Commentary

Optimizing Patient Selection to Maximize Drug Efficacy: the Expanding Role of Pharmacogenomics in the Clinical Development of Pembrolizumab for the Treatment of Non-small Cell Lung Cancer

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ABSTRACT

Pembrolizumab (MK-3475) is a potent and highly selective humanized monoclonal antibody of the immunoglobulin G4κ class directed against the immune checkpoint programmed cell death protein-1 (PD-1). Binding to PD-1 prevents its interaction with natural ligands and allows for the reactivation of the immune response against cancer cells. The list of approved indications of pembrolizumab is fast expanding, including its use as first-line treatment of metastatic non−small cell lung cancer (NSCLC), which is a complex and evolving disease. Pharmacogenomics significantly contributed to understanding this complexity, allowing for the identification of molecular biomarkers and novel pharmacologic targets. This approach has delivered more effective and less toxic drugs for advanced NSCLC. In our opinion, pharmacogenomics played a key role in the approval of pembrolizumab as frontline therapy for NSCLC with high expression of the PD-1 ligand, which occurs in ~30% of patients. Moreover, an analysis conducted on the ongoing clinical trials sponsored by the drug's patent holder shows that the characterization and validation of additional pharmacogenomic biomarkers of response has the potential to extend the frontline clinical use of pembrolizumab in NSCLC. (*Clin Ther*. 2019;41:982−991)

Keywords: non−small cell lung cancer, PD-1/PD-L1, pembrolizumab, pharmacogenomics, predictive gene signature, tumor mutational burden.

INTRODUCTION

Lung cancer is a prevalent disease with high mortality rate in both male and female subjects.[1] The most frequent histologic type is non−small cell lung cancer (NSCLC), accounting for ~85% of all lung tumors.[2] NSCLC is often diagnosed at an advanced stage and in patients aged ≥60 years. Until recently, therapeutic options for advanced disease were scarce, limited to platinum-based combination cytotoxic regimens with a progression-free survival of 4−6 months and an overall survival of 12−18 months.[3] Marginal improvements have been observed with the addition of bevacizumab, an antiangiogenic therapy, for patients with nonsquamous cell carcinoma.[4,5] Over the last decade, pharmacogenomics contributed to the identification of different tumor subtypes based on the expression of specific molecular and genomic biomarkers. These include activating mutations of the epidermal growth factor receptor (EGFR), rearrangements of the anaplastic lymphoma kinase (ALK), and the ROS proto-oncogene 1 receptor tyrosine kinase. The characterization of these molecular biomarkers guided the successful development of selective tyrosine kinase inhibitors for tumors harboring such oncogenic driver mutations.[6] Treatment with these targeted drugs is effective in >50% of patients, nearly doubling median

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progression-free survival.[6,7] However, despite these improvements, the disease eventually progresses after or during first-line platinum-based combination therapy or targeted agents.[8] Few therapeutic options are available after progression, thus underscoring the need for more effective frontline treatments.

In the last few years, immunotherapeutic agents, particularly those targeting the pathway driven by the immune checkpoint programmed cell death protein-1/ligand-1 (PD-1/PD-L1), have shown great promise in clinical trials and have been progressively adopted as second-line therapies for the management of advanced stage NSCLC.[8,9] First discovered in 1992, PD-1 is a receptor involved in classic programmed cell death.[10] The receptor expression is absent on resting T cells but readily induced upon T-cell activation.[11] Persistent T-cell stimulation, as it occurs during chronic viral infections and cancer, further increases the expression level of PD-1, leading to T-cell exhaustion, and inhibits their effector functions. Activation of the PD-1/PD-L1 pathway is a mechanism by which cancer cells can elude the immune system's surveillance.[12] PD-1 has 2 natural ligands: PD-L1 and PD-L2.[11] PD-L1 is constitutively expressed in various cells of the immune system and in nonhematopoietic cells, including cancer cells. In NSCLC, for example, PD-L1 is often overexpressed in response to the pro-inflammatory cytokine interferon gamma.[13] Conversely, the expression of PD-L2 is restricted to antigen-presenting cells and occasionally upregulated in tumor cells, including ~2% of melanoma cases.[11]

