The progress and challenge of anti-PD-1/PD-L1 immunotherapy in treating non-small cell lung cancer

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Abstract: **Abstract**

The use of programmed cell-death protein 1 (PD-1)/programmed cell-death ligand 1 (PD-L1) inhibitors is the standard therapy for the first-line or second-line treatment of patients with non-small-cell lung cancer (NSCLC). In contrast to current traditional treatments such as chemotherapy or radiotherapy, anti-PD-1 and anti-PD-L1 treatments can directly attenuate tumour-mediated exhaustion and effectively modulate the host anti-tumour immune response *in vivo*. In addition, compared with traditional therapy, PD-1/PD-L1 inhibitor monotherapy can significantly prolong survival without obvious side effects in the treatment of advanced NSCLC. Ideally, several biomarkers could be used to monitor the safety and effectiveness of anti-PD-1 and anti-PD-L1 treatments; however, the current lack of optimal prognostic markers remains a widespread limitation and challenge for further clinical applications, as does the possibility of immune-related adverse events and drug resistance. In this review, we aimed to summarise the latest progress in anti-PD-1/anti-PD-L1 treatment of advanced NSCLC, worldwide, including in China. An exploration of underlying biomarker identification and future challenges will be discussed in this article to facilitate translational studies in cancer immunotherapy.

Keywords: immunotherapy, non-small cell lung cancer (NSCLC), PD-1/PD-L1 inhibitors, predictive biomarkers, progress and challenges

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Introduction

Lung cancer is the leading cause of cancer-related deaths and is associated with a poor prognosis.[1,2] Non-small-cell lung cancer (NSCLC) accounts for 85% of all lung cancers, 15–30% of which are squamous-cell carcinomas (SQCs) of the lung.[3,4] The pathogenic genes of NSCLC have been discovered and widely investigated, and genotyping-based targeted therapy has been developed as one of the most successful therapeutic methods.[5–8] At present, with a greater understanding of tumour immunity, targeted molecular therapy and immunotherapy have become important strategies for the conventional treatment of NSCLC.[9–12] In recent years, the successful application of immune-checkpoint inhibitors (ICIs) of cytotoxic T-lymphocyte antigen-4 (CTLA-4), and anti-programmed cell-death protein 1 (PD-1) and programmed cell-death protein ligand 1 (PD-L1) in various advanced cancers has attracted widespread attention in the field of immuno-oncology.[13–18] Two antibodies against PD-1, nivolumab and pembrolizumab, and two antibodies against PD-L1, atezolizumab and durvalumab, have been approved by the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) for treating advanced NSCLC.[19–24] The PD-1/PD-L1 inhibitors licensed for NSCLC treatment are listed in Table 1.

In this review, we aimed to outline the recent development of PD-1/PD-L1 inhibitors as first-,

Table 1. The PD-1/PD-L1 inhibitors licensed for NSCLC treatment.

Checkpoint	Blocking agent	IgG isotype and characteristics	Clinical stage	Manufacturer
PD-1	Pembrolizumab	Humanized IgG4 mAb	EMA, FDA approved for first-line and second-line NSCLC treatment	Merck
	Nivolumab	Humanized IgG4 mAb	FDA approved for second-line NSCLC	Bristol-Myers Squibb
	Toripalimab	Humanized IgG4 mAb	Clinical trial ongoing	Shanghai Junshi
	Sintilimab	Fully human IgG4 mAb	Clinical trial ongoing	Innovent Biologics
	Camrelizumab	Humanized IgG4 mAb	Clinical trial ongoing	Hengrui Medicine
	Tislelizumab	Humanized IgG4 mAb	Clinical trial ongoing	BeiGene
	PDR001	Humanized IgG4 mAb	Clinical trial ongoing	Novartis
	REGN2810	Humanized IgG4 mAb	Clinical trial ongoing	Regeneron-Sanofi
PD-L1	Atezolizumab	High-affinity human IgG1	FDA approved for second-line NSCLC	Roche
	Durvalumab	Human IgG1 mAb	FDA approved for treatment of unresectable stage III NSCLC without relapse after chemoradiation	AstraZeneca
	BMS-936559	Fully high-affinity human IgG4	Clinical trial ongoing	Bristol-Myers Squibb

EMA, European Medicines Agency; FDA, US Food and Drug Administration; IgG, immunoglobulin G; mAb, monoclonal antibody; NSCLC, non-small-cell lung cancer; PD-1, programmed cell-death protein 1; PD-L1, programmed cell-death ligand 1.

second-, and third-line treatments, and to discuss driver-gene mutation in NSCLC patients. Moreover, we summarised current and ongoing clinical trials of PD-1 and PD-L1 inhibitors in China. In the absence of optimal prognostic biomarkers, we elaborate on the presence of immune-related adverse events (irAEs), primary and acquired drug resistance, and the challenge of administering immunotherapy for the treatment of advanced NSCLC.

Regulatory mechanisms of PD-1/PD-L1 in NSCLC

Modulation of immune checkpoints is an important mechanism for evading immune surveillance by inhibiting activated T cells.[25] For example, PD-1$^+$ immune cells can interact with PD-L1$^+$ malignant tumour cells or other host cells. Mechanistically, immune escape might occur by interfering with related anti-tumour functions.[26] In addition, PD-1 is expressed by CD4$^+$ T cells, CD8$^+$ T cells and some other immune cell types. PD-1 interaction with its ligand, PD-L1, can weaken the effector functions of T lymphocytes such as activation, proliferation and survival,

promote antigen-specific T-cell apoptosis, and affect CD4$^+$ and CD8$^+$ T cells, natural killer (NK) cells, and CD11c$^+$ M1 macrophage functions to promote tumour development.[27–29] Antibodies that suppress the interaction between PD-1 and PD-L1 can largely improve and restore the functionality of exhausted T cells and enhance their anti-tumour immune response.[30] PD-1 is highly expressed by regulatory T cells (Tregs) and has a significant inhibitory effect on tumour immunity.[31] The immune-related regulatory mechanism of action of PD-1/PD-L1 inhibitors is shown in Figure 1. Modulation of PD-1/PD-L interactions effectively inhibits the anti-tumour response by weakening the inhibitory activity of Tregs.[32] In addition to T-cell immunity, tumour-associated macrophages,[33,34] as well as NK cells and dendritic cells in the tumour microenvironment (TME) can enhance anti-tumour effects.[35–37]

PD-1/PD-L1 inhibitor therapy in advanced NSCLC

Although there are many ways to treat NSCLC, such as radiotherapy, platinum-based chemotherapy, surgical resection and targeted molecular
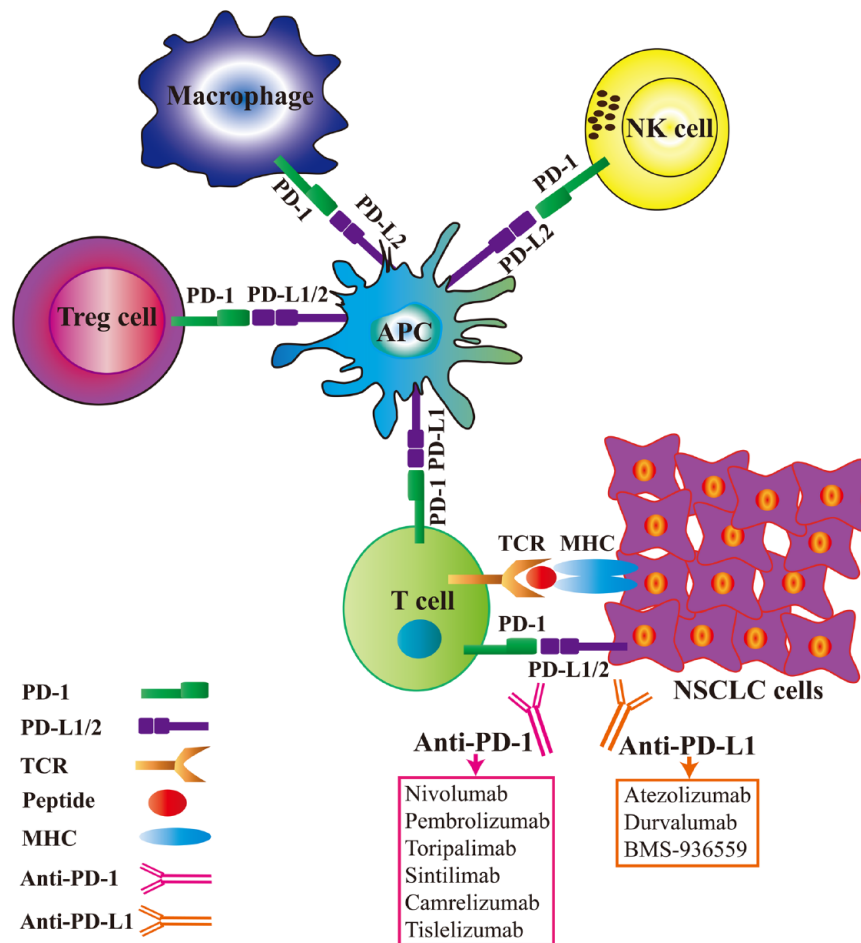
Figure 1. Regulatory mechanism of programmed cell-death protein 1 (PD-1)/programmed cell-death ligand 1 (PD-L1) inhibitors.
The PD-1 receptor is expressed on activated T cells, macrophages, regulatory T cells (Tregs) and natural killer (NK) cells. Binding of PD-1 to its ligands, PD-L1 or PD-L2, results in suppression of proliferation and inhibition of the immune response of T cells. Anti-PD-1 antibodies or PD-L1 inhibitors reverse this process, resulting in enhanced anti-tumour immune responses. APC, antigen-presenting cell; MHC, major histocompatibility complex; NSCLC, non-small-cell lung cancer; TCR, T cell receptor.

therapy, the long-term efficacy of these treatments is still not ideal, and the 5-year survival rate of patients with NSCLC is less than 18%.[38] Thus, there is an urgent demand for novel clinical therapies that are associated with less toxicity and higher efficacy to further prolong the survival time of patients. At present, some anti-PD-1/PD-L1 antibodies have been approved by the FDA for clinical treatment of advanced NSCLC;[39] these clinical studies will have a very important role in guiding long-term clinical practice.

PD-1/PD-L1 inhibitors as first-line treatment of advanced NSCLC
Multiple KEYNOTE trials (either alone or as part of a combined study) are being investigated using PD-1/PD-L1 inhibitors in various tumour types, with a focus on NSCLC. In the open-label phase Ib KEYNOTE-001 trial, patients with advanced NSCLC expressing PD-L1 in tumour tissues who were randomly assigned to the pembrolizumab group exhibited an objective response rate (ORR) of 27%, a median progression-free survival (mPFS) of 6.2 months [95% confidence interval (CI) 4.1–8.6], and a median overall survival (mOS) of 22.1 months (95% CI 17.1–27.2). The ORR, 1-year mPFS, and 1-year mOS were significantly higher in patients with a PD-L1 tumour proportion score (TPS) ⩾50% than in the overall population. The results showed that pembrolizumab was well tolerated; only 12 cases (11.9%) experienced grade 3 treatment-related adverse events (AEs), and there

were no treatment-induced deaths.[40] The 3 years of data from KEYNOTE-001 showed that 79.7% patients with a PD-L1 TPS ≥50% reached the mOS of 34.9 months (95% CI 20.3–not reached).[41] The KEYNOTE-189 study is a phase III clinical trial of non-squamous NSCLC patients without first-line sensitised epidermal growth-factor receptor (EGFR) or anaplastic lymphoma kinase (ALK) mutations. These patients received platinum-based drugs plus either pembrolizumab or placebo. The mPFS of the monoclonal antibody (mAb) group was significantly longer than the control group (8.8 *versus* 4.9 months, respectively; *p* < 0.001), and the incidences of grade 3 or higher AEs in the mAb group and the control group were 67.2% and 65.8%, respectively.[42] The update data from the KEYNOTE-189 study (23.1 months follow up) showed mOS was 22.0 months (95% CI 19.5–25.2) in the pembrolizumab–combination group *versus* 10.7 months (95% CI 8.7–13.6) in the placebo–combination group [hazard ratio (HR) 0.56; 95% CI 0.45–0.70]; the mPFS were 9.0 months (95% CI 8.1–9.9) and 4.9 months (95% CI 4.7–5.5), respectively (HR 0.48; 95% CI 0.40–0.58).[43]

For patients with advanced squamous NSCLC, the KEYNOTE-407 trial indicated that concomitant administration of carboplatin plus paclitaxel and pembrolizumab provided longer mOS and mPFS than traditional chemotherapy alone.[44] The efficacy outcome from the protocol-specified final analysis of KEYNOTE-407 showed that, after a median follow up of 14.3 months, pembrolizumab plus chemotherapy exhibited a clinically meaningful improvement over placebo plus chemotherapy for mOS (17.1 *versus* 11.6 months) and mPFS (8.0 *versus* 5.1 months).[45]

Another phase I/II trial, KEYNOTE-021, was designed to assess the efficacy and frequency of grade 3–4 AEs associated with combination treatment with platinum-based doublet chemotherapy with pembrolizumab. The results demonstrated that combination chemotherapy with pembrolizumab was associated with fewer grade 3–4 AEs compared with platinum-based chemotherapy or pembrolizumab monotherapy.[46] A randomised, open-label, phase II, multi-cohort study, KEYNOTE-021, was performed with 123 patients with primary stage IIIb, or IV NSCLC without EGFR or ALK mutations. The mPFS of the pembrolizumab plus chemotherapy group was 13.0 months (95% CI 8.3–not reached), whereas the mPFS of the group receiving chemotherapy

alone was only 8.9 months (95% CI 4.4–10.3).[47] After a median time of 49.4 months from KEYNOTE-021, ORR (58% *versus* 33%) and mPFS (24.5 *versus* 9.9 months; HR 0.54; 95% CI 0.35–0.83) was improved with the pembrolizumab combination compared with chemotherapy, regardless of PD-L1 status; the median OSs were, respectively, 34.5 *versus* 21.1 months (HR 0.71; 95% CI 0.45–1.12).[48]

In the KEYNOTE-024 trial, compared with platinum-based chemotherapy alone, pembrolizumab significantly improved OS (30.0 *versus* 14.3 months), PFS (10.3 *versus* 6.0 months), and ORR (44.8% *versus* 27.8%) in the first-line therapy of advanced NSCLC cases with a PD-L1 TPS ≥50%.[49,50] Moreover, pembrolizumab was associated with longer mOS and mPFS in contrast to chemotherapy alone in patients with a high PD-L1 TPS ≥50% in the KEYNOTE-042 trial.[51] A real-world study in Japan, HOPE-001, analysed clinical data from patients receiving pembrolizumab as first-line treatment for NSCLC. The ORR, mPFS, and mOS were 51.2%, 8.3 months (95% CI 6.0–10.7), and 17.8 months (95% CI 17.8–not reached), respectively,[52] similar to the results of the KEYNOTE-024 and KEYNOTE-042 trials.

