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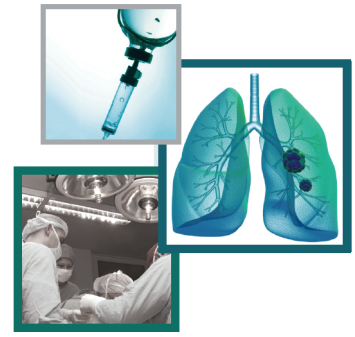
Lung Cancer Management



Choosing the best first-line therapy: NSCLC with no actionable oncogenic driver

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Combination platinum-based therapy has been the standard of care for the treatment of advanced non-small-cell lung cancer (NSCLC). Immunotherapy has emerged and demonstrated to show benefit in the treatment of patients with advanced NSCLC. In this review, we discuss the pivotal trials that led to the US FDA approval of specific immunotherapy regimens in particular patient populations. We discuss the optimal use of immunotherapy as monotherapy based on the KEYNOTE-024, KEYNOTE-042 and IMpower110 trials, chemo-immunotherapy based on KEYNOTE-189, KEYNOTE-407, IMpower150 and IMpower130 trials, and as doublet immunotherapy based on CheckMate-227. We also discuss the role and limitations of PD-L1 expression and tumor mutational burden as predictive biomarkers in response to single-agent immunotherapy and combination chemoimmunotherapy. Furthermore, we discuss emerging resistance markers such as *STK11* and *KEAP1* mutations in immunotherapy response and briefly discuss the role of immunotherapy in elderly patients and in patients with actionable mutations.

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Background

Historically, chemotherapy has long been the standard of care for the treatment of advanced/metastatic lung cancer. Compared with patients who receive the best supportive care, patients receiving platinum-based chemotherapy have an improved 1-year survival rate from 20 to 29% (hazard ratio [HR]: 0.77, 95% CI: 0.71–0.83) [1]. The choice of platinum-based doublet has generally been influenced by the histologic subtype. For example, in a randomized study of advanced non-small-cell lung cancer (NSCLC), in patients with adenocarcinoma and large cell carcinoma histology cisplatin/pemetrexed showed favorable overall survival compared with cisplatin/gemcitabine while for squamous cell carcinoma, cisplatin/gemcitabine demonstrated improved overall survival, speculated to be related to differential expression of thymidylate synthase- the target of pemetrexed [2]. The addition of EGFR antibody, necitumumab to cisplatin/gemcitabine, especially in tumors expressing high EGFR expression demonstrated enhanced benefit with significantly longer overall survival compared with cisplatin/gemicitabine alone (11.5 vs 9.9 months, HR: 0.84, 95% CI: 0.74–0.96) in squamous cell patients [3]. For both squamous and nonsquamous NSCLC, nab-paclitaxel and carboplatin, which has been shown to have improved and comparable overall response rate, respectively, compared with solvent-based paclitaxel and carboplatin is also approved as first-line regimen for advanced NSCLC [4]. The use of bevacizumab, however, is restricted to nonsquamous tumors in light of excessive toxicity, namely severe, potentially fatal hemoptysis in this subset of patients. For the subgroup of patients with adenocarcinoma and large cell carcinoma, molecular testing is standard of care to determine treatment strategy since targeted therapy against *EGFR, ALK* and *ROS* is widely accepted to be superior to conventional chemotherapy in patients with *EGFR*/*ALK*/*ROS*-aberrant tumors [5]. In addition, recently novel highly actionable additional targets have also been recognized, such as *BRAF* mutations, principally *V600E*, *NTRK* and *RET* translocations, *MET* exon 14 skipping and *ERBB2* mutations [6,7]. Last, excitement surrounds the development of *K-Ras* targeting agents, namely *K-Ras G12C* inhibitors – as *K-Ras* mutations are the most common oncogenic drivers in lung adenocarcinoma, and the development of such drugs is anticipated to be a major advancement [8]. While the proper sequencing is not clearly yet defined for these additional targets, it is pivotal that tissue and/or circulating tumor

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Table 1. Single-agent immunotherapy in advanced non-small-cell lung cancer patients in frontline setting.

Trial	Patient (n)	PD-L1 IHC	Agent	Primary end point (months)	Outcome by PD-L1 (months or HR)	Outcome by TMB (months)	US FDA approval
Single IO							
KEYNOTE-024 (NCT02142738)	n = 305	≥50%, by Dako 22C3	Pembrolizumab vs platinum-doublet	PFS: 10.3 (95% CI: 6.7, not reached) v. 6.0 (95% CI: 4.2–6.2)	NA	NA	October 2016
KEYNOTE-042 (NCT02220894)	n = 1274	≥1% by 22C3 by Agilent	Pembrolizumab vs platinum-doublet	OS: ≥50%: 20 vs 12.2 ≥20%: 17.7 vs 13 ≥1%: 16.7 vs 12.1	OS: ≥50%: HR: 0.69 (95% CI: 0.56–0.85); ≥20%: HR: 0.77 (95% CI: 0.64–0.92); ≥1%: HR: 0.81 (95% CI: 0.71–0.93)	OS: tTMB ≥175: 21.9 (95% CI: 17.0–26.7) vs 11.6 (95% CI: 9.9–14.2) tTMB <175: 12 (95% CI: 9.2–14.8) vs 12.3 (95% CI: 11.3–16.2)	April 2019
Checkmate-026 (NCT02041533)	n = 423	PD-L1 ≥5% 28–8 antibody by Dako	Nivolumab vs platinum-based chemotherapy	PFS: 4.2 vs 5.9 (HR: 1.15, 95% CI: 0.91–1.45; p = 0.25)	PFS: ≥50%: HR: 1.07 (95% CI: 0.77–1.49)	PFS: TMB >243: HR: 0.62 (95% CI: 0.38–1) TMB <243: HR: 1.82 (95% CI: 1.3–2.55)	Currently under US FDA review
IMpower-110 (NCT02409342)	n = 572	PD-L1 ≥1% by SP142 by Ventana	Atezolizumab vs platinum-based chemotherapy	OS: TC3 or IC3: 20.2 vs 13.1 TC2/3 or IC2/3: 18.2 vs 14.9 TC1/2/3 or IC1/2/3: 17.5 vs 14.1	OS: TC3 or IC3: HR: 0.60 (95% CI: 0.40–0.89) TC2/3 or IC2/3: HR: 0.72 (95% CI: 0.52–0.99) TC1/2/3 or IC1/2/3: HR: 0.83 (95% CI: 0.65–1.07)	NA	Currently under FDA review

95% CI – TC3 or IC3: PD-L1 ≥50% or ≥10% tumor-infiltrating immune cell; TC2/3 or IC 2/3: PD-L1 ≥5% on tumor cell or tumor-infiltrating immune cell; TC1/2/3 or IC 1/2/3: PD-L1 expression ≥1% on tumor cell or tumor-infiltrating immune cell.
HR: Hazard ratio; IO: Immunotherapy; NA: Not available; OS: Overall survival; PFS: Progression-free survival; TMB: Tumor mutational burden.