Pembrolizumab (MK-3475) is a potent and highly selective humanized monoclonal antibody of the immunoglobulin G4κ class directed against the PD-1 receptor. By binding to the PD-1 receptor, pembrolizumab prevents the interaction with its natural ligands, thus restoring an effective and durable antitumor immune response. Recently, pembrolizumab was approved as frontline treatment for advanced NSCLC tumors that express high levels of PD-L1, which occurs in ~30% of patients.[14] In our opinion, pharmacogenomics played a key role in the clinical development of pembrolizumab in helping to identify patients with a higher probability of response to this treatment as an initial therapeutic option. Moreover, we believe that the research on additional genomic biomarkers holds the potential to effectively extend the use of pembrolizumab as frontline treatment for advanced NSCLC. Evidence supporting this hypothesis is reported.

Clinical Development of Pembrolizumab for Advanced NSCLC: The Role of Pharmacogenomics

Pembrolizumab was first tested in humans in a large, international, multicohort, Phase I study (Table I) sponsored by the patent holder (Merck Sharp & Dohme Corp). The study, identified in the ClinicalTrials.gov database as KEYNOTE-001 (NCT01295827), was conducted in 6 parts. It was designed to investigate the safety and tolerability of pembrolizumab; efficacy in participants with melanoma; efficacy in participants with NSCLC; and efficacy in those patients whose tumors expressed PD-L1. In the initial dose expansion cohort, 30 patients with a histologically or cytologically confirmed diagnosis of melanoma or any type of carcinoma were enrolled. This cohort included 6 patients with NSCLC and 1 patient with squamous cell lung cancer. The dose of intravenous pembrolizumab was escalated to determine the maximum tolerated dose and recommended Phase II dose. It was shown that the drug was, in general, well tolerated. Treatment-related adverse events of grade 1 to 2 severity occurred in 70% of the patients. Among all treated patients, 30% discontinued therapy due to adverse events and 17% experienced immunologic-related adverse events. During dose escalation, no dose-limiting toxicities were observed, and no maximum tolerated dose was found. Per protocol, the predetermined maximum administered dose was 10 mg/kg every 2 weeks.[15] Even though efficacy was not a primary outcome, antitumor activity of pembrolizumab was evaluated by using Response Evaluation Criteria in Solid Tumors version 1.1. This analysis revealed 2 complete responses and 3 partial responses to treatment, with an overall response rate of 16.67%. In addition, 15 patients across all doses and schedules tested reported stable disease as their best response. These included patients with NSCLC. Interestingly, 14 biopsy results were used to test the expression level of PD-L1 and correlate this finding with the observed clinical response. PD-L1—expressing tumors were considered

Table I. Clinical development of pembrolizumab in Japan (additional trials).

Merck Sharp & Dohme Corp— Sponsored Trial	ClinicalTrials.gov Identifier	Phase	Dose/Schedule	PD-L1 Status (TPS)	Primary Outcomes	Reference
KEYNOTE-011 (trial active, not recruiting)	NCT01840579	I	2 mg/kg every 2 wk 10 mg/kg every 2 wk 200 mg every 3 wk	Not required	Safety and tolerability in Japanese patients	28
KEYNOTE-025* (trial completed)	NCT02007070	Ib	10 mg/kg every 3 wk	≥1% of tumor cells (determined by the clinical trial IHC assay, using the Merck 22C3 antibody)	Safety and tolerability; ORR per RECIST version 1.1 in PD-L1 strongly positive patients	29

IHC = immunohistochemistry; ORR = overall response rate; PD-L1 = programmed cell death ligand-1; RECIST v1.1 = Response Evaluation Criteria in Solid Tumors version 1.1; TPS = tumor proportion score.

*The KEYNOTE-025 study enrolled 38 patients, 5 of whom completed the 2-year treatment. Among the secondary outcomes, it included the evaluation of PD-L1 expression as biomarker of response. Twelve patients (31.6%) had tumors strongly positive for PD-L1 expression (TPS ≥50%), 11 of which were evaluated for the primary outcome of efficacy showing an ORR of 27.3 % (95% CI, 6.0—61.0). All patients experienced treatment-related adverse events, but only 10.5% discontinued the drug due to adverse events.