In the CheckMate 012 trial, 52 patients received nivolumab until disease progression or toxicity was experienced. The ORR was 28% in patients regardless of the tumour expression level of PD-L1, and the mPFS and mOS were 3.6 months (95% CI 0.1–28.0) and 19.4 months (95% CI 0.2–35.8), respectively.[53] In another CheckMate 012, open-label, phase I trial, 78 patients who received either nivolumab every 2 weeks plus ipilimumab every 12 weeks, or nivolumab every 2 weeks plus ipilimumab every 6 weeks, were assessed. The use of nivolumab plus ipilimumab as first-line treatment resulted in good tolerability and safety, and encouraging clinical efficacy, with a better ORR in each group.[54] Based on CheckMate 012, in the CheckMate 227 trial, nivolumab plus ipilimumab as first-line therapy significantly prolonged the mPFS compared with chemotherapy alone in patients with advanced NSCLC.[55,56] CheckMate 568, a phase II trial of nivolumab plus ipilimumab in NSCLC patients, defined a cut-off of the tumour mutational burden (TMB) of at least 10 mutations per base pair for selecting patients most likely to respond to treatment, regardless of the PD-L1 expression level.[57] A TMB ≥10 was associated with a high

ORR and mPFS. However, in the CheckMate 026 study, compared with first-line chemotherapy alone in advanced NSCLC patients, patients that were treated with antibody therapy did not obtain significant survival benefits.[58]

Atezolizumab and durvalumab are engineered and humanised mAbs against PD-L1 immunoglobulin G1 (IgG1), which can combine with PD-L1 to block the PD-1/PD-L1-mediated signalling pathway. BIRCH was designed to measure the efficacy of atezolizumab in advanced NSCLC as first-, second- and third-line therapies. Of 139 patients with advanced NSCLC who underwent first-line treatment with atezolizumab, the ORR, mPFS and mOS were 22%, 5.4 months (95% CI 3.0–6.9) and 20.1 months (95% CI 20.1–not reached), respectively.[59] IMpower150 was an open-label phase III study that aimed to assess the therapeutic efficacy of atezolizumab combined with bevacizumab and chemotherapy as first-line treatment in patients with advanced NSCLC. Patients received a combination therapy of atezolizumab + carboplatin + paclitaxel (ACP), bevacizumab + carboplatin + paclitaxel (BCP), or atezolizumab + BCP (ABCP) every 3 weeks for a total of four to six cycles. Following this, they then received atezolizumab, bevacizumab, or both, for maintenance treatment. Regardless of the expression levels and genetic status of PD-L1, the mPFS and mOS of patients with metastatic non-squamous NSCLC in the ABCP group were significantly improved when compared with the ACP or BCP group.[60] In the IMpower132 trial, atezolizumab combined with carboplatin and the chemotherapeutic agent pemetrexed significantly decreased mortality and improved the mPFS and mOS compared with chemotherapy alone in patients with advanced non-squamous NSCLC.[61] The IMpower130 trial reported an obvious improvement in mOS and mPFS, and no change in AEs were found in the group receiving atezolizumab plus chemotherapy compared with the group receiving chemotherapy alone as the first-line treatment of patients with metastasised non-squamous NSCLC without driver gene mutations.[62] The IMpower131 study showed that an atezolizumab plus platinum-based chemotherapy significantly improved mPFS in patients with first-line squamous NSCLC; however, the mOS was similar between groups.[63] Impower110 was performed to analyse the efficacy and safety of atezolizumab, as compared with platinum-based chemotherapy, as a first-line treatment for patients with metastatic NSCLC. The primary measurement was the OS among patients in the intention-to-treat population. This study found that the mOS was longer in the atezolizumab group than in the chemotherapy group (20.2 *versus* 13.1 months; HR for death 0.59; 95% CI 0.40–0.89; $p = 0.01$) as was mPFS (8.1 *versus* 5.0 months; HR for death 0.63; 95% CI 0.45–0.88) in the population with the highest expression of PD-L1. This study indicated that atezolizumab treatment results in significantly longer OS than platinum-based chemotherapy among patients with NSCLC with high PD-L1 expression, regardless of histologic type.[64] Recently, an ongoing APPLE study (WJOG11218L) was performed in Japan to evaluate the additional effect of bevacizumab administered with platinum combination therapy and atezolizumab in patients with advanced non-squamous NSCLC.[65]

In a phase Ib trial, durvalumab and the anti-CTLA-4 antibody tremelimumab were administered to patients with advanced NSCLC. Regardless of the level of PD-L1 expression, the ORR was 23% in the group that received durvalumab combined with tremelimumab.[66] The MYSTIC trial was an open-label, phase III trial of first-line treatment with durvalumab with or without tremelimumab *versus* platinum-based chemotherapy alone in patients with advanced NSCLC. Compared with chemotherapy alone, the durvalumab group exhibited a clinically significant improvement in mOS when tumour cell PD-L1 expression was ≥25%.[67] The SAKK study was performed to evaluate the safety of first-line durvalumab in patients with advanced NSCLC and a performance status of 2. The data demonstrate that using first-line durvalumab in patients with PS2 and metastatic NSCLC with PD-L1 expression ≥25% resulted in an unexpectedly high number of fatal early events due to rapid tumour progression.[68] All the clinical data described here are summarised in Table 2.

PD-1/PD-L1 inhibitors as second-line treatment in advanced NSCLC
The human anti-PD-1 mAb nivolumab was the first to be approved as second-line therapy for stage IV NSCLC.[69] CheckMate 017, a randomised, open-label, phase III study assessed the efficacy and safety of nivolumab for advanced squamous NSCLC; greater efficacy was obtained during or after first-line chemotherapy. Regardless of PD-L1 expression levels, the mOS, ORR, and mPFS were markedly better in the nivolumab

Table 2. Efficacy of anti-PD-1/PD-L1 agents for advanced NSCLC in first line.

Study	Histology	Phase	PD-L1 TPS (%)	Arms	Case number	ORR% (95% CI or p value)	mPFS (95% CI or p value, months)	mOS (95% CI or p value, months)	Grade 3–5 AE%	References
KEYNOTE-001	Squamous or non-squamous	Ib	≥50	Pembrolizumab	27	51.9 (31.9–71.3)	12.5 (6.2–NR)	NR (22.1–NR)	11.9	Hui et al.[40]
			1–49		52	17.3 (8.2–30.3)	4.2 (3.1–6.4)	19.5 (10.7–22.2)		
			<1		12	8.3 (0.2–38.5)	3.5 (2.1–19.0)	14.7 (3.4–NR)		
KEYNOTE-189	Non-squamous	III	Any	Pembrolizumab plus chemotherapy	410	47.6 (42.6–52.5)	8.8 (7.6–9.2)	NR	67.2	Gandhi et al.[42]
				Chemotherapy	206	18.9 (13.8–25.0) p<0.001	4.9 (4.7–5.5) p<0.001	11.3 (8.7–15.1)	65.8	
KEYNOTE-189	Non-squamous	III	Any	Pembrolizumab plus chemotherapy	410	48.0 (43.1–53.0)	9.0 (8.1–9.9)	22.0 (19.5–25.2)	71.9	Gadgeel et al.[43]
				Chemotherapy	206	19.4 (14.2–22.0)	4.9 (4.7–5.5) HR: 0.48; (0.40–0.58)	10.7 (8.7–13.6) HR: 0.56; (0.45–0.70)	66.8	
KEYNOTE-407	Squamous	III	Any	Pembrolizumab plus chemotherapy	278	57.9 (51.9–63.8)	6.4 (6.2–8.3)	15.9 (13.2–NR)	69.8	Paz-Ares et al.[44]
				Chemotherapy	281	38.4 (32.7–44.4)	4.8 (4.3–5.7) p<0.001	11.3 (9.5–14.8) p<0.001	68.2	
KEYNOTE-407	Squamous	III	Any	Pembrolizumab plus chemotherapy	278	62.6 (56.6–68.3)	8.0 (6.3–8.4)	17.1 (14.4–19.9)	74.1	Paz-Ares et al.[45]
				Chemotherapy	281	38.4 (32.7–44.4)	5.1 (4.3–6.0) HR: 0.57; (0.47–0.69)	11.6 (10.1–13.7) HR: 0.71; (0.58–0.88)	69.6	
KEYNOTE-021	Squamous or non-squamous	I/II	Any	Pembrolizumab plus carboplatin plus paclitaxel	25	48	10.3 (6.1–14.6)	21.4 (10.5–NR)	40	Gadgeel et al.[46]
				Carboplatin plus paclitaxel plus bevacizumab	25	56	7.1 (4.2–14.3)	16.7 (8.5–NR)	42	
				Carboplatin plus pemetrexed	24	75	10.2 (6.5–13.9)	16.7 (13.9–29.2)	46	
KEYNOTE-021	Non-squamous	II	Any	Pembrolizumab and chemotherapy	60	55 (42.0–68.0)	13.0 (8.3–NR)	NR	39	Langer et al.[47]
				Chemotherapy alone	63	29 (18.0–41.0) p=0.0016	8.9 (4.4–10.3) p=0.010	NR p=0.39	26	

(Continued)

Table 2. (Continued)

Study	Histology	Phase	PD-L1 TPS (%)	Arms	Case number	ORR% (95% CI or p value)	mPFS (95% CI or p value, months)	mOS (95% CI or p value, months)	Grade 3–5 AE%	References
KEYNOTE-021	Non-squamous	II	Any	Pembrolizumab and chemotherapy	60	58	24.5 (9.7–36.3)	34.5 (24.0–NR)	39	Awad et al.[48]
				Chemotherapy alone	63	33	9.0 (6.2–15.2) HR: 0.54; (0.35–0.83)	21.1 (14.9–35.6) HR: 0.71; (0.45–1.12)	31	
KEYNOTE-024	Squamous or non-squamous	III	≥50	Pembrolizumab	154	44.8 (36.8–53.0)	10.3 (6.3–NR)	30.0 (18.3–NR)	31.2	Reck et al.[49,50]
				Chemotherapy	151	27.8 (20.8–35.7)	6.0 (4.2–6.2) p<0.001	14.2 (9.8–19.0) p=0.002	53.3	
KEYNOTE-042	Squamous or non-squamous	III	≥50	Pembrolizumab	299	39	7.1 (5.9–9.0)	20.0 (15.4–24.9)	18	Mok et al.[51]
				Chemotherapy	300	32	6.4 (6.1–6.9) p=0.0170	12.2 (10.4–14.2) p=0.0003	41	
HOPE-001	Squamous or non-squamous	Real-word study	≥50	Pembrolizumab	213	51.2	8.3 (6.0–10.7)	17.8 (17.8–NR)	18.3	Tamiya et al.[52]
CheckMate 012	Squamous or non-squamous	I	Any	Nivolumab	52	23 (12.0–52.0)	3.6 (0.1–28.0)	19.4 (0.2–35.8)	19	Gettinger et al.[53]
CheckMate 012	Squamous or non-squamous	I	Any	2 weeks nivolumab plus 12 weeks ipilimumab	38	47 (31–64)	NA	NR	37	Hellmann et al.[54]
				2 weeks nivolumab plus 6 weeks ipilimumab	40	38 (23–55)	NA	NR	33	
CheckMate 568	Squamous or non-squamous	II	Any	Nivolumab (TMB ≥10)	48	44	7.1 (3.6–11.3)	NR	29	Ready et al.[57]
				Nivolumab (TMB <10)	50	12	2.6 (1.4–5.4)	NR		
CheckMate 026	Squamous or non-squamous	III	≥5	Nivolumab	211	26 (20–33)	4.2 (3.0–5.6)	14.4 (11.7–17.4)	18	Carbone et al.[58]
				Chemotherapy	212	33 (27–40)	5.9 (5.4–6.9) p=0.25	13.2 (10.7–17.1) HR: 1.01; (0.80–1.30)	51	

(Continued)

Table 2. (Continued)

Study	Histology	Phase	PD-L1 TPS (%)	Arms	Case number	ORR% (95% CI or p value)	mPFS (95% CI or p value, months)	mOS (95% CI or p value, months)	Grade 3–5 AE%	References
BIRCH	Squamous or non-squamous	NA	⩾5	Atezolizumab	139	22	5.4 (3.0–6.9)	20.1 (20.1–NR)	40	Peters et al.[59]
IMpower150	Non-squamous	III	Any	ACP	402	49.2	7.0	18.6	NA	Socinski et al.[60]
				BCP	400	53.5	6.8	14.7	47.7	
				ABCP	400	69.3	8.3 (p < 0.001)	19.2 (p = 0.02)	57.7	
IMpower132	Non-squamous	III	Any	APP	292	49.6	7.6 (6.6–8.5)	18.1 (13.0–NR)	53.6	Papadimitrak-opoulou et al.[61]
				PP	286	32.2	5.2 (4.3–5.6) p < 0.0001	13.6 (11.4–15.5) p = 0.0797	39.1	
IMpower130	Non-squamous	III	Any	Atezolizumab plus chemotherapy	451	49.2	7.0 (6.2–7.3)	18.6 (16.0–21.2)	81.0	Planchard et al.[62]
				Chemotherapy	228	31.9	5.5 (4.4–5.9) p < 0.0001	13.9 (12.0–18.7) p = 0.033	71.0	
IMpower131	Squamous	III	Any	Atezolizumab + CnP	343	49.7	6.3	14.2	69.2	Jotte et al.[63]
				CnP	340	41.0	5.6 (p = 0.0001)	13.5 (p = 0.16)	58.4	
Impower110	Squamous or non-squamous	III	⩾50	Atezolizumab	107	38.3	8.1	20.2 (16.5–NR)	33.9	Herbst et al.[64]
				Chemotherapy	98	28.6	5.0 HR: 0.63; (0.45–0.88)	13.1 (7.4–16.5) p = 0.01)	56.7	