DNA (ctDNA) based approaches are pursued to identify the group of patients who should be able to benefit from targeted therapy along the treatment continuum.

No actionable oncogenic driver

Recent research has drastically shaped the understanding of cancer biology and immunology with the recognition of active immune response being present and actively evaded by tumor cells in a large proportion of lung tumors. The development of checkpoint inhibitors of the first CTLA4 checkpoint engaged in lymph nodes and even more importantly, anti-PD1/PD-L1 inhibitors blocking the second checkpoint actively engaged within the tumor microenvironment rapidly re-shaped our treatment paradigms and improved patient outcomes. With the advent of immunotherapy, the landscape of advanced lung cancer treatment for patients without driver mutations has shifted from traditional doublet chemotherapy to immunotherapy-based treatments with and without chemotherapy.

Single-agent immunotherapy

The first pivotal trial that led to the FDA approval of the PD-1 inhibitor, pembrolizumab, as front-line therapy for advanced lung cancer patients was the KEYNOTE-024 trial. In this Phase III trial, 305 patients with stage IV treatment-naive NSCLC of all histology (*EGFR*/*ALK* negative), with at least 50% PD-L1 expression were randomized to pembrolizumab or platinum doublet therapy (Table 1). Patients who received pembrolizumab monotherapy had a superior progression-free survival (PFS; median PFS: 10.3 months, 95% CI: 6.7, not reached vs 6.0 months, 95% CI: 4.2–6.2) and at 2-year follow-up, improved overall survival (OS; median OS: 30.0 months, 95% CI: 18.3, not reached vs 14.2 months, 95% CI: 9.8–19.0 months) compared with patients who received platinum doublet therapy [9,10]. The FDA approval of pembrolizumab as monotherapy was extended to patients with PD-L1 tumor proportion score (TPS) ≥1% based on KEYNOTE-042, a multicenter trial of 1274 patients randomized to pembrolizumab or doublet chemotherapy, stratified by TPS ≥50, ≥20 and ≥1% [11]. The trial demonstrated improved OS with pembrolizumab monotherapy in all three TPS groups, but with the greatest OS observed in subgroup of patients with TPS ≥50% [11]. The use of single-agent immunotherapy in the less

than <50% TPS score subset remains controversial as a specific look at the TPS score 1–49% versus 50%+ subsets suggests that greatest benefit is noted in the group of high PD-L1 expressors and best might be preserved for patients who are not good candidates for chemotherapy [11]. Most recently, atezolizumab, a PD-L1 inhibitor, was also shown in an interim analysis to have superior OS when used as a monotherapy compared with doublet chemotherapy in the Phase III IMpower110 trial in 572 patients, in the highest PD-L1 expression subgroup defined by the TC/IC score (where TC = tumor cells and IC = tumor-infiltrating immune cells; cut-offs used TC3 ≥50% or IC3 ≥10%; median OS 20.2 months in atezolizumab monotherapy vs 13.1 months in chemotherapy, HR: 0.595, 95% CI: 0.398–0.890) [12]. Atezolizumab is currently under review for use as monotherapy for metastatic treatment-naive NSCLC patients.

Though KEYNOTE-024, KEYNOTE-42 and IMpower-110 showed benefit of immunotherapy as single-agent, Checkmate-026, a Phase III trial comparing single-agent nivolumab once every 2 weeks with platinum chemotherapy once every 3 weeks for up to six cycles in untreated stage IV or recurrent NSCLC patients with PD-L1 ≥5%, did not demonstrate favorable overall survival of nivolumab monotherapy: median OS 14.4 months with nivolumab versus 13.2 months with chemotherapy, HR: 1.02, 95% CI: 0.8–1.3) [13]. Of note, as cross-over was allowed, 60% of patients in the chemotherapy group received nivolumab as subsequent therapy after disease progression and also had slightly more favorable baseline characteristics, including fewer patients with metastasis, smaller tumor burden and increased female proportion, and thus, the lack of overall survival benefit of nivolumab may have been the result of significant cross-over and differences in baseline characteristics [13].

Chemoimmunotherapy

The benefit of pembrolizumab used in the frontline setting in combination with chemotherapy versus chemotherapy alone has also been demonstrated (Table 2). In the Phase III KEYNOTE-189 trial, 616 patients with nonsquamous untreated advanced NSCLC without *EGFR* or *ALK* mutations were randomized 2:1 to pemetrexed and platinum therapy along with pembrolizumab or placebo every 3 weeks for four cycles followed by pembrolizumab or placebo up to 35 cycles and pemetrexed maintenance therapy, with crossover allowed from placebo to pembrolizumab in the control group for patients who had disease progression [14]. Improved overall survival with chemoimmunotherapy was demonstrated compared with the chemotherapy-placebo group: median OS was not reached in pembrolizumab-chemotherapy group versus 11.3 months, (95% CI: 8.7–15) in chemotherapy-placebo group [14]. In contrast to the KEYNOTE-042 trial, superior OS was observed not only in TPS ≥50%, but also in TPS score 1–49% and TPS-negative subgroups which suggests synergy of chemoimmunotherapy compared with immunotherapy alone, highlighting the preferential treatment of chemoimmunotherapy in patients with low TPS scores.