those with >5% of PD-L1–positive cells. According to this criterion, 2 tumors were considered PD-L1 positive, and these patients, both diagnosed with advanced melanoma, showed partial response to the drug. Among the 12 PD-L1–negative patients, 6 had stable disease and 6 progressed. Regrettably, the 2 patients with a complete response did not have evaluable biopsy specimens. Nevertheless, this might be considered the first experimental evidence that the level of expression of PD-L1 is a predictor of likelihood of response to pembrolizumab.[15]

The next step was testing the safety and efficacy of pembrolizumab in advanced stage NSCLC. This analysis was performed in conjunction with the validation of PD-L1 as a genomic biomarker of response. Subsequently, the trial enrolled patients with PD-L1–positive locally advanced or metastatic NSCLC; that is, tumors with PD-L1 expression >1%.[16] In parallel, Merck actively developed a pharmacogenetic test to be used in this trial and during the subsequent phases of drug development to screen patients with higher probability of response. For this part of the study, a prototype test was initially used for patient enrollment. PD-L1 expression was detected by using immunohistochemistry, using a mouse anti-human PD-L1 immunoglobulin G1κ antibody (clone 22C3) generated at Merck Research Laboratories (Kenilworth, New Jersey).[17] NSCLC tumor biopsy samples were considered PD-L1 positive whenever a membranous staining was found in at least 1% of cells, including both neoplastic and intercalated inflammatory cells. As an alternative, PD-L1–positive tumors were considered as those with a distinctive banding pattern adjacent to tumor nests caused by infiltration of mononuclear inflammatory cells in the stroma. With this test, 495 patients were enrolled to receive pembrolizumab, at a dose of either 2 mg/kg every 3 weeks or 10 mg/kg every 2 weeks.[16]

Results from this study largely confirmed the safety profile of pembrolizumab, with treatment-related adverse events observed in 70.9% of patients independently from the dose or the schedule adopted. The most common adverse events were fatigue, pruritus, and decreased appetite. In addition, immunologic-related adverse events observed in >2% of patients were infusion-related reactions, hypothyroidism, and pneumonitis. The overall response rate was 19.4% (95% CI, 16.0–23.2), with stable disease observed in 21.8% of patients as best overall response. Interestingly, the overall response rate was 24.8% (95% CI, 16.7–34.3) in 101 previously untreated patients. After an initial observation of a correlation between PD-L1 expression and the efficacy of pembrolizumab, the protocol was amended to include the evaluation of drug efficacy in patients with previously treated NSCLC with tumors expressing high levels of PD-L1. In parallel, a process was implemented to standardize the immunohistochemistry assay for PD-L1 assessment.[18] The prototype test was automated on the Autostainer Link 48 platform (Dako, an Agilent Technologies Company, Santa Clara, California). Further effort was devoted to standardization of the scoring method and selection of the appropriate cut-off tumor proportion score (TPS) that would best predict response to pembrolizumab. Comparison of scoring methods by using receiver-operating characteristic analysis using both the prototype and automated assays led to standardized conditions for the clinical trial assay. Thus, the assessment of PD-L1 expression would be performed by including exclusively tumor cells with partial or complete membrane staining, using biopsy specimens with ≥100 viable tumor cells. The interface pattern due to infiltrating inflammatory cells was excluded because it increased the false-positive rate.

In these conditions, the cut-off that best predicted the response to pembrolizumab was found to be a TPS ≥50%.[18] Indeed, reanalyzing the data and considering only 73 patients with a TPS ≥50%, the overall response rate increased to 45.2% (95% CI, 33.5–57.3).[16] Median progression-free survival among patients with a TPS ≥50% was 6.3 months (95% CI, 2.9–12.5), whereas median overall survival was not reached in the total population, but it was significantly higher than the overall survival observed in patients with lower TPS. Based on these results, on October 2, 2015, the US Food and Drug Administration (FDA) approved pembrolizumab for the treatment of patients with metastatic NSCLC and tumors expressing PD-L1 with TPS ≥50%, as determined according to an FDA-approved test. The drug was approved as second-line treatment (after disease progression on or after a platinum-containing chemotherapy). On the same day, the FDA approved the Dako immunohistochemistry 22C3 pharmDx test (Dako, an Agilent Technologies Company) as the companion diagnostic for

pembrolizumab in advanced NSCLC. On August 15, 2016, pembrolizumab was approved by the European Medicines Agency for the same indication. Further studies to test safety and efficacy of pembrolizumab in Japanese ethnicity (Table I) resulted in the drug's approval by the Pharmaceuticals and Medical Devices Agency in Japan on December 19, 2016.