ABCP, atezolizumab plus BCP; AE, adverse event; ACP, atezolizumab plus carboplatin plus paclitaxel; APP, atezolizumab plus pemetrexed plus carboplatin or cisplatin; BCP, bevacizumab plus carboplatin plus paclitaxel; CI, confidence interval; CnP, carboplatin + nab-paclitaxel; HR, hazard ratio; mOS, median overall survival; mPFS, median progression-free survival; NA, not applicable; NSCLC, non-small-cell lung cancer; NR, not reached; ORR, objective response rate; PD-1, programmed cell-death protein 1; PD-L1, programmed cell-death ligand 1; PP, pemetrexed plus carboplatin or cisplatin; TMB, tumour mutational burden; TPS, tumour proportion score.

group than in the docetaxel group.[69,70] The CheckMate 057 trial evaluated the efficacy of nivolumab in patients with advanced non-squamous NSCLC who experienced obvious disease progression after first-line treatment. Compared with docetaxel, nivolumab treatment resulted in a remarkably longer mOS, with a more favourable safety profile.[71,72] In both the CheckMate 017 and CheckMate 057 trials, the 2-year OS rates of patients with squamous NSCLC treated with nivolumab compared with docetaxel were about 23% and 8%, respectively. In those with non-squamous NSCLC, the 2-year OS rates of those treated with nivolumab were 29% and 16% in the CheckMate 017 and CheckMate 057 trials, respectively.[73] The CheckMate 078 trial compared the safety and efficacy of nivolumab with docetaxel in patients with squamous or non-squamous NSCLC.[74] The results were consistent with those of the CheckMate 017 and CheckMate 057 trials. Another Argentinian study was conducted to explore the efficacy and safety of nivolumab in previously treated patients with advanced NSCLC; the results showed that nivolumab was well tolerated, with promising prognostic outcomes in patients with NSCLC who had undergone previous treatment.[75] A multicentre retrospective observational study in Japan was performed to evaluate the efficacy of nivolumab in second-line treatment in NSCLC patients. The mOS was 14.6 months (95% CI 12.3–15.9); 1-year survival rate was 54.3%; mPFS was 2.1 months (95% CI 1.9–2.4); and ORR was 20.5%. Subgroup analysis showed that PS, EGFR mutation status, smoking status, and PD-L1 were associated with the effectiveness of nivolumab.[76]

KEYNOTE-010, an open-label, randomised phase II/III trial, included pretreated NSCLC patients with an expression level of PD-L1 ≥1% in tumour cells who had received treatment with pembrolizumab or docetaxel. This study showed that treatment with pembrolizumab significantly prolonged mOS in patients with previously treated, advanced NSCLC, especially in those with high PD-L1 expression levels.[77] Patients in the KEYNOTE-010 trial were followed up to assess long-term outcomes. At a median follow-up time point of 42.6 months (range 35.2–53.2 months), those treated with pembrolizumab experienced a significantly improved mOS compared with those treated with docetaxel. The 3-year OS rates were 34.5% *versus* 12.7% (for those with PD-L1 TPS ≥50%) and 22.9% *versus* 11.0% (for those with PD-L1 TPS ≥1%),

respectively.[78] The KEYNOTE-025 trial assessed the safety and efficacy of pembrolizumab in patients with NSCLC who had been previously treated in Japan.[79] The results showed that pembrolizumab was associated with a generally high ORR in those with a PD-L1 expression level ≥50%. The phase I/II KEYNOTE-021 study evaluated combination therapy with pembrolizumab plus ipilimumab in patients with previously treated advanced NSCLC. This study showed that among patients who received pembrolizumab (2 mg/kg) plus ipilimumab (1 mg/kg), ORR was 30%, mPFS was 4.1 months (95% CI 1.4–5.8), and mOS was 10.9 months (95% CI 6.1–23.7) but was associated with meaningful toxicity.[80]

A randomised phase III clinical trial, OAK, also showed that regardless of the expression level of PD-L1, the survival rate following atezolizumab treatment was significantly improved compared with docetaxel. Based on the OAK findings, atezolizumab has been approved by the FDA and the EMA for further treatment of those previously treated for advanced NSCLC. For long-term survivors in the atezolizumab group, the ORR was 14% and the mOS was 13.8 months (95% CI 11.8–15.7).[81,82] In the POPLAR study, an open-label, phase II trial, atezolizumab treatment significantly improved OS compared with docetaxel in patients with NSCLC who were previously treated.[83] The BIRCH study also proved the efficacy of atezolizumab in patients with advanced NSCLC as second-line therapy. The ORR, mPFS, and mOS were 19%, 2.8 months (95% CI 1.5–3.9), and 15.5 months (95% CI 12.3–not reached), respectively.[59] It is well known that approximately 10% of patients with NSCLC are complicated with comorbid interstitial pneumonia, with a poor prognosis. The Thoracic Oncology Research Group 1936/AMBITIOUS study is an ongoing, multicentre, single-arm, phase II trial to assess the safety and efficacy of atezolizumab for pretreated advanced/recurrent patients with NSCLC complicated with idiopathic chronic fibrotic interstitial pneumonia. We are looking forward to the results of this clinical study.[84] Another multicentre, phase II, open-label, single-arm VinMetAtezo trial was designed to assess the safety and efficacy of metronomic oral vinorelbine in combination with atezolizumab in advanced NSCLC progressing after first-line platinum-based chemotherapy. The primary outcome was PFS at 4 months.[85] In France, Marine *et al*. conducted a study to evaluate the

cost effectiveness of atezolizumab *versus* docetaxel as a second-line treatment for stage IV NSCLC. Their results indicated that atezolizumab was more efficacious and more costly than docetaxel in treating advanced NSCLC.[86] All studies that have assessed PD-1/PD-L1 inhibitors as second-line therapies are summarised in Table 3.

PD-1/PD-L1 inhibitors as third-line or greater treatment in advanced NSCLC

The BIRCH study examined the clinical efficacy of atezolizumab as third-line or greater therapy in advanced NSCLC. The mOS, mPFS, and ORR were 13.2 months (95% CI 10.3–17.5), 2.8 months (95% CI 1.5–3.9), and 18%, respectively as third-line treatment, suggesting that atezolizumab has good clinical efficacy as third-line therapy for advanced NSCLC.[59]

The ATLANTIC trial was an open-label, phase II, multicentre clinical trial conducted to assess the efficacy of durvalumab as third-line or greater therapy for advanced NSCLC. The ORR was 12.2% in EGFR[+] and ALK[+] patients who had expression levels of PD-L1 ≥25% and received durvalumab, which was lower than that of patients who were EGFR[−] and ALK[−] with the same expression level of PD-L1 ≥25% (16.4%) or those with expression of PD-L1 ≥90% (30.9%). The mPFS rates in the three groups were 1.9 months, 3.3 months, and 2.4 months, respectively.[87]

ARCTIC was a phase III, randomised, open-label study that evaluated the efficacy of durvalumab with or without tremelimumab *versus* the standard of care for advanced NSCLC as a greater than third-line therapy. For patients with expression levels of PD-L1 ≥25%, durvalumab resulted in significant improvements in mOS and mPFS compared with the standard of care. For patients with expression of PD-L1 < 25%, durvalumab combined with tremelimumab resulted in obvious improvements in mOS compared with the standard of care.[88] A detailed summary of the clinical trials of third-line or greater treatment of advanced NSCLC is shown in Table 3.

PD-1/PD-L1 inhibitors in cases of advanced NSCLC with driver-gene mutations

Inhibition of the PD-1/PD-L1 axis has resulted in favourable responses in NSCLC patients with EGFR mutations; however, the efficacy was significantly lower in these patients compared with those without EGFR mutations.[89,90] Although high expression levels of PD-L1 in patients with advanced NSCLC may be associated with EGFR mutations,[91] studies have shown that EGFR-tyrosine kinase inhibitors (TKIs) downregulate the messenger ribonucleic acid (mRNA) expression of PD-L1, suggesting that EGFR-TKIs may activate T-cell immunocompetence in the TME of NSCLC patients with EGFR-mutations.[92–94] A phase II clinical trial showed that pembrolizumab was less effective in advanced NSCLC patients who were naïve to TKIs and were PD-L1[+], with EGFR mutations, even those with a PD-L1 expression level ≥50%; this indicated that anti-PD-1/PD-L1 treatment was not the ideal first choice for patients with EGFR mutations.[95]

The phase I/II KEYNOTE-021 trial analysed the feasibility of administering erlotinib or gefitinib combined with pembrolizumab as first-line treatment in patients with stage IIIb/IV EGFR-mutant NSCLC. Pembrolizumab plus gefitinib combination therapy had to be continued because of severe AEs, and another study showed that pembrolizumab plus erlotinib administration did not improve the ORR when compared with pembrolizumab plus gefitinib.[96] The CheckMate 370 trial evaluated the safety of first-line treatment with nivolumab combined with crizotinib in patients with ALK[+] NSCLC. Again, the combination treatment had to be discontinued, and enrolment was closed because of observed grade ≥3 hepatic toxicities.[97]

A subgroup analysis of advanced NSCLC patients with EGFR mutations and ALK rearrangements was performed to assess the use of anti-PD-1/PD-L1 therapy as second-line treatment in the CheckMate 057, KEYNOTE-010, POLAR and OAK studies. Their results revealed that EGFR mutations and ALK rearrangements in patients with advanced NSCLC were associated with beneficial outcomes and prolonged mOS from anti-PD-1/PD-L1 treatment compared with chemotherapy when administered as second-line treatment.[71,72,77,78,81–83] In a study assessing the effects of nivolumab, the ORR was significantly lower in patients with EGFR-mutant non-squamous NSCLC than in patients without the mutation (8.8% *versus* 19.6%, $p = 0.007$).[98] It is well known that the acquired EGFR-T790M mutation is a major reason for patients developing resistance to EGFR-TKIs. A clinical study showed that of 25 NSCLC patients who received nivolumab following previous TKI administration, 8 patients

Table 3. Efficacy of anti-PD-1/PD-L1 agents for advanced NSCLC in second line and third line.

Study	Histologies	Phase	PD-L1 TPS (%)	Arms	Case number	ORR (95% CI or *p* value)	mPFS (95% CI or *p* value, months)	mOS (95% CI or *p* value, months)	Grade 3–4 AE%	Refences
Checkmate 017 (second)	Squamous	III	Any	Nivolumab	135	20 (14–28)	3.5 [2.1–4.0]	9.2 [7.3–13.3]	7	Brahmer et al.[69]; Reck et al.[70]
				Docetaxel	137	9 (5–15) p=0.008	2.8 [2.1–3.5] p<0.01	6.0 [5.1–7.3] p<0.001	55	
Checkmate 057 (second)	Non-squamous	III	Any	Nivolumab	292	19 (15–24)	2.3 [2.2–3.3]	12.2 [9.7–15.0]	10	Borghaei et al.[71]; Reck et al.[72]
				Docetaxel	290	12 [7–17] p=0.02	4.2 [3.5–4.9] p=0.39	9.4 [8.1–10.7] p=0.002	54	
Checkmate 078 (second)	Squamous or non- squamous	III	Any	Nivolumab	338	16.6 (12.2–21.0)	2.8 [2.4–3.4]	12.0 [10.4–14.0]	10	Wu et al.[74]
				Docetaxel	166	4.2 (1.7–8.5) p<0.0001	2.8 [1.6–2.9] p=0.0147	9.6 [7.6–11.2] p=0.0006	48	
Keynote-010 (second)	Squamous or non- squamous	II/III	≥1%	Pembrolizumab 2 mg/kg	345	18	3.9 [3.1–4.1] p=0.07	10.4 [9.4–11.9] p=0.0008	13	Herbst et al.[77,78]
				Pembrolizumab 10 mg/kg	346	18	4.0 [2.7–4.3] p=0.004	12.7 [10.0–17.3] p<0.0001	16	
				Docetaxel	343	9 (p=0.0005)	4.0 [3.1–4.2]	8.5 [7.5–9.8]	35	
			≥50%	Pembrolizumab 2 mg/kg	139	30	5.0 [4.0–6.5] p=0.0002	14.9 [10.4–NR] p=0.0002	NA	
				Pembrolizumab 10 mg/kg	151	29	5.2 [4.1–8.1] p<0.0001	17.3 [11.8–NR] p<0.0001	NA	
				Docetaxel	152	8 (p<0.0001)	4.1 [3.6–4.3]	8.2 [6.4–10.7]	NA	
Keynote-025 (second)	Squamous or non- squamous	Ib	≥1%	Pembrolizumab	38	22 [10–38]	3.9 [2.0–6.2]	19.2 [8.0–26.7]	29	Nishio et al.[79]
			≥50%		12	27 [6–61]	4.1 [1.6–19.1]	17.9 [5.9–NR]	NA	
Keynote-021 (≥2)	NSCLC	I/II	Any	Pembrolizumab plus ipilpmumab	44	30	4.1 [1.4–5.8]	10.9 [6.1–23.7]	29	Gubens et al.[80]
OAK (second)	Squamous or non- squamous	III	Any	Atezolizumab	425	14	2.8 [2.6–3.0]	13.8 [11.8–15.7]	15	Rittmeyer et al.[81]; von Pawel et al.[82]

(Continued)

Table 3. (Continued)