The KEYNOTE-407 trial also demonstrated improved overall survival of chemoimmunotherapy compared with chemotherapy in patients with squamous histology in the frontline setting [15]. 559 patients with untreated metastatic squamous cell NSCLC were randomized 1:1 to carboplatin and paclitaxel or nano-particle albumin-bound [nab]-paclitaxel for four cycles and either pembrolizumab or placebo irrespective of TPS score for up to 35 cycles and it was demonstrated that regardless of TPS score or use of paclitaxel versus nab-paclitaxel, patients who received taxane/platinum/pembrolizumab had superior overall survival than patients who received taxane-platinum-placebo: median OS 15.9 months, (95% CI: 13.2, not reached) versus 11.3 months, (95% CI: 9.5–14.8) [15]. Furthermore, the benefit of the addition of the PD-L1 inhibitor, atezolizumab to frontline triple therapy with bevacizumab, carboplatin, plus paclitaxel (ABCP) versus bevacizumab, carboplatin, plus paclitaxel (BCP) was observed in patients with metastatic nonsquamous NSCLC in the IMpower150 trial (median OS 19.2 months with ABCP vs 14.7 months with BCP, HR: 0.78, 95% CI: 0.64–0.96; p = 0.02) [16]. Subgroup analyses have shown improved survival in patients with baseline liver metastases and potential benefits for the combination in the patient subset with sensitizing EGFR mutations as well [16].

Based on the positive results of KEYNOTE-189 and KEYNOTE-407, pembrolizumab is FDA approved for use in first-line metastatic EGFR/ALK wild-type nonsquamous NSCLC with pemetrexed and carboplatin or cisplatin and in first-line metastatic squamous NSCLC with carboplatin and paclitaxel or nab-paclitaxel. The FDA has also approved atezolizumab and its use with bevacizumab, carboplatin and paclitaxel, for first-line treatment in metastatic nonsquamous NSCLC patients based on the IMpower150 trial, however, the approval is limited to patients with wild-type EGFR and ALK though the trial had suggested intriguing benefits with the ABCP regimen in patients with sensitizing EGFR mutations [17]. Most recently, the FDA has also approved the use of atezolizumab with carboplatin and nab-paclitaxel in nonsquamous NSCLC based on IMpower-130. 724 patients were randomized 2:1 to atezolizumab, nab-paclitaxel and carboplatin followed by atezolizumab versus nab-paclitaxel and carboplatin

Table 2. Chemo-immunotherapy in advanced non-small-cell lung cancer patients in frontline setting.

Trial	Patient (n)	PD-L1 IHC	Agent	Primary end point (months)	Outcome by PD-L1 (months or HR)	Outcome by TMB (months)	US FDA approval
Chemo IO							
KEYNOTE-189 (NCT02578680)	n = 616, nonsquamous	PD-L1 by 22C3 by Agilent	Pemetrexed-platinum + pembrolizumab vs pemetrexed-platinum + placebo	OS: not reached vs 11.3 (95% CI: 8.7–15) PFS: 8.8 (95% CI: 7.6–9.2) vs 4.9 (95% CI: 4.7–5.5)	OS: <1%: HR: 0.59 (95% CI: 0.38–0.92) ≥1–49%: HR: 0.55 (95% CI: 0.34–0.90) ≥50%: HR: 0.42 (95% CI: 0.26–0.68)	OS: TMB ≥175: HR: 0.64 (95% CI: 0.38–1.07) TMB <175: 0.64 (95% CI: 0.42–0.97)	August 2018
KEYNOTE-407 (NCT02775435)	n = 559, squamous	PD-L1 by 22C3 by Agilent	Carboplatin + paclitaxel or nab-paclitaxel + pembrolizumab/ placebo	OS: 15.9 (95% CI: 13.2, not reached) vs 11.3 (95% CI: 9.5–14.8) PFS: 6.4 (95% CI: 6.2–8.3) vs 4.8 (95% CI: 4.3–5.7)	OS: <1%: HR: 0.61 (95% CI: 0.38–0.98) ≥1–49%: HR: 0.57 (95% CI: 0.36–0.9) ≥50%: HR: 0.64 (95% CI: 0.37–1.10)	OS: TMB ≥175: HR: 0.74 (95% CI: 0.5–1.08) TMB <175: HR: 0.86 (95%: 0.57–1.28)	October 2018
IMpower-150 (NCT02366143)	n = 1202 nonsquamous	PD-L1 by SP142 by Ventana	ACP vs BCP vs ABCP	PFS: 8.3 ABCP vs 6.8 BCP, HR: 0.62 (95% CI: 0.52–0.74) OS: 19.2 ABCP vs 14.7 BCP, HR: 0.78 (95% CI: 0.64–0.96)	PFS: TC3 or IC3: HR: 0.39 (95% CI: 0.25–0.60) TC 1/2/3 or IC 1/2/3: HR: 0.50 (95% CI: 0.39–0.64) TC0 and IC0: HR: 0.77 (95% CI: 0.61–0.99)	NA	December 2018
IMpower-130 (NCT02367781)	n = 724 nonsquamous	PD-L1 by VENTANA SP142 assay	Atezolizumab + carboplatin + nab-paclitaxel followed by atezolizumab vs carboplatin + nab-paclitaxel followed by pemetrexed	PFS: 7.0 vs 5.5, HR: 0.64 (95% CI: 0.54–0.77) OS: 18.6 vs 13.9, HR: 0.79 (95% CI: 0.64–0.98)	OS: PD-L1 high: 17.3 vs 16.9, HR: 0.84 (95% CI: 0.51–1.39) PD-L1 low: 23.7 vs 15.9, HR: 0.70 (95% CI: 0.45–1.08) PD-L1 neg: 15.2 vs 12.0, HR: 0.81 (95%: 0.61–1.08) PFS: PD-L1 high: 6.4 vs 4.6, HR: 0.51 (0.34–0.77) PD-L1 low: 8.3 vs 6.0, HR: 0.61 (0.43–0.85) PD-L1 neg: 6.2 vs 4.7, HR: 0.72 (0.56–0.91)	NA	December 2019

TC3 or IC3: PD-L1 ≥50% or ≥10% tumor-infiltrating immune cell; TC 1/2/3 or IC 1/2/3: PD-L1 expression ≥1% of tumor cell or tumor-infiltrating immune cell; TC0 and IC0: PD-L1 expression <1% of tumor cell and tumor-infiltrating immune cell.
ABCP: Atezolizumab/bevacizumab/carboplatin/paclitaxel; ACP: Aztezolizumab/carboplatin/paclitaxel; BCP: Bevacizumab/carboplatin/paclitaxel; HR: Hazard ratio; IO: Immunotherapy; NA: Not available; OS: Overall survival; PFS: Progression-free survival; TMB: Tumor mutational burden.

followed by pemetrexed and it was shown that the atezolizumab combination group had improved PFS (HR: 0.75, 95% CI: 0.63–0.91; p = 0.0024) and OS (HR: 0.80, 95% CI: 0.64–0.99; p = 0.0384) [18].