Interestingly, from the previous analysis it emerged that the overall response rate was 50.0% (95% CI, 24.7−75.3) in a subgroup of patients with a TPS ≥50% in which pembrolizumab was administered as first-line treatment.[16] Median progression-free survival was 12.5 months (95% CI, 2.4−12.5) in these patients[16], thus suggesting a potential use of pembrolizumab as frontline therapy. This hypothesis was tested in the KEYNOTE-025 (NCT02142738) clinical trial. This Phase III, open-label, randomized clinical trial enrolled 305 participants with histologically or cytologically confirmed stage IV NSCLC without sensitizing EGFR mutations or ALK translocations and a PD-L1 TPS ≥50%. In this study, pembrolizumab was tested at a fixed dose of 200 mg every 3 weeks and compared with cytotoxic chemotherapy, at investigators' choice among the approved frontline platinum-based combination regimens. The primary outcome was progression-free survival, as assessed by using Response Evaluation Criteria in Solid Tumors version 1.1. The study produced remarkable results. In the intention-to-treat population, on the basis of 189 total events of progression or death, median progression-free survival was 10.3 months (95% CI, 6.7 to not reached) in the

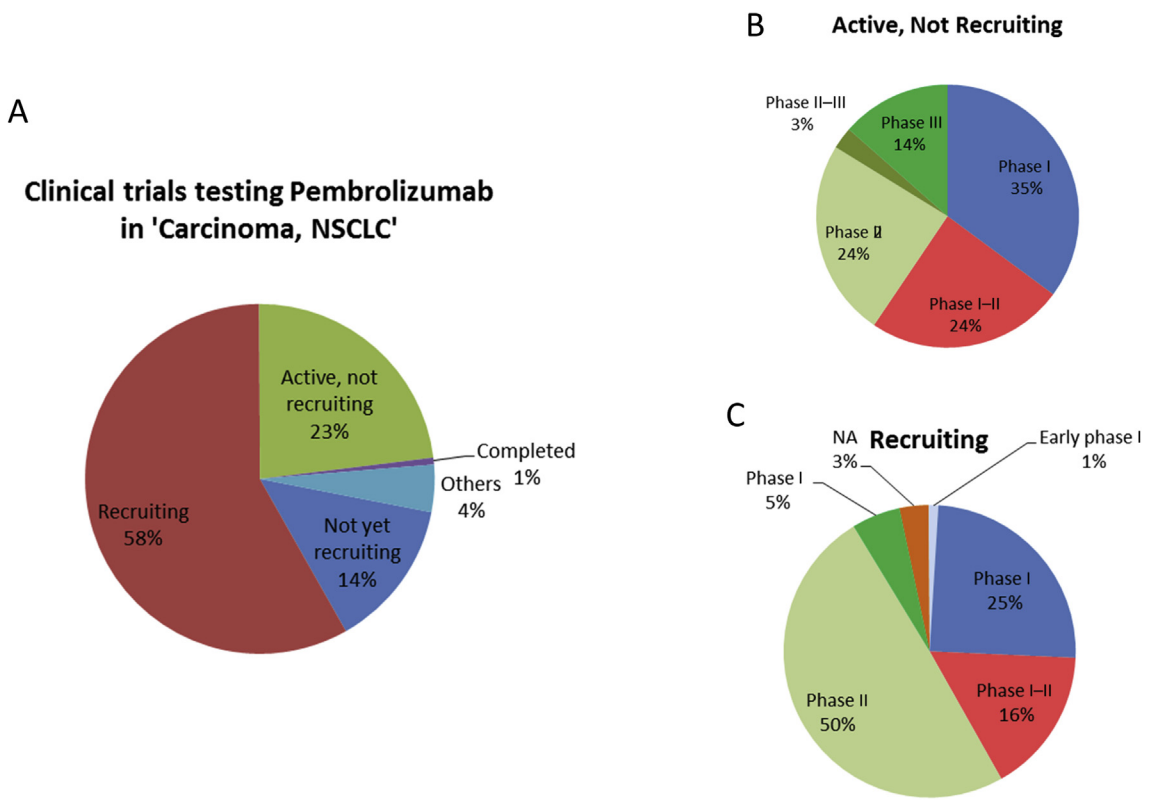

Figure 1. Clinical trials registered in the ClinicalTrial.gov database on pembrolizumab in non−small cell lung cancer (NSCLC) (A) Studies are divided according to status. (B) Studies active, not recruiting are reported, divided according to clinical phases of development. (C) Studies recruiting are reported, divided according to clinical phases of development. NA = not applicable.

