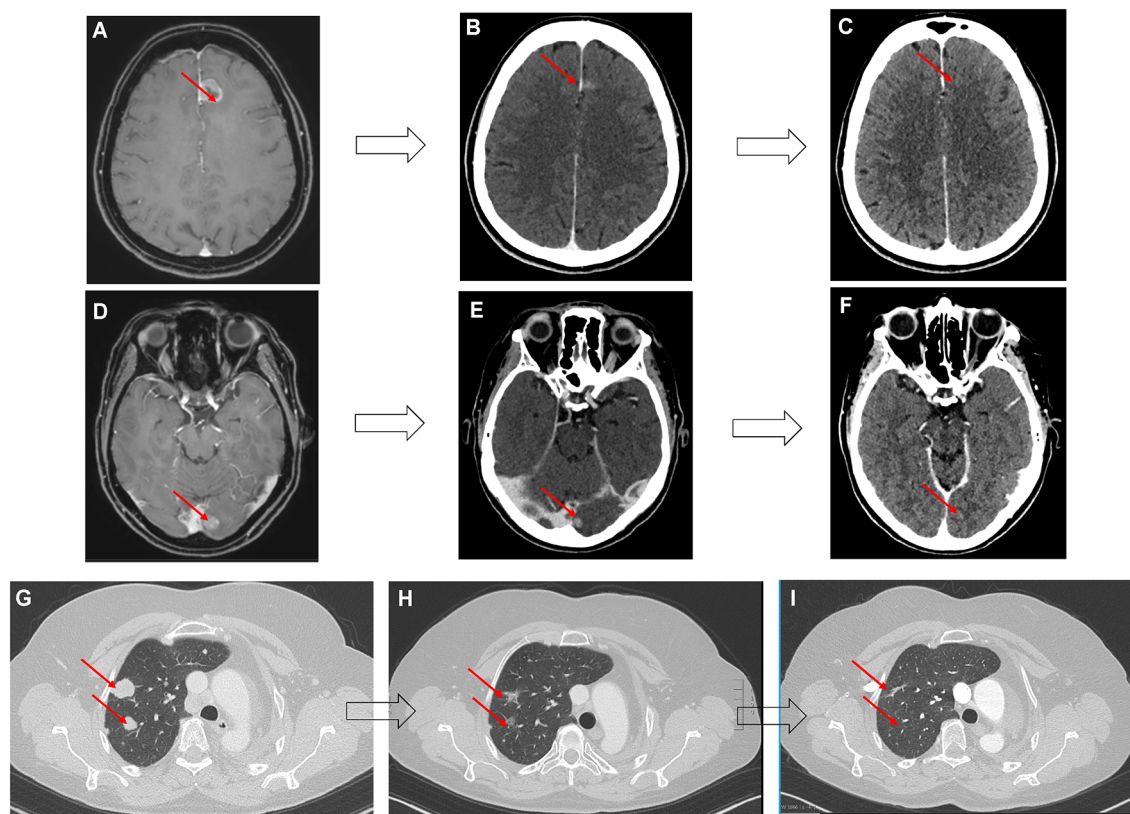
Fig. 3 MRI of the brain (T1 gad sequence) and CT scan of the lung performed right before the start of immunotherapy, showing two centimetric metastatic lesions in the left frontal and occipital lobes (**A**, **D**) and pulmonary metastases in the right lung (**G**); CT scan of the brain and the lung performed after 2 months of treatment with pembrolizumab, showing reductions in the sizes of brain lesions (**B**, **E**) and pulmonary metastases (**H**); CT scan of the brain and the lung performed 9 months after the initiation of pembrolizumab, showing a complete response in the brain (**C**, **F**) and lung (**I**)

TATTON study, which evaluated the safety and tolerability of osimertinib plus the anti-PD-L1 agent durvalumab in *EGFR*-mutated NSCLCs who progressed on prior TKI, documented an unacceptable 38% incidence of interstitial lung disease (ILD) [6]. These disappointing results imply that the role of ICI therapy for the treatment of patients with *EGFR*-mutated NSCLC is limited, being confined to patients who progress on an EGFR-TKI and for which no other effective therapies are present.

On the other hand, the fact that occasional responses to immunotherapy have still been reported suggests that proper patient selection is needed to identify the *EGFR*-mutated NSCLCs who could benefit from ICI therapy. Table 1 shows the anti-tumor response in patients with an *EGFR* mutation treated in clinical trials employing anti-PD-1/PD-L1 agents either as monotherapy or in combination with CTLA-4 blockade. Although responses were highly variable, they did not exceed 14% in the trials that evaluated more than 10 patients. However, it is important to note that firm conclusions cannot be drawn for this clinical scenario given the generally low number of patients that have been included in these studies as well as the heterogeneous types of *EGFR* mutations (sensitizing common and uncommon and non-sensitizing) that were considered (Table 1).