Combination immunotherapy

Most recently, combination immunotherapy has also demonstrated superior overall survival in untreated metastatic NSCLC patients regardless of PD-L1 expression in CheckMate-227 (Table 3) [19]. In this complex study, 1739 patients with treatment-naive metastatic or recurrent NSCLC were randomized 1:1:1 to receive combination nivolumab and the anti-CTLA-4 antibody, ipilumumab versus nivolumab versus platinum-based doublet chemotherapy if PD-L1 ≥1% or nivolumab/ipilumumab versus nivolumab and chemotherapy versus chemotherapy if PD-L1 <1% [19]. Primary end point was progression-free survival with nivolumab/ipilumumab compared with chemotherapy in patients with high tumor mutational burden (TMB), (≥10 mutations per Mb) and overall survival in patients with PD-L1 ≥1% [19,20]. Cross-over was not permitted. Patients who received combination immunotherapy demonstrated improved PFS compared with chemotherapy in patients with high TMB (7.2 vs 5.5 months, HR: 0.58, 98% CI: 0.41–0.81) [20]. Patients with PD-L1 ≥1% who received combination immunotherapy indeed demonstrated improved median overall survival and duration of response compared with chemotherapy

Table 3. Doublet immunotherapy in advanced non-small-cell lung cancer patients in frontline setting.

Trial	Patient (n)	PD-L1 IHC	Agent	Primary end point (months)	Exploratory analysis	Outcome by TMB (months) and PD-L1	US FDA approval
Doublet IO							
CheckMate-227 (NCT02477826)	Part 1a: 1189 PD-L1 ≥1% Part 1b: 550 PD-L1 <1% Part 2: 755 (any PD-L1)	PD-L1 by Dako 28–8 antibody	Part 1a: Nivolumab+ ipilumumab vs Nivolumab vs doublet chemotherapy (PD-L1 ≥1%) Part 1b: Nivolumab + ipilimumab vs chemotherapy+ nivolumab vs doublet chemotherapy (PD-L1 <1%) Part 2: Nivolumab + doublet chemotherapy vs doublet chemotherapy	Part 1: PFS in high TMB (≥10 mut/Mb) for all PD-L1 Nivo/ipi vs chemo: PFS 7.2 vs 5.5 (HR: 0.58, 98% CI: 0.41–0.81) OS in PD-L1 ≥1% Nivo/ipi vs chemo: median OS 17.1 vs 14.9 (HR: 0.79, 98% CI: 0.65–0.96) Part 2: did not meet primary end point for OS in nonsquamous patients	Part 1: PD-L1<1%: Nivo/ipi vs chemo: 17.2 vs 12.2 (HR: 0.62, 95% CI: 0.48–0.78)	≥10 mut/Mb: Nivo/ipi vs chemo: OS – 23 vs 16.4, HR: 0.68 (95% CI: 0.51–0.91) <10 mut/Mb: Nivo/ipi vs chemo: OS – 16.2 vs 12.6, HR: 0.75 (95% CI: 0.59–0.94) PD-L1 ≥50%, TMB ≥10 vs PD-L1 <1%, TMB <10: HR: 0.63 (0.37–1.07) vs HR: 0.69 (0.46–1.05)	Currently under FDA review

chemo: Chemotherapy; HR: Hazard ratio; IO: Immunotherapy; ipi: Ipilumumab; Mb: Megabase; mut: Mutation; Nivo: Nivolumab; ORR: Objective response rate; OS: Overall survival; PFS: Progression-free survival; TMB: Tumor mutational burden.

alone (median OS: 17.1 months, 95% CI: 15.0–20.1 with combination immunotherapy vs 14.9 months, CI: 12.7–16.7 in chemotherapy, HR: 0.79, 95% CI: 0.65–0.96) [19]. In addition, in the PD-L1 <1% subset, patients who received combination immunotherapy also appeared to have superior overall survival compared with chemotherapy (median OS: 17.2 months, 95% CI: 12.8–22.0 with combination immunotherapy vs 12.2 months, 95% CI: 9.2–14.3 with chemotherapy, HR: 0.62, 95% CI: 0.49–0.79) [19]. The similarity in median overall survival between two PD-L1 groups with combination immunotherapy, in contrast to improved response with higher PD-L1 expression in immunotherapy used as monotherapy, suggests possibly synergistic antitumor immune effects of CTLA-4 and PD-L1 inhibition in patients with low PD-L1 expression where single-agent immunotherapy otherwise is expected to have little activity. This synergistic effect is further supported by longer duration of response and higher complete response rates seen in patients receiving nivolumab/ipilumumab compared with patients receiving nivolumab alone, though study was not powered to assess these findings [19].

Selection of patients

Identifying subsets of patients who would best benefit from frontline immunotherapy has been of great interest and is suggested to be associated with specific biomarkers involving both tumor and host factors, including PD-L1 expression, tumor mutational burden and factors reflective of an immune-permissive or refractory tumor microenvironment. Predictive biomarkers have been hypothesized based on the biology of immune surveillance. It is well known that the innate and adaptive immune system provide immune surveillance to remove malignant cells through activation of T cells, which occur by T-cell receptor binding of tumor neo-antigens presented on antigen presenting cells (APC). Logically, recognition of tumor cells as foreign might depend on the number of neoantigens generated – reflected grossly by TMB. To inhibit activation of immune cells against self-antigens, immune tolerance is regulated by co-stimulation of T-cell CD28 by B7 ligand on APC for T-cell activation, and thus absence of the CD28-B7 complex results in T-cell anergy. Activated T cells then enter the microenvironment. Here immune breaks also occur through interaction of T-cell CTLA4 receptor and APC B7 ligand and interaction of T-cell PD-1 and APC PD-L1. Tumor cells have adapted several mechanisms to evade immune tolerance, for example, by overexpressing inhibitory PD-L1 receptors, typically in an inducible manner upon IFN-γ-induced pathway activation, inhibiting IL-2 production, and inducing apoptosis of CD8 T cells [21]. Thus, patients with tumors exhibiting high PD-L1 may benefit best from immunotherapy as the PD-L1 expression both defines tumors actively engaged by T cells in the microenvironment and identifies treatment targets for anti-PD1/PD-L1 antibodies.