pembrolizumab group and 6.0 months (95% CI, 4.2−6.2) in the chemotherapy group, with a 50% reduction of the risk of progression or death in the group treated with pembrolizumab. In addition, the overall survival was significantly higher in the pembrolizumab group than in the chemotherapy group. Finally, pembrolizumab was better tolerated than cytotoxic chemotherapy.[19]

These results are in sharp contrast with negative findings reported using nivolumab, a different PD-1 checkpoint inhibitor, as a frontline treatment for advanced NSCLC.[20] The CheckMate 026 (NCT02041533) trial, funded by Bristol-Myers Squibb and others, enrolled a broader population and thus likely included poor responders. In addition, the detection of PD-1 expression for patient enrollment was based on a different immunohistochemistry assay, using a rabbit monoclonal anti-human PD-L1 antibody (clone 28-8) on the same Dako platform.[21] Finally, the subgroup analysis showed similar efficacy of nivolumab and chemotherapy in patients with PD-L1 expression >50%. Therefore, it is possible that this cut-off point that was accurately selected and validated to identify responders to pembrolizumab is not predictive of response to nivolumab.[22] Taken together, this evidence further underscores the relevance of a validated pharmacogenomic biomarker and a robust companion diagnostic test for the successful development of more effective drugs in oncology.

Based on the results of the KEYNOTE-024 clinical trial, the FDA label was changed on October 24, 2016, and pembrolizumab was approved as frontline treatment for patients with metastatic NSCLC whose tumors have high PD-L1 expression (TPS ≥50%), as determined by an FDA-approved test. Meanwhile, the KEYNOTE-010 (NCT01905657) study, a Phase II/III randomized trial, confirmed the superiority of pembrolizumab versus docetaxel as second-line

Table II. Clinical trials registered in the ClinicalTrials.gov database, testing the drug pembrolizumab in "Carcinoma, NSCLC." The database was accessed on November 8, 2018. All studies in the status "Active, Not Recruiting," and "Recruiting" are reported and divided according to phases of clinical development. Studies sponsored by Merck Sharp & Dohme Corp are reported in different columns.

Phase	Active, Not Recruiting (ANT)	Merck Sponsored (ANT)	Merck Study Code (ANT)	Recruiting (R)	Merck Sponsored (R)	Merck Study Code (R)
NA	—	—	—	3	—	
Early	—	—	—	1		
I	13	3/13 (23.07%)	KEYNOTE-001 KEYNOTE-011 KEYNOTE-032	23	1/23 (4.3%)	KEYNOTE-434
I/II	9	1/9 (11.11%)	KEYNOTE-021	15	—	
II	9	—	—	46	4/46 (8.6%)	KEYNOTE-034 KEYNOTE-495 KEYNOTE-782 KEYNOTE-799
II/III	1	1/1 (100%)	KEYNOTE-010	—		
III	5	5/5 (100%)	KEYNOTE-024 KEYNOTE-033 KEYNOTE-042 KEYNOTE-189 KEYNOTE-407	5	4/5 (80%)	KEYNOTE-598 KEYNOTE-671 KEYNOTE-789 KEYNOTE-091
Total	37	10/37 (27.02%)		93	9 (9.68%)	

ANT = active, not recruiting; NA= not applicable; R= recruiting.

Table III. Pembrolizumab as first-line monotherapy or in combination with currently approved first-line therapies for advanced non–small cell lung cancer (NSCLC).