Study	Histologies	Phase	PD-L1 TPS (%)	Arms	Case number	ORR (95% CI or p value)	mPFS (95% CI or p value, months)	mOS (95% CI or p value, months)	Grade 3–4 AE%	Refences
				Docetaxel	425	13	4.0 [3.3–4.2] p=0.049	9.6 [8.6–11.2] p=0.0003	43	Fehrenbacher et al.[83]
POPLAR (second)	Squamous or non-squamous	II	Any	Atezolizumab	144	17 [11.0–23.8]	2.7	12.6 [9.7–16.4]	11	
				Docetaxel	143	15 [9.3–21.4]	3.0 HR: 0.94; (0.72–1.23)	9.7 [8.6–12.0] p=0.04	39	
BIRCH (second)	Squamous or non-squamous	NA	≥5%	Atezolizumab	268	19	2.8 [1.5–3.9]	15.5 [12.3–NR]	NA	Peters et al.[59]
BIRCH (third)	Squamous or non-squamous	NA	≥5%	Atezolizumab	252	18	2.8 [1.5–3.9]	13.2 [10.3–17.5]	NA	Peters et al.[59]
ATLANTIC (third)	Squamous or non-squamous	II	≥25%	EGFR and ALK positivity (durvalumab)	74	12.2 [5.7–21.8]	1.9 (1.803.6)	13.3 [8.1–NR]	6	Garassino et al.[87]
				EGFR and ALK negativity (durvalumab)	146	16.4 [10.8–23.5]	3.3 [1.9–3.7]	10.9 [8.6–13.6]	9	
				≥90% PD-L1 expression (durvalumab)	68	30.9 [20.2–43.3]	2.4 [1.8–5.5]	NR [9.5–NR]	18	
ARCTIC (≥3)	Squamous or non-squamous	III	≥25	durvalumab	62	35.5	3.8 [1.9–5.6]	11.7 [8.2–17.4]	9.7	Planchard et al.[88]
				Standard of care	64	12.5	2.2 [1.9–3.7] HR: 0.71 (0.49–1.04)	6.8 [4.9–10.2] HR: 0.63 (0.42–0.93)	44.4	
			<25	Durvalumab + tremelimumab	174	14.9	3.5 [2.3–4.6]	11.5 [8.7–14.1]	22.0	
				Standard of care	118	6.8	3.5 [1.9–3.9] p=0.056	8.7 [6.5–11.7] p=0.109	36.4	

AE, adverse event; ALK, anaplastic lymphoma kinase; CI, confidence interval; EGFR, epidermal growth-factor receptor; HR, hazard ratio; mOS, median overall survival; mPFS, median progression-free survival; NA, not applicable; NSCLC, non-small-cell lung cancer; NR, not reached; ORR, objective response rate; PD-1, programmed cell-death protein 1; PD-L1, programmed cell-death ligand 1; TMB, tumour mutational burden; TPS, tumour proportion score.

exhibited the T790M mutation, rendering them resistant to EGFR-TKI treatment, whereas 17 patients did not. The mPFS times were 1.3 months (95% CI 0.1–1.8) and 2.1 months (95% CI 1.3–3.4) for the T790M-positive and T790M-negative patients, respectively ($p = 0.099$), and the mOS was not achieved at the follow up at 7.3 months. Overall, this study indicated that patients resistant to EGFR-TKI with negative expression of T790M probably benefited from subsequent treatment with nivolumab.[99] Hence, some studies have explored combination therapy in advanced NSCLC patients who are resistant to TKIs.[100,101] Gettinger et al. performed a study to assess the efficacy of nivolumab combined with erlotinib in advanced NSCLC patients with EGFR mutations who were treated with an EFGR-TKI but no chemotherapy.[102] Another multicentre, open-label, phase Ib dose-escalation clinical trial was performed with patients previously treated with an ALK-TKI or chemotherapy who had advanced, ALK-rearranged NSCLC.[103] The patients received either nivolumab plus ceritinib (450 mg daily) or nivolumab plus ceritinib (300 mg daily). The results showed that the ORR and mPFS were improved in the nivolumab plus ceritinib (450 mg) group; however, this combination of drugs also associated with increased toxicity. Based on safety considerations, an alternative regimen is being investigated in which ceritinib is administered as a monotherapy for two cycles before the initiation of combination treatment with nivolumab.

One of the studies intended to identify a biomarker for patients most likely to positively respond to immunotherapy. Tumour deoxyribonucleic acid (DNA) was derived from advanced NSCLC patients receiving nivolumab or pembrolizumab therapy as second- or third-line treatment. The most common mutated genes observed in these patients were tumour protein p53 (TP53; 49%), Kirsten rat sarcoma 2 viral oncogene homolog (KRAS; 43%), and v-erb-b2 erythroblastic leukaemia viral oncogene homolog 2 (ERBB2; 13%). This study confirmed that patients with KRAS mutations experienced a longer mPFS and mOS than patients without KRAS mutations. Furthermore, patients with ERBB-family mutations failed to respond to treatment with PD-1 antibodies.[104] The efficacy data of anti-PD-1/PD-L1 for advanced NSCLC with driver gene mutations is shown in Table 4.

Application of PD-1/PD-L1 inhibitors in advanced NSCLC cases in China

Toripalimab (also known as JS001 or TAB001) is a recombinant human anti-PD-1 mAb developed by Shanghai Junshi Biotechnology Co., Ltd. that selectively inhibits PD-1 and PD-L1/PD-L2 interactions to facilitate T-cell activation.[105] Yang et al. conducted a phase I dose escalation and expansion clinical study to evaluate the safety and tolerability of toripalimab in patients with advanced or recurrent malignancies in China.[106] Thirty-three patients participated in this study, seven of which had NSCLC. The results showed that toripalimab was well tolerated at a dose of 10 mg/kg every 2 weeks, and no dose-limiting toxicity was apparent; in addition, long-term anti-tumour activity was achieved in patients with alveolar soft-part sarcomas.[106]

Sintilimab is a human IgG4 mAb coupled to PD-1; it was jointly developed by Innovent Biologics and Eli Lilly and Company. Sintilimab was approved for treating patients with classical Hodgkin's lymphoma who had relapsed or were refractory after at least two rounds of systemic chemotherapy in China.[107] A phase Ib study was performed to assess the safety and efficacy of sintilimab as a neoadjuvant therapy for NSCLC; the results showed that sintilimab was well tolerated in NSCLC patients, with an encouraging pathologic response rate.[108]

Camrelizumab, a PD-1 inhibitor, developed by Jiangsu Hengrui Pharmaceutical Co., Ltd., has recently been approved for the treatment of relapsed or refractory classical Hodgkin's lymphoma in China.[109] A study evaluated the efficacy and safety of camrelizumab combined with microwave ablation (MWA) in patients with metastatic NSCLC. The results indicated that the ORR was 33.3%, with two patients achieving a complete response (CR) and five patients achieving a partial response (PR). The mPFS was 5.1 months; OS was not reached. The most common AE related to camrelizumab administration was reactive capillary hyperplasia of the skin[110] that could be alleviated by substitution with apatinib.[111]

The anti-PD-1 antibody, tislelizumab, has demonstrated clinical tolerability and safety in treating solid tumours, including NSCLC in Chinese patients.[112] Toripalimab, sintilimab, camrelizumab and tislelizumab are all currently undergoing clinical trials for the treatment of various solid

Table 4. Efficacy of anti-PD-1/PD-L1 agents for advanced NSCLC with driver gene mutations.

Study	Therapy line	Phase	Driver gene mutant	PD-L1 TPS (%)	Arms	Case number	ORR (95% CI or p value)	mPFS (95% CI or p value, months)	mOS (95% CI or p value, months)	Grade 3–4 AE%	References
NCT02879994	First line	II	EGFR	≥1%	Pembrolizumab	11	9	3.9	NR	NA	Lisberg et al.[95]
KEYNOTE-021 (NCT02039674)	First line	I/II	EGFR	Any	Pembrolizumab plus gefitinib	7	14.3	1.4 (0.2–13.0)	13.0 (0.2–NR)	71.5	Yang et al.[96]
					Pembrolizumab plus erlotinib	12	41.7	19.5 (3.0–19.5)	NR	33.3	
Checkmate 370	First line	I/II	ALK	Any	Nivolumab Plus Crizotinib	13	38	NA	NA	38	Spigel et al.[97]
Garassino et al.[98]	≥2	NA	WT-EGFR	Any	Nivolumab	1293	19.6 (17.4–21.7)	3.0 (2.8–31)	11.0 (10.0–12.0)	6	Garassino et al.[98]
			EGFR-mutant			102	8.8 (3.3–14.3) p=0.007	3.0 (2.7–3.3) p=0.004	8.3 (2.2–14.4) p=0.046	7	
Haratani et al.[99]	Second line	NA	T790M-negative	Any	Nivolumab	17	24	2.1 (1.3–3.4)	NR	NA	Haratani et al.[99]
			T790M-positive			8	13 p=1.000	1.3 (0.1–1.8) p=0.099	NR	NA	
Gettinger et al.[102]	Second line	I	EGFR	Any	Nivolumab Plus erlotinib	21	15	5.1 (2.3–12.2)	18.7 (7.3–NR)	24	Gettinger et al.[102]
Felip et al.[103]	Second line	Ib	ALK	Any	Ceritinib 450 mg plus nivolumab	8	50 (16–84)	6.4 (0.8–13.7)	NA	93	Felip et al.[103]
					Ceritinib 300 mg plus nivolumab	12	25 (6–57)	3.7 (1.8–NR)	NA	82	

ABCP, atezolizumab plus bevacizumab plus carboplatin plus paclitaxel; AE, adverse event; ALK, anaplastic lymphoma kinase; CI, confidence interval; EGFR, epidermal growth-factor receptor; mOS, median overall survival; mPFS, median progression-free survival; NA, not applicable; NSCLC, non-small-cell lung cancer; NR, not reached; ORR, objective response rate; PD-1, programmed cell-death protein 1; PD-L1, programmed cell-death ligand 1; TPS, tumour proportion score; WT, wild type.

tumours, including advanced NSCLC; these are summarised in Table 5.

Predictive biomarkers in anti-PD-1/PD-L1 immunotherapy

In order to predict the response to immunotherapy, the biomarkers initially used by the researchers were the expression of PD-1 receptor and PD-L1, and the prediction method was assessed only by immunohistochemistry.[113] More recently, with the development with newer technology, there are many available biomarkers that could be used to further predict the efficacy and prognosis of immunotherapy.[113–117] These include PD-L1 expression, the number of tumour-infiltrating lymphocytes (TILs), the TMB, mutations in certain driver genes, microsatellite instability (MSI)/ defective mismatch repair (dMMR), peripheral blood-based biomarkers and interferon gamma (IFN-γ) levels (Figure 2).

Level of PD-L1 expression and the number of TILs

Currently, the level of PD-L1 is commonly used as a tumour biomarker to evaluate the efficacy of anti-PD-1/PD-L1 therapy in patients with NSCLC.[34,118] The KEYNOTE-024 and KEYNOTE-042 trials[49–51] suggested that pembrolizumab could significantly improve the survival rate in patients with high expression levels of PD-L1. The KEYNOTE-001 study also indicated that patients with a PD-L1 TPS ≥ 50% would likely experience better outcomes, including improved ORR, mPFS, and mOS.[40] Therefore, pembrolizumab has been approved by the FDA for advanced NSCLC patients without driver gene mutations as a first-line treatment. In a single-arm study assessing early ICIs in selected patients with high expression levels of PD-L1, Ito *et al.* found that ORR and mPFS could be used as an endpoint to predict the survival of patients with high PD-L1 expression levels.[119] Teramoto *et al.* analysed PD-L1 immunohistochemistry in tumour patients at stage I–IIIa NSCLC; they found that high PD-L1 expression is strongly related to early-stage NSCLC and, therefore, is a representative biomarker for predicting a good prognosis.[120] Recently, Ricci *et al.* investigated 150 advanced NSCLC patients, they suggested PD-L1 expression with a cut-off of 50% can be effectively evaluated by the cytological smears.[121] Another study showed that PD-L1 expression, CD8+ T cell infiltration, the TMB, and the

human leukocyte antigen (HLA) class I were outstanding predictive biomarkers of the anticipated response to anti-PD-1 immunotherapy.[122] The mPFS was significantly improved in patients with both a high level of PD-L1 expression and TMB or in those without a loss of HLA class I molecules.

The TME plays a vital role in the growth and progression of cancer cells and distant metastasis, and affects the efficacy of immunotherapy in clinical oncology.[123] TILs mainly include NK cells and cytotoxic T cells, both of which are routinely assessed in histopathology reports as predictive biomarkers.[124,125] In hyperprogressive disease patients, a high number of CD2− CD4 T lymphocytes during the first and second line of immunotherapy was associated with a lower mOS.[126] A retrospective study by Chen *et al.*, assessed tumour PD-L1 expression and T-cell infiltration in NSCLC patients with rare EGFR mutations and found that the frequency of a high PD-L1 expression level and CD8+ TIL infiltration was 36.7%, with a worse prognosis.[127]

TMB

The TMB is representative of the number of tumour mutations; the higher the frequency of mutations in somatic exonic regions, the greater the number of corresponding tumour-related antigens that are expressed.[128,129] For patients with advanced NSCLC, a high TMB detected by next-generation sequencing seems to predict the lasting clinical effects of PD-1 or PD-L1 blockades.[130] This results in the subsequent increase in activation of lymphocytes to maintain an immunological anti-tumour response. A previous study evaluated the predictive role of the TMB in 76 NSCLC patients treated with anti-PD-1/PD-L1 therapy. Patients with a high TMB exhibited significantly longer mPFS and mOS.[131] In a Chinese study, the TMB was analysed in NSCLC patients receiving anti-PD-1/PD-L1 treatment; the mPFS was significantly longer at 10.6 months in the high TMB group compared with just 3.9 months in the low TMB group ($p = 0.0007$), and the mOS was 21.0 months in the high TMB group and just 11.6 months in the low TMB group ($p = 0.0126$).[132] The circulating tumour DNA (ctDNA)-related TMB was also assessed in NSCLC patients who received anti-PD-1 or anti-PD-L1 therapy;[133] however, higher ctDNA-related TMBs were obviously related to shorter mOS and mPFS, which indicated that a higher ctDNA TMB

Table 5. Application of PD-1/PD-L1 inhibitors in the advanced NSCLC in China.