PD-L1 as selection biomarker

PD-L1 as a biomarker for response to immunotherapy has been extensively evaluated and currently is the only validated albeit inconsistent marker for immunotherapy selection. One reason for this inconsistency lies in the various antibodies used for immunohistochemical staining. There are currently at least five commercially available assays for PD-L1 staining, including Dako 22C3 for pembrolizumab, Dako 28–8 for nivolumab, Ventana SP 142 for atezolizumab, Ventana SP263 for durvalumab and Dako 73–10 for avelumab. Each of these assays were developed with different scoring systems as well adding further confounding. The Blueprint PD-L1 immunohistochemistry comparability project set out to compare these assays and demonstrated that of the 81 lung cancer specimens stained in this project, 22C3, 28–8, and SP263 assays were highly comparable on tumor cell staining, with inter-reliability among trial pathologists [22]. SP142 was noted to have decreased sensitivity while the 73–10 assay was noted to have increased sensitivity [22]. Of note, inter-reliability of pathologists on PD-L1 staining in immune cell scoring was poor [22]. The next part of the study, Phase IIB, is underway to compare PD-L1 scores across types of biopsies, including fine needle aspirate, core biopsy and surgical samples. An additional reason for inconsistency of PD-L1 as a biomarker lies in patient factors of heterogeneity of PD-L1 expression as PD-L1 expression can vary based on cancer stage, prior chemotherapy, gender and heterogeneous expression based on tissue sampling [23].

Despite the variability in assays and heterogeneous expression, PD-L1 expression remains a fair predictor of single agent immunotherapy benefit based on the KEYNOTE-042, KEYNOTE-024 and IMpower 110 trials. PD-L1 expression as a good predictor for benefit of immunotherapy was also observed in a retrospective study of 187 patients with NSCLC in which patients with very high PD-L1 $\geq$90% receiving frontline pembrolizumab had a superior response rate, PFS, and OS than PD-L1 50–89%, (ORR: 60.0 vs 32.7%; $p < 0.001$, mPFS: 14.5 vs 4.1 months, HR: 0.50, 95% CI: 0.33–0.74, mOS not reached vs 15.9 months, HR: 0.39, 95% CI: 0.21–0.70) [24].

In contrast to single-agent immunotherapy, PD-L1 expression has not been shown to be a robust predictor for chemo-immunotherapy benefit based on KEYNOTE-189 and KEYNOTE-407. Thus, in clinical practice, PD-L1 expression may help direct clinicians between immunotherapy or chemo-immunotherapy, where single-agent immunotherapy is preferentially chosen for patients high PD-L1-positive tumors while chemoimmunotherapy for lower PD-L1 expression.

TMB as predictive biomarker

TMB is a reflection of the number of somatic nonsynonymous coding mutations in a tumor and has also been evaluated as a possible biomarker for immunotherapy response. High TMB correlates with increased antigenic neoepitopes that drive immunogenic host response, and is hypothesized to associate with improved outcomes with immunotherapy. Whole-exome sequencing is the gold standard for quantifying TMB, but due to long turnaround time and cost, alternative methods such as targeted enrichment sequencing using specific gene panels, such as FoundationOne, MSK-IMPACT, Guardant360 and Trusight170 have also been demonstrated to be reliable estimates, with larger gene panels of 1.5–3Mb of sequencing providing more robust estimates of TMB [25].

The value of TMB as a predictive biomarker was demonstrated in recently presented exploratory analyses of KEYNOTE-010 and KEYNOTE-042. 24% of specimens from KEYNOTE-010 and 62% of specimens from KEYNOTE-042 were available for whole-exome sequencing. In the exploratory analysis of KEYNOTE-010 (pivotal Phase III study comparing standard of care docetaxel chemotherapy with single-agent pembrolizumab in the second line advanced NSCLC setting) of NSCLC patients with PD-L1 $\geq$1%, patients with TMB $\geq$175 mutations per exome had more favorable OS (HR: 0.56, 95% CI: 0.38–0.83), PFS and overall response rate with use of pembrolizumab as second line of therapy compared with chemotherapy, where in contrast, in patients with TMB $<$175 mutations per exome, there was no statistical difference between OS (HR: 0.85, 95% CI: 0.56–1.30) and PFS of pembrolizumab compared with chemotherapy [26]. Similarly, in the exploratory analysis of KEYNOTE-042, where pembrolizumab was used in the frontline setting in NSCLC patients with PD-L1 $\geq$1%, patients with TMB $\geq$175 mutations per exome had improved OS (HR: 0.62, 95% CI: 0.48–0.80) and PFS (HR: 0.75, 95% CI: 0.59–0.95) with pembrolizumab versus chemotherapy as opposed to patients with $<$175 mutations per exome where no such benefits were seen (OS HR: 1.09, 95% CI: 0.88–1.36, PFS HR: 1.27, 95% CI: 1.04–1.55) [26]. CheckMate-026, which was a negative study of nivolumab used as monotherapy in frontline metastatic or recurrent setting compared with chemotherapy, demonstrated in a *post hoc* analysis that when NSCLC patients with PD-L1 $\geq$5% were stratified by TMB, patients with high TMB ($>$243 mutations per exome) measured by whole exome sequencing, had superior PFS compared with chemotherapy [13].