Merck-Sponsored Trial	ClinicalTrials.gov Identifier	Phase	Pembrolizumab	PD-L1 Status (TPS)	Primary Outcomes	Comments
KEYNOTE-042 (trial active, not recruiting)	NCT02220894	III	Monotherapy	PD-L1 TPS > 1%	OS (time frame: up to 2.5 y)	This study aims to explore efficacy in patients with PD-L1 TPS 1%–50%
KEYNOTE-021 (trial active, not recruiting)	NCT02039674	I/II	In combination with: chemotherapy; EGFR TKIs erlotinib or gefitinib; or ipilimumab	Not required	ORR (time frame: up to 2 y) Safety and tolerability	These 3 studies aim to extend the use of pembrolizumab in first-line therapy, in association with currently approved treatments. Increased efficacy is expected. For these participants, treatment with pembrolizumab as monotherapy (PD-L1 TPS <50%; or PD-L1–negative tumors [ie, EGFR or ALK mutated]).
KEYNOTE-189* (trial active, not recruiting)	NCT02578680	III	In combination with: pemetrexed/ platinum chemotherapy	Not required	PFS and OS (time frame: up to ~21 mo)	
KEYNOTE-407† (trial active, not recruiting)	NCT02775435	III	In combination with: carboplatin-paclitaxel/nab-paclitaxel chemotherapy	Not required	PFS and OS (time frame: up to ~2 y)	

ALK = anaplastic lymphoma kinase; EGFR = epidermal growth factor receptor; ORR = overall response rate; OS = overall survival; PD-L1 = programmed cell death ligand-1; PSF = progression-free survival; TKI = tyrosine kinase inhibitor; TPS = tumor proportion score.

* Participants with metastatic nonsquamous NSCLC. The study reported significantly longer OS and PFS in patients treated with pembrolizumab in combination with standard chemotherapy.[30] On May 10, 2017, pembrolizumab gained US Food and Drug Administration approval as first-line treatment of patients with metastatic nonsquamous NSCLC in combination with pemetrexed and carboplatin. A similar extension of indication was approved by the European Medicines Agency on July 26, 2018. Approval was based on the efficacy and safety data from KEYNOTE-189, supported by data from KEYNOTE-021 cohorts C and G.

† Participants with metastatic squamous NSCLC.

treatment in patients with advanced NSCLC after disease progression.[23] Participants had PD-L1–positive tumors with TPS ≥1%. Therefore, on October 24, 2016, the FDA label was changed accordingly, and pembrolizumab was indicated as second-line treatment for patients with metastatic NSCLC expressing PD-L1 with TPS ≥1% as determined by an FDA-approved test. Thus, disease progression should be diagnosed on or after platinum-containing chemotherapy. Patients with EGFR or ALK genomic tumor aberrations should progress on FDA-approved targeted therapy before receiving pembrolizumab. Similar changes to the terms of the marketing authorization were approved by the European Medicines Agency on December 15, 2016.

Future Directions in Pembrolizumab Clinical Development for NSCLC: Novel Pharmacogenetic Biomarkers of Response

The clinical development of pembrolizumab in NSCLC is ongoing. A search on the ClinicalTrials.gov database (accessed on November 1, 2018) using "pembrolizumab" as a key word retrieved 894 registered clinical trials. Among these, 191 were registered on the topic "lung neoplasm," including 160 on the topic "Carcinoma, NSCLC." The majority of these studies (93 of 160) are currently recruiting, 37 are active but not recruiting, and only the aforementioned KEYNOTE-025 study was completed (Figure 1). Among the active but not recruiting studies, 59% are Phase I (13 of 37) and Phase I/II (9 of 37) studies. In addition, 5 are Phase III clinical trials, all sponsored by Merck (Table II). A total of 4 active studies continue to evaluate the efficacy of pembrolizumab as first-line therapy, alone or in combination with other approved frontline therapies (Table III). Among studies that are currently recruiting, there is still a considerable number of Phase I (23 of 93) and Phase I/II (15 of 93) studies, which account for 41% of all recruiting studies. Approximately 50% of these studies are Phase II, whereas only 5 are Phase III clinical trials. With the exception of 1 trial, all the Phase III clinical trials are sponsored by Merck.