Importantly, given the impressive and well-established activity of targeted therapy in the first-line setting of *EGFR*-mutated NSCLC, the question of whether immunotherapy should be
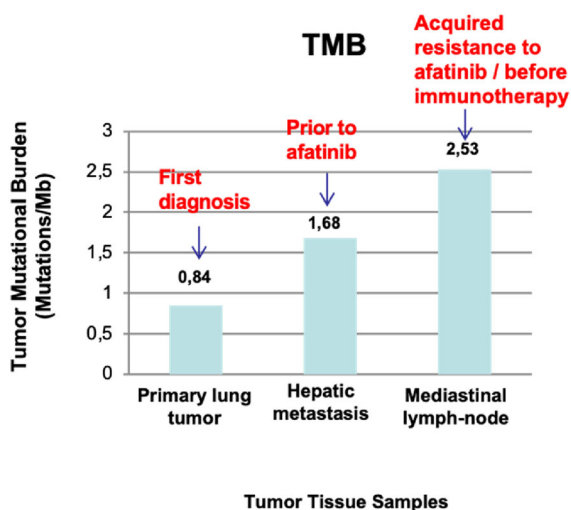
Fig. 4 Assessment of tumor mutation burden (TMB) on the primary lung tumor at the time of the first diagnosis, on hepatic metastasis prior to the start of treatment with afatinib, and on a mediastinal lymph node at the time of acquired resistance to afatinib before the start of immunotherapy. TMB levels, defined as the total number of nonsynonymous mutations within a tumor genome/per megabase (Mb), were progressively increased at the times of first and second disease progression (1.68 and 2.53 mutations/Mb, respectively) as compared to surgery (0.84 mutations/Mb)

administered to patients with an *EGFR* mutation is particularly relevant for those individuals who develop acquired resistance to an EGFR-TKI (i.e., response or stable disease for ≥ 6 months with subsequent development of disease progression while still on EGFR-TKI). In fact, limited treatment options exist for these patients, and the true effectiveness of immunotherapy is uncertain.

Our case is exceptional, since the patient experienced a deep and durable response (brain included) to ICI therapy despite having a sensitizing *EGFR* mutation. According to a study, only 2% of patients with an *EGFR* sensitizing mutation treated with anti-PD1/PD-L1 agents achieve a long-term response (defined as a complete or partial response lasting for more than 24 months) [16]. The constantly high PD-L1 levels expressed by the tumor of the patient (60%), even at the time of progression on afatinib, were crucial, along with the demonstration of T790M-negative acquired resistance, for selecting pembrolizumab as the next line of therapy following afatinib. Importantly, while de novo PD-L1 expression is commonly present in *EGFR*-mutated NSCLCs, likely reflecting a constitutive activation rather than a marker of

Table 1 Activity of ICI therapy in *EGFR*-mutated advanced NSCLC patients treated in clinical trials with either an anti-PD-1/PD-L1 as monotherapy or in combination with CTLA-4 blockade

Trial	Drug	Overall response	
		EGFR-TKI naïve	**EGFR-TKI pretreated**
Garon et al. [9]	Pembrolizumab	2 out of 3 pts – 67%	1 out of 26 pts – 4%
Lisberg et al. [10]	Pembrolizumab	0 out 11 pts – 0%*	–
Gettinger et al. [11]	Nivolumab	1 out of 7 pts – 14%**	
Peters et al. [12]	Atezolizumab	4 out of 45 pts – 9%**	
Garassino et al. [13]	Durvalumab		9 out 64 pts – 14%***
Hellmann et al. [14]	Nivolumab + ipilimumab	4 out of 8 pts – 50%**	
Gubens et al. [15]	Pembrolizumab + ipilimumab	-	1 out of 10 pts – 10%

EGFR-TKI epidermal growth factor-tyrosine kinase inhibitors, *pts* patients
*PD-L1 ≥ 1%
**Data on pretreatment with an EGFR-TKI are not available
***PD-L1 ≥ 25%

adaptive immune response, high levels of PD-L1 expression at the time of acquired resistance to EGFR-TKI treatment might imply true dependence on the PD-1/PD-L1 axis as an escape mechanism [17–21]. Therefore, PD-L1 reassessment through re-biopsy at the time of acquired resistance to an EGFR-TKI could be warranted in EGFR-mutated patients with acquired resistance to an EGFR-TKI, as this might have important clinical implications. With regard to this, the importance of reassessing PD-L1 expression after EGFR-TKI treatment was elegantly shown by Isomoto et al. in a retrospective study [21]. They demonstrated that 138 EGFR-mutated patients who underwent re-biopsy at the time of acquired resistance to an EGFR-TKI showed a significant increase in PD-L1 expression $\geq$ 50% from 14 to 28%. Even more importantly, a higher PD-L1 expression appeared to correlate with an improved clinical outcome in patients who received subsequent ICI treatment.