Similar to PD-L1 where expression level did not correlate with outcome with chemoimmunotherapy, there was no TMB correlation noted with chemoimmunotherapy based on subgroup analyses of the KEYNOTE-189 and KEYNOTE 407 [27,28]. Patients who received chemoimmunotherapy had superior PFS compared with chemotherapy alone regardless of TMB. In contrast, it was observed in the first part of CheckMate-227 that patients with TMB ≥10 mutations per megabase who received nivolumab and ipilumumab had superior PFS compared with chemotherapy alone, which was not observed in patients with TMB <10 mutations per megabase in patients with PD-L1 ≥1, which suggests overall PFS benefit with higher TMB in patients who receive doublet immunotherapy [20]. In the follow-up part of CheckMate-227, however, there was no differential TMB benefit in OS with doublet immunotherapy compared with chemotherapy when all PD-L1 groups were combined [19]. When stratified by PD-L1, only patients with PD-L1 <1%, a subgroup with limited benefit from single-agent immunotherapy, had differential TMB demonstrated favorable overall survival with nivolumab and ipilumumab compared with chemotherapy where higher OS was observed with TMB ≥10 [19]. It appears that PD-L1 and TMB as biomarkers lose their predictive power in the context of chemo-immunotherapy. While this is not fully explained, the loss of predictive power may be related to the strong synergy noted in PD-L1 low patients, with chemotherapy enhancing immunotherapy benefits in this particular patient subset.

The current use of TMB by whole exome sequencing is limited by turnaround time of several weeks, making practical integration into typical clinical decision workflows tedious [29]. The utility of blood TMB (bTMB) from circulating tumor DNA (ctDNA), which has a shorter turnaround time compared with tissue TMB, was demonstrated in the MYSTIC trial [30]. 1118 treatment-naive metastatic NSCLC patients were randomized to receive durvalumab (D, anti-PD-L1) versus durvalumab and tremelimumab (D + T, anti-CTLA-4) versus platinum chemotherapy (CT). It was observed that bTMB correlated with tissue TMB and that high bTMB (≥20) was associated with superior OS and PFS with combination immunotherapy compared with platinum chemotherapy (median OS: D + T vs CT, HR: 0.49, 95% CI: 0.32–0.74; median PFS: D + T vs CT, HR: 0.53, 95% CI: 0.34, 0.81) [30]. This study supports further development of ctDNA TMB assays in this context.

While PD-L1 and TMB are imperfect markers individually, whether combination of PD-L1 and TMB can be used as a predictive biomarker was analyzed retrospectively in 426 patients with NSCLC. There was no correlation between PD-L1 and TMB suggestive of these being independent markers [31]. When considered together, patients with both high TMB and high PD-L1 had the highest likelihood of realworld tumor response (rwTR), defined by clinician notes and radiology/pathology reports, compared with other permutations, including high TMB/low PD-L1 and low TMB/high PD-L1 [31]. Thus use of PD-L1 and TMB together may be complimentary tools to identify which patients would respond best to immunotherapy with TMB potentially refining the choice of single-agent immunotherapy for PD-L1-positive tumors and tailoring choice of combination immunotherapy for PD-L1-negative tumors.

Immunotherapy in elderly patients

Though lung cancer is a disease of the elderly with the average age of diagnosis 70 years, the elderly population is under-represented in clinical trials. Based on the concept of immunosenescence and thus impaired T-cell activation with older age, immune agents are hypothesized to possibly have lower efficacy in the elderly. In one retrospective study, it was observed that patients ≥70 years of age treated with single-agent immunotherapy had decreased OS of 5.5 months versus 13 months (HR: 3.86) compared with patients <70 years of age [32]. Pooled analysis of randomized trials, KEYNOTE-010, KEYNOTE-024 and KEYNOTE-042, by Nosaki, however, suggested comparable improvement in median OS with single-agent pembrolizumab compared with chemotherapy in patients ≥75 years of age and in patients <75 years of age with TPS of ≥1% (median OS: 15.7 vs 11.7 months, HR: 0.76) [33]. There was enhanced benefit seen in subgroup of patients with PD-L1 ≥50%, which suggests consideration of single-agent immunotherapy in elderly patients who may not otherwise be candidates for chemotherapy or chemoimmunotherapy [33]. More research is needed in the elderly population to better understand the role of immunotherapy in this particular patient subset. The DURATION study is a Phase II trial comparing doublet chemotherapy for four cycles versus single-agent chemotherapy for two cycles followed by two cycles of durvalumab and subsequent durvalumab maintenance treatment every 4 weeks in patients ≥70 years of age, with primary end point including treatment-related grade III/IV adverse events and secondary end point PFS, RR and OS analyzed by PD-L1 expression (NCT03345810) [34].

Resistance to immunotherapy

Data are starting to emerge as to other biological factors impacting primary or acquired resistance to immunotherapy. In human tumor and mouse models, it was observed that with tumoral mutations in *STK11*, a gene that encodes a serine threonine kinase that regulates cell homeostasis and growth, there was increased difficulty of $CD8^+$ lymphocytes to enter the tumor microenvironment, often referred as 'cold tumor immune microenvironment' [35]. Clinical validity of this finding was then corroborated through a cohort study from three institutions that assessed treatment benefits in patients with *k-ras* mutant NSCLC with *k-ras* mutant/*STK11* mutant, *k-ras* mutant/*TP53* mutant and *k-ras* mutant-alone subsets receiving PD-1 inhibitor monotherapy or CTLA-4 monotherapy [35]. It was observed that *k-ras*-mutant/*STK11* mutant had the worst overall response rate (RR) of 7.4% and *k-ras* mutant/TP53 mutant patients had best overall response rate of 35.7% to immunotherapy [35]. OS was also inferior in *STK11* mutant patients compared with wild-type patients, (HR: 1.99; 95% CI: 1.29–3.06) [35]. An international cohort study that collected clinical outcomes of 497 patients treated with first-line platinum therapy/pemetrexed/pembrolizumab (CPP) versus platinum/pemetrexed (CP) alone demonstrated that patients with *STK11*/*LKB1* mutation had shorter PFS, OS and ORR and also demonstrated that addition of pembrolizumab also did not affect PFS or OS [36]. These findings suggest that *STK11* mutation is not only a poor predictive marker for response to chemoimmunotherapy compared with those without the mutation, but also that immunotherapy has no survival benefit added to chemotherapy in *STK11*-mutated patients. *KEAP1*, a gene that encodes for a protein that assists in transportation of NF-E2 related factor 2 to the cell nucleus, has been shown to be co-mutated often with *STK11*, and it has been demonstrated in a small cohort study of 308 patients, those who had co-mutations in *STK11*/*KEAP1* had less favorable OS and PFS compared with *STK11*wt/*KEAP1*wt patients when treated with PD-L1 inhibitor despite high TMB in *STK11*/*KEAP1* mutant patients [37]. Thus, while prior data suggest that *STK11* and *KEAP1* mutations might serve as emerging markers to select patients who may not receive benefit from immunotherapy, more recent data, suggest lack of correlation. In a recent exploratory analysis of KEYNOTE-042, pembrolizumab monotherapy was associated with improved overall response rate compared with chemotherapy regardless of *STK11* and *KEAP1* mutational status [38]. Thus, based on this new observation, TMB, *STK11* and *KEAP1* mutations remain unvalidated biomarkers.