Drug	ClinicalTrials.gov identifier	Phase	Condition	Therapy line	Design	Patients (n)	Recruiting status	Locations
Toripalimab	NCT03513666	II	Advanced NSCLC with EGFR-mutation positive and T790M negative	Second line	Toripalimab combined with pemetrexed plus carboplatin	40	Not yet	Jiangsu Province Hospital
Toripalimab	NCT04316351	II	IIIb/IV NSCLC with T790M-positive mutations failed to osimertinib therapy	Second line	Toripalimab (JS001) combined with pemetrexed and anlotinib	60	Not yet	Guangzhou Institute of Respiratory Disease
Toripalimab	NCT03856411	III	Advanced NSCLC	First line	Toripalimab injection or placebo combined with standard chemotherapy	450	Recruiting	Cancer Hospital Chinese Academy of Medical Sciences
Toripalimab	NCT03924050	III	Advanced NSCLC with TKI-resistant EGFR mutated	Second line	Toripalimab or placebo combined with chemotherapyin	350	Recruiting	Shanghai Pulmonary Hospital
Sintilimab	NCT03798743	II	Advanced NSCLC	Second line	Sintilimab combined with docetaxel	30	Recruiting	Hunan Province Tumor Hospital
Sintilimab	NCT03765775	II	Advanced NSCLC with first-generation EGFR-TKI resistance along with T790M negative	Second line	Anlotinib combined with sintilimab	20	Recruiting	First Hospital of Shijiazhuang
Sintilimab	NCT04252365	II	Advanced NSCLC	First line	Sintilimab and pembrolizumab	20	Not yet	Guangdong Association of Clinical Trials
Sintilimab	NCT03812549	I	Advanced NSCLC	First line	Sintilimab combination with radiation	29	Recruiting	West China Hospital, Sichuan University
Sintilimab	NCT04124731	II	Advanced NSCLC	First line	Sintilimab combined with anlotinib	98	Recruiting	Tianjin Medical University Cancer Institute and Hospital
Sintilimab	NCT03802240	III	EGFR-mutant advanced non-squamous NSCLC	Second line	Sintilimab ± IBI305 combined with pemetrexed and cisplatin	600	Recruiting	Shanghai Chest Hospital
Sintilimab	NCT04213170	II	Advanced NSCLC with brain matastases	NA	Sintilimab combined with bevacizumab	60	Recruiting	Sun Yat-sen University
Sintilimab	NCT03830411	II	Nonsquamous NSCLC with wild-type EGFR	Second line	Sintilimab compared with docetaxel or pemetrexed	76	Recruiting	China Three Gorges University
Camrelizumab	NCT04211090	II	Nonsquamous NSCLC with brain matastases and without driver gene mutations	First line	Camrelizumab combined with pemetrexed/carboplatin	64	Recruiting	Sun Yat-sen University
Camrelizumab	NCT04203485	III	PD-L1-positive advanced NSCLC	First line	Camrelizumab combined with apatinib mesylate or camrelizumab alone	762	Recruiting	Jiangsu HengRui Medicine Co., Ltd
Camrelizumab	NCT04303130	II	Advanced squamous NSCLC	First line	Camrelizumab combined with endostar	52	Recruiting	Beijing Cancer Hospital
Camrelizumab	NCT04167774	II	Advanced NSCLC	Second line	Camrelizumab combined with paclitaxel	62	Recruiting	Sun Yat-sen University
Tislelizumab	NCT03663205	III	Advanced non-squamous NSCLC	First line	Tislelizumab with chemotherapy *versus* chemotherapy	334	Not yet	Anhui Provincial Hospital
Tislelizumab	NCT03594747	III	Advanced squamous NSCLC	First line	Tislelizumab with chemotherapy *versus* chemotherapy	360	Not yet	Anhui Provincial Hospital

EGFR, epidermal growth-factor receptor; NSCLC, non-small-cell lung cancer; PD-1, programmed cell-death protein 1; PD-L1, programmed cell-death ligand 1; TKI, tyrosine kinase inhibitor.
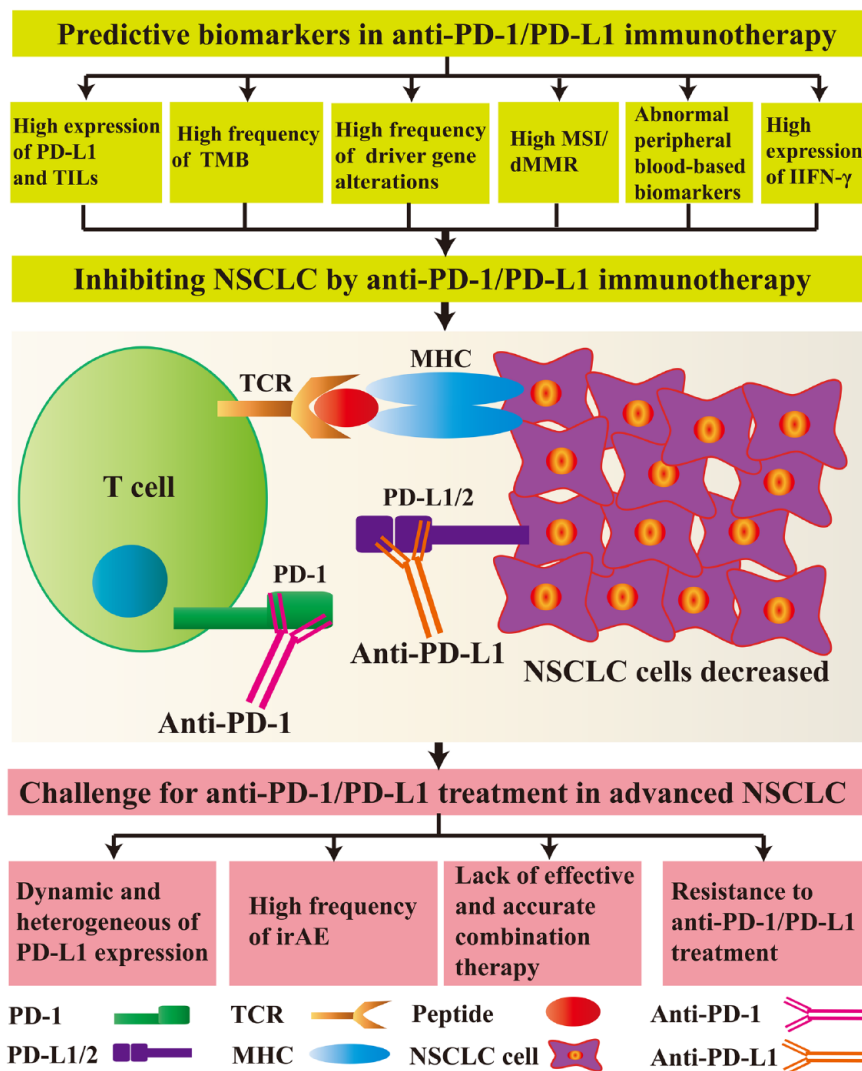
Figure 2. Predictive biomarkers and challenges facing anti–programmed cell-death protein 1 (PD-1)/programmed cell-death ligand 1 (PD-L1) treatment in advanced non-small-cell lung cancer (NSCLC). For inhibiting NSCLC cells, the predictive biomarkers include high expression levels of PD-L1, high numbers of tumour infiltrating lymphocytes (TILs), high tumour mutational burden (TMB), a high frequency of driver-gene alterations, high microsatellite instability (MSI)/defective mismatch repair (dMMR), abnormal peripheral blood-based biomarkers, and high expression levels of interferon gamma (IFN-γ). After administration of anti-PD-1 or anti-PD-L1 antibodies, the interaction between PD-1 and PD-L1/L2 is disrupted, inhibiting cell signalling pathways. T cells and other immune-related cells exert anti-tumour effects; however, there are still many unresolved problems in the clinical use of anti-PD-1/PD-L1 therapies, such as dynamic changes and heterogeneous differences in PD-L1 expression, a high frequency of immune-related adverse events (irAEs), the lack of effective and accurate combination therapies, and resistance to anti-PD-1/PD-L1 treatment. MHC, major histocompatibility complex; TCR, T cell receptor.

reflected worse clinical outcomes.[134] Another group detected TMB in plasma through blood-based cell-free DNA (cfDNA), and they were able to successfully identify NSCLC patients whose PFS was significantly improved after anti-PD-L1 therapy.[135] Chen *et al.* collected tumour tissue samples from 183 patients with NSCLC, and then assessed PD-L1 expression levels by immunohistochemical analysis, as well as TMB status to assess correlations between PD-L1 expression, TMB, and clinical pathological characteristics.[136] They found that in addition to quantifying existing PD-L1 expression levels, monitoring the TMB could be a valuable non-invasive biomarker for predicting the success of chemotherapy and more targeted treatments. This difference may be associated with the large amount of tumour heterogeneity in NSCLCs. A meta-analysis also found that the combination of CD8+ T-cell TILs, PD-L1 expression and TMB

was strongly related to a reliable prognosis for advanced NSCLC.[137] On the other hand, the precise cut-off values and methods of optimally monitoring TMBs need to be further explored in future clinical studies.

Driver-gene alterations

Next-generation sequencing technology has been able to identify thousands of mutations in large cohorts for various cancers.[138] Ono *et al.* investigated 242 patients with NSCLC to assess driver mutations using whole exome sequencing; they found that the existence of driver-gene alterations might be an independent factor that affects prognosis in NSCLC.[139] KRAS is one of the most common driver oncogenes associated with NSCLC.[140] A study investigated NSCLC-specific driver-gene alterations in the hope of identifying potential immunotherapy biomarkers. The most common driver genes were TP53 (49%), KRAS (43%) and ERBB2 (13%). Patients with KRAS mutations responded better to PD-1 inhibitors and exhibited improved mPFS and mOS than those without such mutations.[104] However, patients with ERBB-family mutations failed to respond to anti-PD-1 therapy. There was a case report of a 64-year-old non-smoking man with squamous NSCLC with co-mutations of TP53 and KRAS who received pembrolizumab combined with gemcitabine treatment; this patient achieved PR, which had persisted for over 7 months at the time of publication.[141] However, another study retrospectively identified patients with NSCLC who were harbouring KRAS mutations who were treated with immunotherapy, and there was no significant difference between patients with or without KRAS mutations in terms of mPFS.[142] Another study showed that the reason patients with KRAS mutations responded better to PD-1/PD-L1 inhibitors was related to tumour immunogenicity. NSCLC patients with KRAS mutations show good clinical outcomes following treatment with PD-1/PD-L1 inhibitors.[143]

MSI/dMMR

In addition to PD-1/PD-L1 expression analysis, the presence of MSI is also a key reaction marker.[144] Studies have shown that genetic mutations in the dMMR pathway can lead to MSI, and high MSI is closely related to the increased immunogenicity and immune response produced by tumours. MSI is an excellent predictive biomarker. Therefore, the FDA has approved pembrolizumab to treat unresectable solid tumours with high MSI or dMMR.[145] The DNA MMR pathway includes the mutL homolog 1 (MLH1), MSH (mutS homolog)-2, postmeiotic segregation increased 2 (PMS2) and MSH-6 proteins. When this pathway is defective (dMMR), it can be used as a means to select patients for immunotherapy. In addition, MSI frequency can also be used to predict NSCLC.[117] Similarly, NSCLC patients with DNA polymerase epsilon (POLE) and DNA polymerase delta 1 (POLD1) gene mutations also have an objective clinical response to pembrolizumab treatment and a corresponding degree of association.[146,147] Therefore, clinicians can further determine whether a patient can use the epidemiology of the patient's MSI/dMMR to predict NSCLC prognosis.[148]

Peripheral blood-based biomarkers

Because biopsies of lung cancer tissue samples are difficult to obtain, some alternative methods include minimally invasive biopsy of peripheral blood for practical reasons.[149] Peripheral blood contains DNA, RNA, and proteins released from tumour tissues reflecting the dynamic changes in the TME. The level of ctDNA in the peripheral blood in the early stage of treatment provides valuable clues to the therapeutic effect. In various cancer types, including NSCLC, the detection of highly mutated ctDNA is positively correlated with a better clinical response to immune-checkpoint blockade.[150] Zhang *et al.* analysed individual peripheral T-cell clones of 110 peripheral T cells of NSCLC patients before and after the initiation of PD-1 blockade.[151] They found that 25 genes were significantly changed during tumour progression. These analyses help to understand the dynamic changes of T-cell clones in the peripheral blood during PD-1 blockade of NSCLC. Tanizaki *et al.* investigated 134 patients with advanced or recurrent NSCLC treated with nivolumab to evaluate the association between survival and peripheral blood parameters; they found that a low absolute neutrophil count, high absolute lymphocyte count, and high absolute eosinophil count were closely related to a better clinical outcome in patients with NSCLC treated with nivolumab.[152] In addition, Jiang *et al.* investigated 76 NSCLC patients to evaluate the relationship between survival and peripheral blood markers with ICI monotherapy; they found that a high platelet-to-lymphocyte ratio and low albumin in peripheral blood might be important indicators in

advanced NSCLC patients treated with nivolumab and durvalumab monotherapy.[153]

IFN-γ

IFN-γ is a vital immune-regulatory molecule in the TME released by tumour-reactive T cells.[154] Recent studies have suggested that IFN-γ is an important driver of PD-L1 expression in tumours, and it can significantly improve the response to anti-PD-1 therapy.[155] The increased IFN-γ levels at baseline and at 3 months after anti-PD-1 therapy were significantly correlated with improved mOS.[156] Recent studies have shown that in 17 NSCLC patients treated with nivolumab, mPFS was obviously prolonged in those with high expression levels of IFN-γ, in contrast to those with low expression of IFN-γ (5.1 *versus* 2 months, respectively; $p = 0.0124$). This indicated that IFN-γ is a vital biomarker for NSCLC patients treated with anti-PD-1 therapy.[157]

Other potential biomarkers

Other potential biomarkers being investigated in anti-PD-1/PD-L1 immunotherapy on NSCLC include the pretreatment haemoglobin level,[158] serum vascular endothelial growth factor (VEGF) concentration,[159] serum lactate dehydrogenase level,[160] gut microbiome composition[116] and neoantigen load.[161]

Challenges for anti-PD-1/PD-L1 treatment in advanced NSCLC

Currently, there are still many unresolved problems associated with the clinical use of anti-PD-1/PD-L1 therapies (Figure 2). First, PD-L1 expression is a predictive biomarker of outcomes of anti-PD-1/PD-L1 treatment, as it is correlated with the efficacy of PD-1/PD-L1 inhibitor therapy in NSCLC; however, this association can be quite variable. For example, the improvement in mOS by nivolumab and durvalumab treatment for NSCLC was independent of PD-L1 expression levels.[71,162] In immunohistochemical analysis, the expression levels of PD-L1 are dynamic and heterogeneous, and this is further complicated by issues related to antibody specificity and detection techniques.[163] In the future, more valuable biomarkers may be identified by neoantigen prediction techniques, microbiota signatures, or high microsatellite instability (MSI), all of which should be further explored in clinical medicine.