To identify the future implications of pharmacogenomics in the clinical development of pembrolizumab for the treatment of NSCLC, we analyzed in more detail studies sponsored by Merck that are currently recruiting. Briefly, 9 studies were identified (Table II). Among these, two Phase II trials are designed to characterize additional genomic biomarkers of response to pembrolizumab. In both studies, pembrolizumab will be added to standard first-line chemotherapy, which means that the use of pembrolizumab will be extended to patients with low or absent PD-L1 expression. The KEYNOTE-782 (NCT03664024) trial will test as its primary hypothesis whether total baseline tumor mutational burden (TMB) in circulating free DNA is predictive of objective response to pembrolizumab. This hypothesis is based on evidence that tumors with high mutational burden express more neo-antigens and are more immunogenic, and thus are more likely to respond to immunotherapy.[24] This was apparent in the CheckMate 026 clinical trial, in which patients with high TMB (>243 mutations) were identified as the only group more responsive to nivolumab than to chemotherapy.[20] This accounted for 58% of enrolled patients, thus suggesting that stratification of patients based on this biomarker can effectively extend the use of immune checkpoint inhibitors as a frontline therapeutic option. The KEYNOTE-782 study is also looking at the validation of the assessment of TMB in liquid biopsy specimens, implying the characterization of a specific pharmacogenomic test, together with the selection of a cut-off point best predictive of response.[25]

Taken together, both PD-L1 TPS and TMB are pharmacogenomic biomarkers of response based on tumor biology. However, a crucial determinant of the clinical outcome of immunotherapy is the patient's immune system and, more importantly, the interactions between tumor-infiltrating lymphocytes and cancer cells within the tumor microenvironment. In this scenario, a retrospective analysis conducted on melanoma samples from the KEYNOTE-001 study showed that a genomic signature associated with interferon gamma signaling and other parameters of T-cell biology (including cytolytic activity, antigen presentation, and chemokine production) can be highly indicative of a PD-1 checkpoint inhibition–responsive immune microenvironment in melanoma.[26] This observation, together with a further refinement of the gene expression profile (GEP) signature, was confirmed in other tumors using biopsy specimens and clinical data available from pembrolizumab-treated patients enrolled in the

KEYNOTE-012 (NCT01848834) study. Moreover, a final set of 18 genes in the T-cell inflamed GEP was identified as a pan-tumor predictor marker of pembrolizumab response across 9 tumor types (KEYNOTE-028 [NCT02054806]). Interestingly, a prospective validation of this genomic biomarker in NSCLC will be performed in the KEYNOTE-495 (NCT03516981) clinical trial. In this study, participants will be stratified according to a pan-tumor biomarker-based classifier (including high and low GEP; and high and low TMB).[27] Patients will therefore be divided into 4 groups based on the tumor expression level of these markers. Accordingly, the first group will include tumors with low GEP and low TMB, the second group will include tumors with low GEP and high TMB, the third group will include tumors with high GEP and low TMB, and the fourth group will include tumors with high GEP and high TMB. A higher overall response rate (>45%) was expected in the group of patients with higher expression of both GEP and TMB. A lower response rate (estimated >20%) is expected in patients with only one of these two biomarkers highly expressed, whereas a response >5% is expected among participants with low GEP and low TMB. These data suggest that a broader understanding of tumor biology, including the tumor microenvironment as well, may help to identify the best candidates for immunotherapy. In this context, the successful clinical development of pembrolizumab seems strictly linked to the validation of additional pharmacogenomic biomarkers.

CONCLUSIONS

NSCLC is a complex and evolving disease. A significant part of variability derives from the interactions between cancer cells and the patient's immune system, leading to different clinical outcomes even in the presence of similar initial conditions. This is far truer when it comes to response to immune checkpoint inhibitors such as pembrolizumab. We thus envision that PD-L1 expression, which successfully led to the approval of pembrolizumab as frontline therapy for advanced NSCLC, may not be the only biomarker of response. We expect that a more comprehensive analysis of tumor biology (TMB) and inflammatory microenvironment (GEP of tumor-infiltrating T-lymphocytes) will allow the selection of the best responders to pembrolizumab therapy with higher precision.

CONFLICT OF INTEREST

The authors have indicated that they have no conflicts of interest regarding the content of this article.

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Dr Dello Russo and Professor Navarra conceived the article. Dr Dello Russo performed the review of the literature on pembrolizumab's clinical development in NSCLC, analyzed the data collected by the ClinicalTrials.gov database, and wrote the primary draft. Drs Gagliardi and Ramlogan planned the strategy of data analysis, collected the data, and performed preliminary analysis. Professor Navarra contributed to the literature review and to the study of relevant clinical trials. Drs Gagliardi and Ramlogan, and Professor Navarra contributed to manuscript editing. All authors read and approved the final manuscript.

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