Tumor angiogenesis and expression of VEGF expression by tumor cells, which suppress immune T-cell development and inhibit lymphocyte adhesion, may also play a role in resistance to PD-L1 therapy. In a pre-clinical study, genetically engineered mouse with small cell lung cancer have shown improvement in overall survival with addition of anti-angiogenesis to immunotherapy compared with immunotherapy alone [39]. The mechanism is thought to be secondary to downregulation of PD-1 expression on cytotoxic T-cells with anti-VEGF. The Lung Cancer Master Protocol (Lung-MAP) includes an ongoing trial evaluating the efficacy of pembrolizumab in combination with the anti-VEGFR monoclonal antibody. ramucirumab versus standard of care in previously immunotherapy treated patients with advanced NSCLC (NCT03971474) and CheckMate-012 is evaluating response to nivolumab with bevacizumab in advanced NSCLC (NCT01454102).

Actionable oncogenic drivers & Immunotherapy

Patients with oncogenic drivers, including *EGFR, ALK* and *RET* mutations, have shown to demonstrate poor response to single-agent immunotherapy, thought to be related to low tumor mutation burdens and associated reduced inflamed tumor microenvironment, especially in patients with nonsmoking history. In *EGFR*-mutant patients, single-agent immunotherapy was demonstrated to show no improvement in OS compared with docetaxel in pretreated patients (HR: 1.11; p = 0.54) based on a meta-analysis of CheckMate-057, KEYNOTE-010 and POPLAR, and as demonstrated in the ATLANTIC trial [40,41]. The IMMUNOTARGET registry, which was a retrospective study for single-agent immunotherapy in patients with actionable mutations demonstrated overall low PFS in patients with driver mutations: 2.5 months *EGFR*, 2.5 months *ALK*, 2.1 months *RET* and 3.2 months *KRAS*, with PFS correlating with PD-L1 expression in *KRAS* and *EGFR* patients [42]. This observation was supported prospectively in a Phase II trial of pembrolizumab in *EGFR* mutant treatment naive patients which demonstrated no benefit of pembrolizumab even in patients with PD-L1 expression $\geq$50% leading to early termination of trial [43]. While single agent immunotherapy has not been shown to be effective in *EGFR* mutant patients, subset analysis of IMpower150 suggested that the addition of atezolizumab to frontline bevacizumab, carboplatin, plus paclitaxel (ABCP) may have added benefit in patients with sensitizing *EGFR* mutations [16]. This possible added benefit of combined chemoimmunotherapy in this subset population will need to be further demonstrated in proper, randomized studies several of which are ongoing, including KEYNOTE-789, a Phase III trial evaluating