The second challenge is the risk of irAEs associated with the use of anti-PD-1/PD-L1 monotherapy for advanced NSCLC. Although the safety profile of individual antibodies is favourable compared with standard chemotherapy; the frequency of irAEs in combination therapies was much higher (Tables 2–4). For example, 53.6% of patients who received atezolizumab plus chemotherapy developed irAEs, whereas only 29.1% of patients in the group receiving chemotherapy alone did.[61] Therefore, better strategies to reduce the risks of serious irAEs need to be explored in future studies.

The third challenge of immunotherapy is deciding how to effectively and accurately select the best combination therapy for patients with advanced NSCLC. Anti-PD-1/PD-L1 treatment combined with chemotherapy is proven to have great potential for improving ORRs.[164,165] Apart from chemotherapy, treatments with VEGF, CTLA-4, chimeric antigen receptor T cell, CRISPR-Cas9 gene editing or lymphocyte-activation-protein-3 therapies could provide additional choices for combination strategies in future clinical studies.[17,166–169] Therefore, it is important to establish a standard to guide and determine the most appropriate combination therapy for treating advanced NSCLC.

The fourth challenge is that some patients who are resistant to anti-PD-1/PD-L1 therapy experience a poor prognosis after treatment. The underlying resistance mechanism is a very complicated process, and little is known about the potential tumour-related antigens associated with TMB resistance to anti-PD-1/PD-L1 therapy or the genetic determinants of a good prognosis.

Conclusion

To date, four anti-PD-1/PD-L1 drugs, including pembrolizumab, nivolumab, atezolizumab and durvalumab, have been registered and approved for the neoadjuvant, first-line and second-line treatment of NSCLC. With the approval of ICIs, immune-checkpoint therapy based on modulating PD-1/PD-L1 signalling provides more choices for treating advanced NSCLC patients. However, the ORR after ICI treatment is only 14–20% in NSCLC patients who are not identified beforehand as being likely to experience a good prognosis. Therefore, identification of predictive biomarkers is crucial for selecting patients with NSCLC who would most benefit from

anti-PD-1/PD-L1 therapy. Although the clinical application of anti-PD-1/PD-L1 therapy is still at an early stage, there are an increasing number of ongoing clinical trials to assess the efficacy of anti-PD-1/PD-L1 treatment. EGFR mutation rates and microbiome differences may affect the efficacy of anti-PD-1/PD-L1 treatment. ALK rearrangements in patients with advanced NSCLC are associated with beneficial outcomes and prolonged mOS from anti-PD-1/PD-L1 treatment compared with chemotherapy, when administered as second-line treatment. Patients with KRAS mutations experienced a longer mPFS and mOS than patients without a KRAS mutation using anti-PD-1/PD-L1 treatment, thus immunotherapy may be a promising strategy with KRAS mutation of NSCLC owing to regulating immune response. Therefore, with the continuous development of clinical trials, more retrospective and prospective clinical studies need to comprehensively evaluate more underlying biomarkers to improve treatment efficacy and enable more NSCLC patients to benefit from ICIs for anti-PD-1/PD-L1 therapy.

Authors' contributions
JZ and JQ wrote the manuscript; QM, LC, LL, TC and PW collected the references and modified the manuscript; LC and JZ designed the manuscript and approved the final manuscript for publication.

Availability of data and materials
Please contact the corresponding author for data requests.

Conflict of interest statement
The authors declare that there is no conflict of interest.

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References
1. Duruisseaux M and Esteller M. Lung cancer epigenetics: from knowledge to applications. *Semin Cancer Biol* 2018; 51: 116–128.

2. Alberg AJ, Brock MV, Ford JG, *et al.* Epidemiology of lung cancer: diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest* 2013; 143: e1S–e29S.

3. Siegel RL, Miller KD and Jemal A. Cancer statistics, 2020. *CA Cancer J Clin* 2020; 70: 7–30.

4. Herbst RS, Morgensztern D and Boshoff C. The biology and management of non-small cell lung cancer. *Nature* 2018; 553: 446–454.

5. Ohe Y, Imamura F, Nogami N, *et al.* Osimertinib versus standard-of-care EGFR-TKI as first-line treatment for EGFRm advanced NSCLC: FLAURA Japanese subset. *Jpn J Clin Oncol* 2019; 49: 29–36.

6. Peters S, Camidge DR, Shaw AT, *et al.*; ALEX trial investigators. Alectinib versus crizotinib in untreated ALK-positive non-small-cell lung cancer. *N Engl J Med* 2017; 377: 829–838.

7. Yang CY, Yang JC and Yang PC. Precision management of advanced non-small cell lung cancer. *Annu Rev Med* 2020; 71: 117–136.

8. Aggarwal C, Rolfo CD, Oxnard GR, *et al.* Strategies for the successful implementation of plasma-based NSCLC genotyping in clinical practice. *Nat Rev Clin Oncol* 2021; 18: 56–62.

9. Doroshow DB and Herbst RS. Treatment of advanced non-small cell lung cancer in 2018. *JAMA Oncol* 2018; 4: 569–570.

10. Iams WT, Porter J and Horn L. Immunotherapeutic approaches for small-cell lung cancer. *Nat Rev Clin Oncol* 2020; 17: 300–312.

11. Brozos-Vazquez EM, Diaz-Pena R, Garcia-Gonzalez J, *et al.* Immunotherapy in nonsmall-cell lung cancer: current status and future prospects for liquid biopsy. *Cancer Immunol Immunother*. Epub ahead of print 28 October 2020. DOI: 10.1007/s00262-020-02752-z.

12. Califano R, Kerr K, Morgan RD, *et al.* Immune checkpoint blockade: a new era for non-small cell lung cancer. *Curr Oncol Rep* 2016; 18: 59.

13. Baraibar I, Melero I, Ponz-Sarvise M, *et al.* Safety and tolerability of immune checkpoint inhibitors (PD-1 and PD-L1) in cancer. *Drug Saf* 2019; 42: 281–294.

14. Shin DS and Ribas A. The evolution of checkpoint blockade as a cancer therapy: what's here, what's next? *Curr Opin Immunol* 2015; 33: 23–35.

15. Taghizadeh H, Marhold M, Tomasich E, *et al.* Immune checkpoint inhibitors in mCRPC - rationales, challenges and perspectives. *Oncoimmunology* 2019; 8: e1644109.

16. Walsh RJ and Soo RA. Resistance to immune checkpoint inhibitors in non-small cell lung cancer: biomarkers and therapeutic strategies. *Ther Adv Med Oncol* 2020; 12: 1758835920937902.

17. Chae YK, Arya A, Iams W, *et al.* Current landscape and future of dual anti-CTLA4 and PD-1/PD-L1 blockade immunotherapy in cancer; lessons learned from clinical trials with melanoma and non-small cell lung cancer (NSCLC). *J Immunother Cancer* 2018; 6: 39.

18. Edwards SC, Hoevenaar WHM and Coffelt SB. Emerging immunotherapies for metastasis. *Br J Cancer* 2021; 124: 37–48.

19. Dang TO, Ogunniyi A, Barbee MS, *et al.* Pembrolizumab for the treatment of PD-L1 positive advanced or metastatic non-small cell lung cancer. *Expert Rev Anticancer Ther* 2016; 16: 13–20.

20. Garon EB, Rizvi NA, Hui R, *et al.*; KEYNOTE-001 investigators. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med* 2015; 372: 2018–2028.

21. Lim SH, Sun JM, Lee SH, *et al.* Pembrolizumab for the treatment of non-small cell lung cancer. *Expert Opin Biol Ther* 2016; 16: 397–406.

22. Sul J, Blumenthal GM, Jiang X, *et al.* FDA approval summary: pembrolizumab for the treatment of patients with metastatic non-small cell lung cancer whose tumors express programmed death-ligand 1. *Oncologist* 2016; 21: 643–650.

23. Rimm DL, Han G, Taube JM, *et al.* A prospective, multi-institutional, pathologist-based assessment of 4 immunohistochemistry assays for PD-L1 expression in non-small cell lung cancer. *JAMA Oncol* 2017; 3: 1051–1058.

24. Munoz-Unceta N, Burgueno I, Jimenez E, *et al.* Durvalumab in NSCLC: latest evidence and clinical potential. *Ther Adv Med Oncol* 2018; 10: 1758835918804151.

25. Yang K, Li J, Sun Z, *et al.* Retreatment with immune checkpoint inhibitors in solid tumors: a systematic review. *Ther Adv Med Oncol* 2020; 12: 175883592097535.

26. Noguchi T, Ward JP, Gubin MM, *et al.* Temporally distinct PD-L1 expression by tumor and host cells contributes to immune escape. *Cancer Immunol Res* 2017; 5: 106–117.

27. Machicote A, Belen S, Baz P, *et al.* Human CD8^{+} HLA-DR^{+} regulatory T cells, similarly to classical CD4^{+}Foxp3^{+} cells, suppress immune responses via PD-1/PD-L1 axis. *Front Immunol* 2018; 9: 2788.

28. Yahata T, Mizoguchi M, Kimura A, *et al.* Programmed cell death ligand 1 disruption by clustered regularly interspaced short palindromic repeats/Cas9-genome editing promotes antitumor immunity and suppresses ovarian cancer progression. *Cancer Sci* 2019; 110: 1279–1292.

29. Bally AP, Austin JW and Boss JM. Genetic and epigenetic regulation of PD-1 expression. *J Immunol* 2016; 196: 2431–2437.

30. Kim CG, Kim KH, Pyo KH, *et al.* Hyperprogressive disease during PD-1/PD-L1 blockade in patients with non-small-cell lung cancer. *Ann Oncol* 2019; 30: 1104–1113.

31. Kumagai S, Togashi Y, Kamada T, *et al.* The PD-1 expression balance between effector and regulatory T cells predicts the clinical efficacy of PD-1 blockade therapies. *Nat Immunol* 2020; 21: 1346–1358.

32. Pio R, Ajona D, Ortiz-Espinosa S, *et al.* Complementing the cancer-immunity cycle. *Front Immunol* 2019; 10: 774.

33. Gordon SR, Maute RL, Dulken BW, *et al.* PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. *Nature* 2017; 545: 495–499.

34. Liu Y, Zugazagoitia J, Ahmed FS, *et al.* Immune cell PD-L1 colocalizes with macrophages and is associated with outcome in PD-1 pathway blockade therapy. *Clin Cancer Res* 2020; 26: 970–977.

35. Zheng Y, Fang YC and Li J. PD-L1 expression levels on tumor cells affect their immunosuppressive activity. *Oncol Lett* 2019; 18: 5399–5407.

36. Trefny MP, Kaiser M, Stanczak MA, *et al*. PD-1⁺ natural killer cells in human non-small cell lung cancer can be activated by PD-1/PD-L1 blockade. *Cancer Immunol Immunother* 2020; 69: 1505–1517.

37. Mayoux M, Roller A, Pulko V, *et al*. Dendritic cells dictate responses to PD-L1 blockade cancer immunotherapy. *Sci Transl Med* 2020; 12: eaav7431.

38. Zappa C and Mousa SA. Non-small cell lung cancer: current treatment and future advances. *Transl Lung Cancer Res* 2016; 5: 288–300.

39. Marur S, Singh H, Mishra-Kalyani P, *et al*. FDA analyses of survival in older adults with metastatic non-small cell lung cancer in controlled trials of PD-1/PD-L1 blocking antibodies. *Semin Oncol* 2018; 45: 220–225.

40. Hui R, Garon EB, Goldman JW, *et al*. Pembrolizumab as first-line therapy for patients with PD-L1-positive advanced non-small cell lung cancer: a phase 1 trial. *Ann Oncol* 2017; 28: 874–881.

41. Leighl NB, Hellmann MD, Hui R, *et al*. Pembrolizumab in patients with advanced non-small-cell lung cancer (KEYNOTE-001): 3-year results from an open-label, phase 1 study. *Lancet Resp Med* 2019; 7: 347–357.

42. Gandhi L, Rodriguez-Abreu D, Gadgeel S, *et al*.; KEYNOTE-189 Investigators. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. *N Engl J Med* 2018; 378: 2078–2092.

43. Gadgeel S, Rodriguez-Abreu D, Speranza G, *et al*. Updated analysis from KEYNOTE-189: pembrolizumab or placebo plus pemetrexed and platinum for previously untreated metastatic nonsquamous non-small-cell lung cancer. *J Clin Oncol* 2020; 38: 1505–1517.

44. Paz-Ares L, Luft A, Vicente D, *et al*.; KEYNOTE-407 investigators. Pembrolizumab plus chemotherapy for squamous non-small-cell lung cancer. *N Engl J Med* 2018; 379: 2040–2051.

45. Paz-Ares L, Vicente D, Tafreshi A, *et al*. A randomized, placebo-controlled trial of pembrolizumab plus chemotherapy in patients with metastatic squamous NSCLC: protocol-specified final analysis of KEYNOTE-407. *J Thorac Oncol* 2020; 15: 1657–1669.

46. Gadgeel SM, Stevenson JP, Langer CJ, *et al*. Pembrolizumab and platinum-based chemotherapy as first-line therapy for advanced non-small-cell lung cancer: phase 1 cohorts from the KEYNOTE-021 study. *Lung Cancer* 2018; 125: 273–281.

47. Langer CJ, Gadgeel SM, Borghaei H, *et al*.; KEYNOTE-021 Investigators. Carboplatin and pemetrexed with or without pembrolizumab for advanced, non-squamous non-small-cell lung cancer: a randomised, phase 2 cohort of the open-label KEYNOTE-021 study. *Lancet Oncol* 2016; 17: 1497–1508.

48. Awad MM, Gadgeel SM, Borghaei H, *et al*. Long-term overall survival from KEYNOTE-021 cohort G: pemetrexed and carboplatin with or without pembrolizumab as first-line therapy for advanced nonsquamous NSCLC. *J Thorac Oncol* 2021; 16: 162–168.

49. Reck M, Rodriguez-Abreu D, Robinson AG, *et al*. Updated analysis of KEYNOTE-024: pembrolizumab versus platinum-based chemotherapy for advanced non-small-cell lung cancer with PD-L1 tumor proportion score of 50% or greater. *J Clin Oncol* 2019; 37: 537–546.