Tumor mutation burden is another putative marker of sensitivity to immunotherapy [22]. In fact, a higher number of DNA mutations is associated with an increased probability of successfully presenting neoantigens, which, in turn, induce tumor immunogenicity [23]. Importantly, TMB status does not overlap with PD-L1 expression, and assessing both markers could help increase the identification of patients who are candidates for ICI treatment [24]. However, EGFR-mutated patients tend to have lower levels of TMB as compared with EGFR wild-type patients, which is particularly true for patients who bear a common EGFR mutation (exon 19 deletion/L858R point mutation) [25]. This is because EGFR-mutated NSCLC is associated with less tobacco exposure as compared to NSCLC of smokers, and a significant relationship has been demonstrated between smoking history and TMB in advanced lung adenocarcinoma [26]. In addition, the narrow range of TMB levels of expression in EGFR-mutated patients confirms that it may not be a useful biomarker for a therapeutic decision on ICI treatment in this molecularly selected subgroup of patients [25]. Notwithstanding, similarly to other authors, we found that TMB levels increased at the time of acquired resistance to afatinib in our patient [21]. Therefore,

although current data suggest that TMB is devoid of predictive significance in EGFR-mutated NSCLC, we still believe that its reassessment in conjunction with PD-L1 measurement in the post-EGFR-TKI setting could help clinical decision making.

Importantly, several other mechanisms than those previously mentioned could be put forward in order to explain the poor sensitivity of EGFR-mutated tumors to immunotherapy. Among others, high levels of the CD73 enzyme, which fosters the immunosuppressive adenosine pathway, as well as the upregulation of genes that belong to the CD73/adenosine pathway can be present in EGFR-mutated tumors [27, 28]. Likewise, CD39, another protein involved in the metabolism of tumor-specific CD8+ T cells, is generally found at lower levels in EGFR-mutated tumors [29, 30]. However, the presence of an immune desert tumor microenvironment in NSCLCs with EGFR mutations is probably the most common finding. In a study, it was demonstrated that EGFR-mutated tumors are characterized by the absence of T-cell infiltration and the presence of a poorly immunogenic tumor microenvironment with a higher proportion of PD-L1+ / CD8+ TILs compared to the EGFR wild-type genotype [31]. By contrast, CD8+ TILs were found to be consistently high in our case, with both a peripheral and intratumoral distribution, which, added to a high expression of PD-L1, suggest that the tumor should be classified as inflamed, resulting in a neoplasm that is more likely responsive to ICI therapies [32]. On the other hand, Tregs are regarded as the important suppressive immune cells in the tumor microenvironment, contributing to tumor progression and clinical failure of treatment with ICI [33]. By contrast, low levels of FOXP3+ Tregs could predict a benefit from immunotherapy. Our patient showed the presence of a poor FOXP3+ TIL density, which, coupled with the notion of high peripheral and intratumoral CD8+ cell infiltration, contributed to the decision to propose ICI therapy to this patient at the time of progression on afatinib.

While it is relatively common following treatment with an EGFR-TKI in EGFR-mutated

NSCLCs with brain metastases, the brain response to systemic therapy such as ICI treatment may be impaired [33, 34]. Nevertheless, our group has previously reported an intracranial response of 55.5% in 9 patients with asymptomatic brain metastases and a PD-L1 score of ≥ 50% who were treated with upfront pembrolizumab in the absence of brain radiotherapy [35]. Therefore, even in the presence of asymptomatic brain metastases, ICI treatment should not be delayed, especially in the case of predictors of increased sensitivity, such as high PD-L1 expression.

CONCLUSIONS

In conclusion, we believe that proper patient selection for ICI therapy based on multiple immune biomarkers for a more personalized immunoscoring system could be warranted for *EGFR*-mutated NSCLCs with acquired resistance to an EGFR-TKI. Although barriers linked to cost as well as time necessary to return the test results may exist, it is the author's belief that immunoscoring based on multiple tests should be advocated in this clinical scenario. In the present case, we showed that a composite score based on an increase in TMB levels as well as elevated PD-L1 expression and simultaneous documentation of high CD8+/low FOXP3+ TIL density in the tumor microenvironment was able to identify an *EGFR*-mutated NSCLC patient with acquired resistance to an EGFR-TKI who experienced a complete and long-term response to ICI therapy.

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