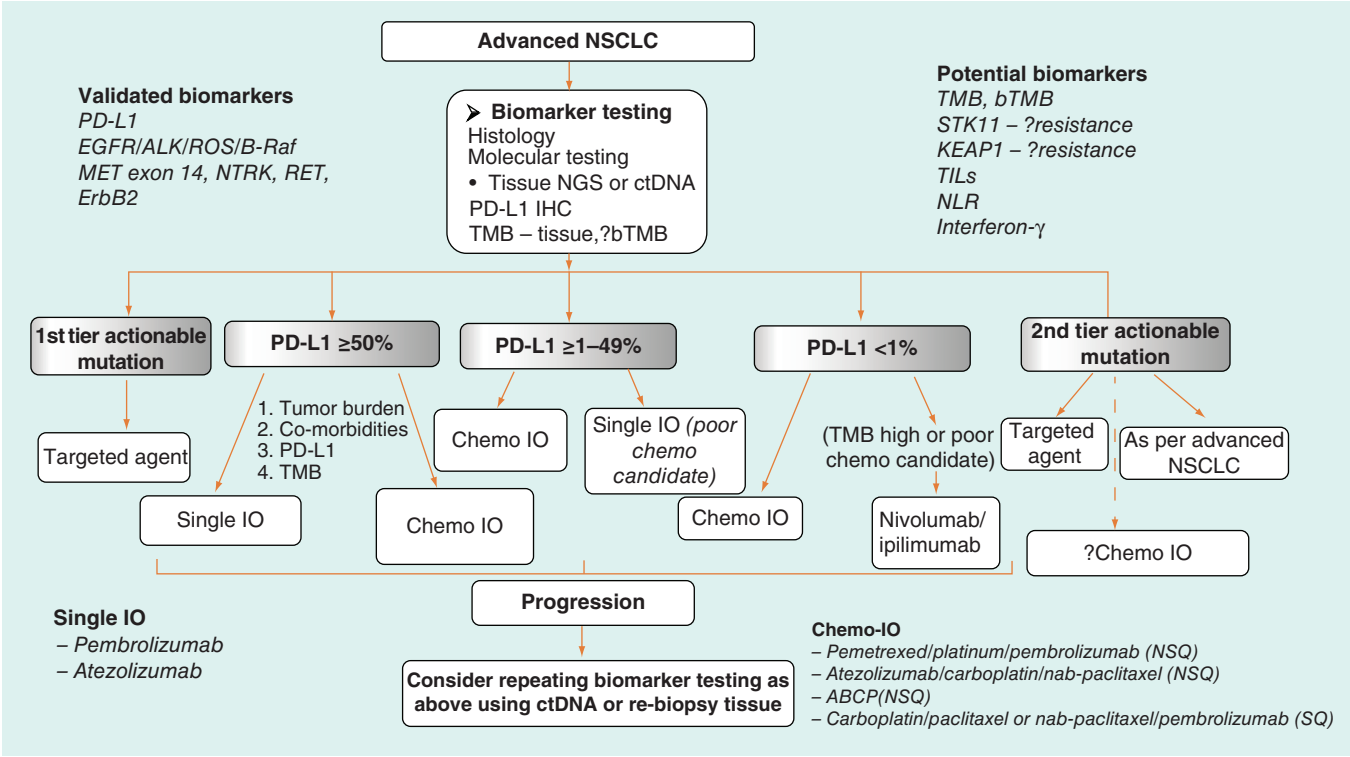

Figure 1. Treatment schema for advanced non-small-cell lung cancer. Treatment in parenthesis represent treatment under review by US FDA for approval. Dotted arrow – cautious consideration.
ABCP: Atezolizumab/bevacizumab/carboplatin/paclitaxel; bTMB: Blood tumor mutational burden; Chemo: Chemotherapy; ctDNA: Circulating tumor DNA; IO: Immunotherapy; NLR: Neutrophil:lymphocyte ratio; NSCLC: Non-small-cell lung cancer; NSQ: Nonsquamous; SQ: Squamous; TIL: Tumor-infiltrating lymphocyte; TMB: Tumor mutational burden.

pemetrexed-platinum with or without pembrolizumab in TKI-resistant or *EGFR*-mutated advanced NSCLC (NCT 03515837). Due to lack of strong evidence on the benefit of immunotherapy in patients with actionable mutations, further research is necessary in defining the role of immunotherapy in patients with *EGFR* mutations and should only be cautiously considered after exhaustion of targeted agents in patients with high PD-L1 expression.

Conclusion

Immunotherapy has completely transformed the treatment landscape in frontline metastatic treatment in NSCLC, in which for *EGFR*/*ALK*-negative advanced NSCLC patients with PD-L1 ≥50%, there is favorable outcome with monotherapy and favorable outcome is also seen irrespective of PD-L1 status with chemoimmunotherapy over chemotherapy alone in both squamous and nonsquamous patients. Current markers for immunotherapy response include the validated marker, PD-L1 and the emerging marker, TMB, both of which are reasonable markers in predicting response to immunotherapy monotherapy but demonstrate no predictive value in the context of chemoimmunotherapy. Choosing chemoimmunotherapy over immunotherapy or *vice versa* in addition to these biomarkers needs to depend on patient related factors such as for example age, performance status and co-morbidities (Figure 1). There has been emerging data on specific immune resistance markers such as *STK11* and *KEAP1* possibly correlating with less favorable outcomes and further novel biomarkers are needed to better understand primary and acquired resistance to immunotherapy. Combination immunotherapy while not FDA approved yet might offer another validated alternative for select patient subgroups, for example, patients who might not be optimal chemotherapy candidates. Tremendous benefits have been gained through the rapid development and integration of checkpoint inhibitors into the management of advanced non-small-cell lung cancer. Broad support for ongoing studies refining these treatment combinations and optimizing biomarkers to assist in patient selection remains pivotal for these advances to continue for the benefit of our patients.

Future perspective

Future evolution of the field of immunotherapy in NSCLC will likely include improved understanding of the tumor microenvironment and neoantigen landscape, paralleled by the development of novel biomarkers for treatment selection. There will likely be increased application of ctDNA as a tool to help monitor treatment response to immunotherapy. Ongoing clinical trials and research will continue to refine our current options in the treatment of advanced NSCLC, and include studies for better patient selection based on biomarkers, and studies for improved outcomes that include a large spectrum of studies assessing combination immunotherapy, vaccine-immunotherapy, radio-immunotherapy, CAR T-cell immunotherapy and bispecific T-cell engager (BitTE) antibodies among others moving immunotherapy into targeted and earlier stage settings.

Executive summary

- Pembrolizumab as monotherapy is a validated first-line therapy choice in advanced non-small-cell lung cancer (NSCLC) patients with PD-L1 TPS $\geq$50% based on KEYNOTE-024.
- Pembrolizumab as monotherapy has been extended for use as first-line therapy in advanced NSCLC patients with PD-L1 TPS $\geq$1% based on KEYNOTE-042; however, data remains controversial as greatest benefit is noted in the group of high PD-L1 expressors and therefore single-agent immunotherapy for PD-L1 TPS score 1–49% is best preserved for patients who are not good candidates for added chemotherapy.
- Atezolizumab and nivolumab as monotherapy are not yet approved for monotherapy for metastatic treatment-naive NSCLC patients.
- PD-L1 expression remains the sole validated predictor for single-agent immunotherapy benefit based on the KEYNOTE-042, KEYNOTE-024, IMpower110 and KEYNOTE-010 trials but demonstrate no predictive potential for chemo-immunotherapy benefit based on KEYNOTE-189 and KEYNOTE-407. TMB remains a promising complementary biomarker in the context of single-agent immunotherapy.
- In patients with TPS <50%, chemoimmunotherapy with pembrolizumab + pemetrexed and carboplatin or cisplatin in nonsquamous advanced NSCLC and pembrolizumab + carboplatin and paclitaxel or nab-paclitaxel in squamous advanced NSCLC is the suggested first-line therapy based on KEYNOTE-189 and KEYNOTE-407.
- Atezolizumab with bevacizumab, carboplatin and paclitaxel (ABCP) or atezolizumab with carboplatin and nab-paclitaxel can also be used as first-line treatment in metastatic nonsquamous NSCLC patients based on IMpower150 and IMpower130, respectively.
- Combination immunotherapy with nivolumab and ipilumumab has also demonstrated superior overall survival compared with chemotherapy in untreated PD-L1 selected metastatic NSCLC patients in CheckMate-227 and regulatory agency decision on its use as first-line regimen is pending.
- Early data suggest that patients with *STK11* and *KEAP1* mutations may receive less benefits from immunotherapy.
- Evidence supporting benefit of immunotherapy as single agent treatment in patients with actionable driver mutations is lacking. There is only weak evidence that chemoimmunotherapy may have some benefit in patients with sensitizing *EGFR* mutations based on the IMpower150 trial and this important area calls for further investigations.

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