50. Reck M, Rodriguez-Abreu D, Robinson AG, *et al*.; KEYNOTE-024 investigators. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. *N Engl J Med* 2016; 375: 1823–1833.

51. Mok TSK, Wu YL, Kudaba I, *et al*.; KEYNOTE-042 investigators. Pembrolizumab versus chemotherapy for previously untreated, PD-L1-expressing, locally advanced or metastatic non-small-cell lung cancer (KEYNOTE-042): a randomised, open-label, controlled, phase 3 trial. *Lancet* 2019; 393: 1819–1830.

52. Tamiya M, Tamiya A, Hosoya K, *et al*. Efficacy and safety of pembrolizumab as first-line therapy in advanced non-small cell lung cancer with at least 50% PD-L1 positivity: a multicenter retrospective cohort study (HOPE-001). *Invest New Drugs* 2019; 37: 1266–1273.

53. Gettinger S, Rizvi NA, Chow LQ, *et al*. Nivolumab monotherapy for first-line treatment of advanced non-small-cell lung cancer. *J Clin Oncol* 2016; 34: 2980–2987.

54. Hellmann MD, Rizvi NA, Goldman JW, *et al*. Nivolumab plus ipilimumab as first-line treatment for advanced non-small-cell lung cancer (CheckMate 012): results of an open-label, phase 1, multicohort study. *Lancet Oncol* 2017; 18: 31–41.

55. Hellmann MD, Ciuleanu TE, Pluzanski A, *et al.* Nivolumab plus ipilimumab in lung cancer with a high tumor mutational burden. *N Engl J Med* 2018; 378: 2093–2104.

56. Reck M, Schenker M, Lee KH, *et al.* Nivolumab plus ipilimumab versus chemotherapy as first-line treatment in advanced non-small-cell lung cancer with high tumour mutational burden: patient-reported outcomes results from the randomised, open-label, phase III CheckMate 227 trial. *Eur J Cancer* 2019; 116: 137–147.

57. Ready N, Hellmann MD, Awad MM, *et al.* First-line nivolumab plus ipilimumab in advanced non-small-cell lung cancer (CheckMate 568): outcomes by programmed death ligand 1 and tumor mutational burden as biomarkers. *J Clin Oncol* 2019; 37: 992–1000.

58. Carbone DP, Reck M, Paz-Ares L, *et al.* First-line nivolumab in stage IV or recurrent non-small-cell lung cancer. *N Engl J Med* 2017; 376: 2415–2426.

59. Peters S, Gettinger S, Johnson ML, *et al.* Phase II trial of atezolizumab as first-line or subsequent therapy for patients with programmed death-ligand 1-selected advanced non-small-cell lung cancer (BIRCH). *J Clin Oncol* 2017; 35: 2781–2789.

60. Socinski MA, Jotte RM, Cappuzzo F, *et al.* Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. *N Engl J Med* 2018; 378: 2288–2301.

61. Papadimitrakopoulou V, Cobo M, Bordoni R, *et al.* OA05.07 IMpower132: PFS and safety results with 1l atezolizumab + carboplatin/cisplatin + pemetrexed in stage IV non-squamous NSCLC. *J Thorac Oncol* 2018; 13: S332–S333.

62. Planchard D, Yokoi T, McCleod MJ, *et al.* A phase III study of durvalumab (MEDI4736) with or without tremelimumab for previously treated patients with advanced NSCLC: rationale and protocol design of the ARCTIC study. *Clin Lung Cancer* 2016; 17: 232–236.e231.

63. Jotte R, Cappuzzo F, Vynnychenko I, *et al.* Atezolizumab in combination with carboplatin and nab-paclitaxel in advanced squamous NSCLC (IMpower131): results from a randomized phase III trial. *J Thorac Oncol* 2020; 15: 1351–1360.

64. Herbst RS, Giaccone G, De Marinis F, *et al.* Atezolizumab for first-line treatment of PD-L1-selected patients with NSCLC. *N Engl J Med* 2020; 383: 1328–1339.

65. Shiraishi Y, Kishimoto J, Tanaka K, *et al.* Treatment rationale and design for APPLE (WJOG11218L): a multicenter, open-label, randomized phase 3 study of atezolizumab and platinum/pemetrexed with or without bevacizumab for patients with advanced nonsquamous non-small-cell lung cancer. *Clin Lung Cancer* 2020; 21: 472–476.

66. Antonia S, Goldberg SB, Balmanoukian A, *et al.* Safety and antitumour activity of durvalumab plus tremelimumab in non-small cell lung cancer: a multicentre, phase 1b study. *Lancet Oncol* 2016; 17: 299–308.

67. Zhang X, Wang C, Wang J, *et al.* PD-1 blockade cellular vesicles for cancer immunotherapy. *Adv Mater* 2018; 30: e1707112.

68. Mark M, Froesch P, Eboulet EI, *et al.*; Swiss Group for Clinical Cancer. SAKK 19/17: safety analysis of first-line durvalumab in patients with PD-L1 positive, advanced nonsmall cell lung cancer and a performance status of 2. *Cancer Immunol Immunother.* Epub ahead of print 1 November 2020. DOI: 10.1007/s00262-020-02757-8.

69. Brahmer J, Reckamp KL, Baas P, *et al.* Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. *N Engl J Med* 2015; 373: 123–135.

70. Reck M, Taylor F, Penrod JR, *et al.* Impact of nivolumab versus docetaxel on health-related quality of life and symptoms in patients with advanced squamous non-small cell lung cancer: results from the CheckMate 017 study. *J Thorac Oncol* 2018; 13: 194–204.

71. Borghaei H, Paz-Ares L, Horn L, *et al.* Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. *N Engl J Med* 2015; 373: 1627–1639.

72. Reck M, Brahmer J, Bennett B, *et al.* Evaluation of health-related quality of life and symptoms in patients with advanced non-squamous non-small cell lung cancer treated with nivolumab or docetaxel in CheckMate 057. *Eur J Cancer* 2018; 102: 23–30.

73. Horn L, Spigel DR, Vokes EE, *et al.* Nivolumab versus docetaxel in previously treated patients with advanced non-small-cell lung cancer: two-year outcomes from two randomized, open-label, phase III trials (CheckMate 017 and CheckMate 057). *J Clin Oncol* 2017; 35: 3924–3933.

74. Wu YL, Lu S, Cheng Y, *et al.* Nivolumab versus docetaxel in a predominantly Chinese patient population with previously treated advanced

NSCLC: CheckMate 078 randomized phase III clinical trial. *J Thorac Oncol* 2019; 14: 867–875.

75. Martin C, Lupinacci L, Perazzo F, *et al*. Efficacy and safety of nivolumab in previously treated patients with non-small-cell lung cancer: real world experience in Argentina. *Clin Lung Cancer* 2020; 21: e380–e387.

76. Morita R, Okishio K, Shimizu J, *et al*. Real-world effectiveness and safety of nivolumab in patients with non-small cell lung cancer: a multicenter retrospective observational study in Japan. *Lung Cancer* 2020; 140: 8–18.

77. Herbst RS, Baas P, Kim DW, *et al*. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet* 2016; 387: 1540–1550.

78. Herbst RS, Garon EB, Kim DW, *et al*. Long-term outcomes and retreatment among patients with previously treated, programmed death-ligand 1positive, advanced nonsmall-cell lung cancer in the KEYNOTE-010 study. *J Clin Oncol* 2020; 38: 1580–1590.

79. Nishio M, Takahashi T, Yoshioka H, *et al*. KEYNOTE-025: phase 1b study of pembrolizumab in Japanese patients with previously treated programmed death ligand 1-positive advanced non-small-cell lung cancer. *Cancer Sci* 2019; 110: 1012–1020.

80. Gubens MA, Sequist LV, Stevenson JP, *et al*. Pembrolizumab in combination with ipilimumab as second-line or later therapy for advanced non-small-cell lung cancer: KEYNOTE-021 cohorts D and H. *Lung Cancer* 2019; 130: 59–66.

81. Rittmeyer A, Barlesi F, Waterkamp D, *et al*.; OAK study group. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. *Lancet* 2017; 389: 255–265.

82. Von Pawel J, Bordoni R, Satouchi M, *et al*. Long-term survival in patients with advanced non-small-cell lung cancer treated with atezolizumab versus docetaxel: results from the randomised phase III OAK study. *Eur J Cancer* 2019; 107: 124–132.

83. Fehrenbacher L, Spira A, Ballinger M, *et al*.; POPLAR study group. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. *Lancet* 2016; 387: 1837–1846.

84. Ikeda S, Kato T, Kenmotsu H, *et al*. A phase II study of atezolizumab for pretreated advanced/recurrent non-small cell lung cancer with idiopathic interstitial pneumonias: rationale and design for the TORG1936/AMBITIOUS study. *Ther Adv Med Oncol* 2020; 12: 1758835920922022.

85. Vergnenegre A, Monnet I, Bizieux A, *et al*. Open-label phase II trial to evaluate safety and efficacy of second-line metronomic oral vinorelbine-atezolizumab combination for stage-IV non-small-cell lung cancer - VinMetAtezo trial, (GFPC‡ 04-2017). *Future Oncol* 2020; 16: 5–10.

86. Marine S, Stephane R, Nicolas P, *et al*. Cost-effectiveness of atezolizumab versus docetaxel and nivolumab in the treatment of non-small cell lung cancer as a second line in France. *J Med Econ* 2020; 23: 464–473.

87. Garassino MC, Cho BC, Kim JH, *et al*. Durvalumab as third-line or later treatment for advanced non-small-cell lung cancer (ATLANTIC): an open-label, single-arm, phase 2 study. *Lancet Oncol* 2018; 19: 521–536.

88. Planchard D, Reinmuth N, Orlov S, *et al*. ARCTIC: durvalumab with or without tremelimumab as third-line or later treatment of metastatic non-small-cell lung cancer. *Ann Oncol* 2020; 31: 609–618.

89. Gainor JF, Shaw AT, Sequist LV, *et al*. EGFR mutations and ALK rearrangements are associated with low response rates to PD-1 pathway blockade in non-small cell lung cancer: a retrospective analysis. *Clin Cancer Res* 2016; 22: 4585–4593.

90. Peters S, Kerr KM and Stahel R. PD-1 blockade in advanced NSCLC: a focus on pembrolizumab. *Cancer Treat Rev* 2018; 62: 39–49.

91. D'Incecco A, Andreozzi M, Ludovini V, *et al*. PD-1 and PD-L1 expression in molecularly selected non-small-cell lung cancer patients. *Br J Cancer* 2015; 112: 95–102.

92. Zhang N, Zeng Y, Du W, *et al*. The EGFR pathway is involved in the regulation of PD-L1 expression via the IL-6/JAK/STAT3 signaling pathway in EGFR-mutated non-small cell lung cancer. *Int J Oncol* 2016; 49: 1360–1368.

93. Abdelhamed S, Ogura K, Yokoyama S, *et al*. AKT-STAT3 pathway as a downstream target of EGFR signaling to regulate PD-L1 expression on NSCLC cells. *J Cancer* 2016; 7: 1579–1586.

94. Jiang XM, Xu YL, Huang MY, *et al*. Osimertinib (AZD9291) decreases programmed

death ligand-1 in EGFR-mutated non-small cell lung cancer cells. *Acta Pharmacol Sin* 2017; 38: 1512–1520.

95. Lisberg A, Cummings A, Goldman JW, *et al.* A phase II study of pembrolizumab in EGFR-mutant, PD-L1+, tyrosine kinase inhibitor naive patients with advanced NSCLC. *J Thorac Oncol* 2018; 13: 1138–1145.

96. Yang JC, Gadgeel SM, Sequist LV, *et al.* Pembrolizumab in combination with erlotinib or gefitinib as first-line therapy for advanced NSCLC with sensitizing EGFR mutation. *J Thorac Oncol* 2019; 14: 553–559.

97. Spigel DR, Reynolds C, Waterhouse D, *et al.* Phase 1/2 study of the safety and tolerability of nivolumab plus crizotinib for the first-line treatment of anaplastic lymphoma kinase translocation - positive advanced non-small cell lung cancer (CheckMate 370). *J Thorac Oncol* 2018; 13: 682–688.

98. Garassino MC, Gelibter AJ, Grossi F, *et al.* Italian nivolumab expanded access program in nonsquamous non-small cell lung cancer patients: results in never-smokers and EGFR-mutant patients. *J Thorac Oncol* 2018; 13: 1146–1155.

99. Haratani K, Hayashi H, Tanaka T, *et al.* Tumor immune microenvironment and nivolumab efficacy in EGFR mutation-positive non-small-cell lung cancer based on T790M status after disease progression during EGFR-TKI treatment. *Ann Oncol* 2017; 28: 1532–1539.

100. Maione P, Sacco PC, Sgambato A, *et al.* Overcoming resistance to targeted therapies in NSCLC: current approaches and clinical application. *Ther Adv Med Oncol* 2015; 7: 263–273.

101. Wu SG and Shih JY. Management of acquired resistance to EGFR TKI-targeted therapy in advanced non small cell lung cancer. *Mol Cancer* 2018; 17: 38.

102. Gettinger S, Hellmann MD, Chow LQM, *et al.* Nivolumab plus erlotinib in patients with EGFR-mutant advanced NSCLC. *J Thorac Oncol* 2018; 13: 1363–1372.

103. Felip E, De Braud FG, Maur M, *et al.* Ceritinib plus nivolumab in patients with advanced ALK-rearranged non-small cell lung cancer: results of an open-label, multicenter, phase 1B study. *J Thorac Oncol* 2020; 15: 392–403.

104. Cinausero M, Laprovitera N, De Maglio G, *et al.* KRAS and ERBB-family genetic alterations affect response to PD-1 inhibitors in metastatic nonsquamous NSCLC. *Ther Adv Med Oncol* 2019; 11: 1758835919885540.

105. Fu J, Wang F, Dong LH, *et al.* Preclinical evaluation of the efficacy, pharmacokinetics and immunogenicity of JS-001, a programmed cell death protein-1 (PD-1) monoclonal antibody. *Acta Pharmacol Sin* 2017; 38: 710–718.

106. Yang J, Dong L, Yang S, *et al.* Safety and clinical efficacy of toripalimab, a PD-1 mAb, in patients with advanced or recurrent malignancies in a phase I study. *Eur J Cancer* 2020; 130: 182–192.

107. Hoy SM. Sintilimab: first global approval. *Drugs* 2019; 79: 341–346.

108. Gao S, Li N, Gao S, *et al.* Neoadjuvant PD-1 inhibitor (sintilimab) in NSCLC. *J Thorac Oncol* 2020; 15: 816–826.

109. Markham A and Keam S. Camrelizumab: first global approval. *Drugs* 2019; 79: 1355–1361.

110. Wei Z, Yang X, Ye X, *et al.* Camrelizumab combined with microwave ablation improves the objective response rate in advanced non-small cell lung cancer. *J Cancer Res Ther* 2019; 15: 1629–1634.

111. Li W, Wei Z, Yang X, *et al.* Salvage therapy of reactive capillary hemangiomas: apatinib alleviates the unique adverse events induced by camrelizumab in non-small cell lung cancer. *J Cancer Res Ther* 2019; 15: 1624–1628.

112. Shen L, Guo J, Zhang Q, *et al.* Tislelizumab in Chinese patients with advanced solid tumors: an open-label, non-comparative, phase 1/2 study. *J Immunother Cancer* 2020; 8: e000437.

113. Darabi S, Braxton DR, Eisenberg BL, *et al.* Predictive biomarkers for immunotherapy response beyond PD-1/PD-L1. *Oncology (Williston Park)* 2020; 34: 321–327.

114. Andrews LP, Yano H and Vignali DAA. Inhibitory receptors and ligands beyond PD-1, PD-L1 and CTLA-4: breakthroughs or backups. *Nat Immunol* 2019; 20: 1425–1434.

115. Guven DC, Sahin TK, Dizdar O, *et al.* Predictive biomarkers for immunotherapy efficacy in non-small-cell lung cancer: current status and future perspectives. *Biomark Med* 2020; 14: 1383–1392.

116. Tray N, Weber JS and Adams S. Predictive biomarkers for checkpoint immunotherapy: current status and challenges for clinical application. *Cancer Immunol Res* 2018; 6: 1122–1128.

117. Ren D, Hua Y, Yu B, *et al.* Predictive biomarkers and mechanisms underlying resistance to PD1/PD-L1 blockade cancer immunotherapy. *Mol Cancer* 2020; 19: 19.

118. Zhang B, Liu Y, Zhou S, *et al*. Predictive effect of PD-L1 expression for immune checkpoint inhibitor (PD-1/PD-L1 inhibitors) treatment for non-small cell lung cancer: a meta-analysis. *Int Immunopharmacol* 2020; 80: 106214.

119. Ito K, Miura S, Sakaguchi T, *et al*. The impact of high PD-L1 expression on the surrogate endpoints and clinical outcomes of anti-PD-1/PD-L1 antibodies in non-small cell lung cancer. *Lung Cancer* 2019; 128: 113–119.

120. Teramoto K, Igarashi T, Kataoka Y, *et al*. Biphasic prognostic significance of PD-L1 expression status in patients with early- and locally advanced-stage non-small cell lung cancer. *Cancer Immunol Immunother*. Epub ahead of print 28 October 2020. DOI: 10.1007/s00262-020-02755-w.

121. Ricci C, Capizzi E, Giunchi F, *et al*. Reliability of programmed death ligand 1 (PD-L1) tumor proportion score (TPS) on cytological smears in advanced non-small cell lung cancer: a prospective validation study. *Ther Adv Med Oncol* 2020; 12: 1758835920954802.

122. Hurkmans DP, Kuipers ME, Smit J, *et al*. Tumor mutational load, CD8+ T cells, expression of PD-L1 and HLA class I to guide immunotherapy decisions in NSCLC patients. *Cancer Immunol Immunother* 2020; 69: 771–777.

123. Chen F, Zhuang X, Lin L, *et al*. New horizons in tumor microenvironment biology: challenges and opportunities. *BMC Med* 2015; 13: 45.

124. Wein L, Savas P, Luen SJ, *et al*. Clinical validity and utility of tumor-infiltrating lymphocytes in routine clinical practice for breast cancer patients: current and future directions. *Front Oncol* 2017; 7: 156.

125. Donnem T, Kilvaer TK, Andersen S, *et al*. Strategies for clinical implementation of TNM-immunoscore in resected nonsmall-cell lung cancer. *Ann Oncol* 2016; 27: 225–232.

126. Arasanz H, Zuazo M, Bocanegra A, *et al*. Early detection of hyperprogressive disease in non-small cell lung cancer by monitoring of systemic T cell dynamics. *Cancers (Basel)* 2020; 12: 344.

127. Chen K, Cheng G, Zhang F, *et al*. PD-L1 expression and T cells infiltration in patients with uncommon EGFR-mutant non-small cell lung cancer and the response to immunotherapy. *Lung Cancer* 2020; 142: 98–105.

128. Chan TA, Yarchoan M, Jaffee E, *et al*. Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic. *Ann Oncol* 2019; 30: 44–56.

129. Fumet JD, Truntzer C, Yarchoan M, *et al*. Tumour mutational burden as a biomarker for immunotherapy: current data and emerging concepts. *Eur J Cancer* 2020; 131: 40–50.

130. Rizvi H, Sanchez-Vega F, La K, *et al*. Molecular determinants of response to anti-programmed cell death (PD)-1 and anti-programmed death-ligand 1 (PD-L1) blockade in patients with non-small-cell lung cancer profiled with targeted next-generation sequencing. *J Clin Oncol* 2018; 36: 633–641.

131. Alborelli I, Leonards K, Rothschild SI, *et al*. Tumor mutational burden assessed by targeted NGS predicts clinical benefit from immune checkpoint inhibitors in non-small cell lung cancer. *J Pathol* 2020; 250: 19–29.

132. Huang D, Zhang F, Tao H, *et al*. Tumor mutation burden as a potential biomarker for PD-1/PD-L1 inhibition in advanced non-small cell lung cancer. *Target Oncol* 2020; 15: 93–100.

133. Fang W, Ma Y, Yin JC, *et al*. Combinatorial assessment of ctDNA release and mutational burden predicts anti-PD(L)1 therapy outcome in nonsmall-cell lung cancer. *Clin Transl Med* 2020; 10: 331–336.

134. Chae YK, Davis AA, Agte S, *et al*. Clinical implications of circulating tumor DNA tumor mutational burden (ctDNA TMB) in non-small cell lung cancer. *Oncologist* 2019; 24: 820–828.

135. Gandara DR, Paul SM, Kowanetz M, *et al*. Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab. *Nat Med* 2018; 24: 1441–1448.

136. Chen Y, Liu Q, Chen Z, *et al*. PD-L1 expression and tumor mutational burden status for prediction of response to chemotherapy and targeted therapy in non-small cell lung cancer. *J Exp Clin Cancer Res* 2019; 38: 193.

137. Yu Y, Zeng D, Ou Q, *et al*. Association of survival and immune-related biomarkers with immunotherapy in patients with non-small cell lung cancer: a meta-analysis and individual patient-level analysis. *JAMA Netw Open* 2019; 2: e196879.

138. Pon JR and Marra MA. Driver and passenger mutations in cancer. *Annu Rev Pathol* 2015; 10: 25–50.

139. Ono A, Isaka M, Serizawa M, *et al*. Genetic alterations of driver genes as independent prognostic factors for disease-free survival in patients with resected non-small cell lung cancer. *Lung Cancer* 2019; 128: 152–157.

140. Roman M, Baraibar I, Lopez I, *et al.* KRAS oncogene in non-small cell lung cancer: clinical perspectives on the treatment of an old target. *Mol Cancer* 2018; 17: 33.

141. Fang C, Zhang C, Zhao WQ, *et al.* Co-mutations of TP53 and KRAS serve as potential biomarkers for immune checkpoint blockade in squamous-cell non-small cell lung cancer: a case report. *BMC Med Genomics* 2019; 12: 136.

142. Gianoncelli L, Spitaleri G, Passaro A, *et al.* Efficacy of anti-PD1/PD-L1 therapy (IO) in KRAS mutant non-small cell lung cancer patients: a retrospective analysis. *Anticancer Res* 2020; 40: 427–433.

143. Liu C, Zheng S, Jin R, *et al.* The superior efficacy of anti-PD-1/PD-L1 immunotherapy in KRAS-mutant non-small cell lung cancer that correlates with an inflammatory phenotype and increased immunogenicity. *Cancer Lett* 2020; 470: 95–105.

144. Dudley JC, Lin MT, Le DT, *et al.* Microsatellite instability as a biomarker for PD-1 blockade. *Clin Cancer Res* 2016; 22: 813–820.

145. Marcus L, Lemery SJ, Keegan P, *et al.* FDA approval summary: pembrolizumab for the treatment of microsatellite instability-high solid tumors. *Clin Cancer Res* 2019; 25: 3753–3758.

146. Rizvi NA, Hellmann MD, Snyder A, *et al.* Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 2015; 348: 124–128.

147. Yao J, Gong Y, Zhao W, *et al.* Comprehensive analysis of POLE and POLD1 gene variations identifies cancer patients potentially benefit from immunotherapy in Chinese population. *Sci Rep* 2019; 9: 15767.

148. Zhao P, Li L, Jiang X, *et al.* Mismatch repair deficiency/microsatellite instability-high as a predictor for anti-PD-1/PD-L1 immunotherapy efficacy. *J Hematol Oncol* 2019; 12: 54.

149. Nassar FJ, Chamandi G, Tfaily MA, *et al.* Peripheral blood-based biopsy for breast cancer risk prediction and early detection. *Front Med (Lausanne)* 2020; 7: 28.

150. Shen H, Yang ES, Conry M, *et al.* Predictive biomarkers for immune checkpoint blockade and opportunities for combination therapies. *Genes Dis* 2019; 6: 232–246.

151. Zhang F, Bai H, Gao R, *et al.* Dynamics of peripheral T cell clones during PD-1 blockade in non-small cell lung cancer. *Cancer Immunol Immunother* 2020; 69: 2599–2611.

152. Tanizaki J, Haratani K, Hayashi H, *et al.* Peripheral blood biomarkers associated with clinical outcome in non-small cell lung cancer patients treated with nivolumab. *J Thorac Oncol* 2018; 13: 97–105.

153. Jiang M, Peng W, Pu X, *et al.* Peripheral blood biomarkers associated with outcome in non-small cell lung cancer patients treated with nivolumab and durvalumab monotherapy. *Front Oncol* 2020; 10: 913.

154. Thibaut R, Bost P, Milo I, *et al.* Bystander IFN-gamma activity promotes widespread and sustained cytokine signaling altering the tumor microenvironment. *Nat Cancer* 2020; 1: 302–314.

155. Ayers M, Lunceford J, Nebozhyn M, *et al.* IFN-gamma-related mRNA profile predicts clinical response to PD-1 blockade. *J Clin Invest* 2017; 127: 2930–2940.

156. Boutsikou E, Domvri K, Hardavella G, *et al.* Tumour necrosis factor, interferon-gamma and interleukins as predictive markers of antiprogrammed cell-death protein-1 treatment in advanced non-small cell lung cancer: a pragmatic approach in clinical practice. *Ther Adv Med Oncol* 2018; 10: 1758835918768238.

157. Karachaliou N, Gonzalez-Cao M, Crespo G, *et al.* Interferon gamma, an important marker of response to immune checkpoint blockade in non-small cell lung cancer and melanoma patients. *Ther Adv Med Oncol* 2018; 10: 1758834017749748.

158. Zhang Z, Zhang F, Yuan F, *et al.* Pretreatment hemoglobin level as a predictor to evaluate the efficacy of immune checkpoint inhibitors in patients with advanced non-small cell lung cancer. *Ther Adv Med Oncol* 2020; 12: 175883592097004.

159. Shibaki R, Murakami S, Shinno Y, *et al.* Predictive value of serum VEGF levels for elderly patients or for patients with poor performance status receiving anti-PD-1 antibody therapy for advanced non-small-cell lung cancer. *Cancer Immunol Immunother* 2020; 69: 1229–1236.

160. Zhang M, Yang J, Hua W, *et al.* Monitoring checkpoint inhibitors: predictive biomarkers in immunotherapy. *Front Med* 2019; 13: 32–44.

161. Rosenthal R, Cadieux EL, Salgado R, *et al.* Neoantigen-directed immune escape in lung cancer evolution. *Nature* 2019; 567: 479–485.

162. Antonia SJ, Villegas A, Daniel D, *et al.*; PACIFIC investigators. Durvalumab after chemoradiotherapy in stage III non-small-cell

lung cancer. *N Engl J Med* 2017; 377: 1919–1929.

163. Hofman P. PD-L1 immunohistochemistry for non-small cell lung carcinoma: which strategy should be adopted? *Expert Rev Mol Diagn* 2017; 17: 1097–1108.

164. Sui H, Ma N, Wang Y, *et al*. Anti-PD-1/ PD-L1 therapy for non-small-cell lung cancer: toward personalized medicine and combination strategies. *J Immunol Res* 2018; 2018: 6984948.

165. Pirker R. Immunotherapy combinations in advanced nonsmall cell lung cancer. *Curr Opin Oncol* 2021; 33: 73–79.

166. Meder L, Schuldt P, Thelen M, *et al*. Combined VEGF and PD-L1 blockade displays synergistic treatment effects in an autochthonous mouse

model of small cell lung cancer. *Cancer Res* 2018; 78: 4270–4281.

167. Qu J, Mei Q, Chen L, *et al*. Chimeric antigen receptor (CAR)-T-cell therapy in non-small-cell lung cancer (NSCLC): current status and future perspectives. *Cancer Immunol Immunother* Epub ahead of print 6 October 2020. DOI: 10.1007/ s00262-020-02735-0.

168. Lu Y, Xue J, Deng T, *et al*. Safety and feasibility of CRISPR-edited T cells in patients with refractory non-small-cell lung cancer. *Nat Med* 2020; 26: 732–740.

169. He Y, Yu H, Rozeboom L, *et al*. LAG-3 protein expression in non-small cell lung cancer and its relationship with PD-1/PD-L1 and tumor-infiltrating lymphocytes. *J Thorac Oncol* 2017; 12: 814–823.