Biomarkers for the response to immunotherapy in patients with non-small cell lung cancer

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2 Pembrolizumab for the treatment of non-small cell lung cancer

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Expert review of anticancer treatment, 2017. 17(5): 399-409

Abstract

Introduction: In the last years, a spectacular development of immunotherapeutic agents aimed at the PD-1/PD-L1 axis has taken place. This development of these checkpoint inhibitors has greatly influenced our approach to the treatment of lung cancer in first and second line. The limited toxicity profile and the ability to treat for prolonged periods, even in smokers, is a welcome expansion of the therapeutic arsenal of the oncologist.
Areas covered: This review highlights the results of recent clinical trials on pembrolizumab for the treatment of non-small cell lung cancer. The authors discuss both first and second line treatment with pembrolizumab as monotherapy and in combination therapies. Additionally, implications of the PD-L1 immunohistochemistry assay with the 22C3 antibody and its use in clinical practice and trials is discussed.
Expert commentary: A higher overall response, overall survival and a moderate toxicity profile is observed with the use of pembrolizumab, compared to chemotherapy, in both first and second line. These promising results have already translated into the registration of pembrolizumab in first and second line in patients with a high expression of PD-L1. However, as PD-L1 staining does not sufficiently discriminate responders from non-responders for all checkpoint inhibitors, there still is a need for a better predictive biomarker.

Key issues

- In the setting of advanced or metastasized non-small cell lung cancer, pembrolizumab is FDA approved for:
    - The treatment of patients who are progressive on or after platinum based chemotherapy and are at least 1% PD-L1 positive.
    - First line treatment of patients whose tumor is ≥50% PD- L1 positive, using the validated IHC assay with the 22C3 antibody.
- Pembrolizumab compared to docetaxel showed a doubling of overall response rate and 24 months overall survival.
- PD-L1 positivity (TPS ≥50%) is associated with higher ORR, PFS and OS for the use of pembrolizumab.
- PD-L1 expression may be highly variable between different tumor sites, leading to a possible false negative result.
- The toxicity profile of pembrolizumab is superior over chemotherapy in both first and second line.
- Pseudoprogression can complicate a proper response evaluation, but is an uncommon event in lung cancer.

1. Introduction

Chemotherapy has been the standard treatment for patients with disseminated non-small cell lung cancer (NSCLC) for many years. In the last two decades, several novel therapeutic agents have been developed [13-15]. Major progress has been made in the field of targeted therapy. These agents have become part of the regular treatment plan for a subgroup of patients. The efficacy of these drugs depends heavily on the presence of oncogenic driver mutations, for example aberrant EGFR or ALK. However, only a minority of patients are candidates for this kind of treatment, underscoring the need for new treatment avenues to be explored [14, 17, 116].

The unraveling of the interaction between various proteins with functions in the immunologic response in the tumor microenvironment, initiated the development of immunotherapeutic drugs aimed at these proteins. In particular, the interaction between programmed cell death 1 (PD-1) and programmed death ligand-1 (PD-L1, also known as B7 homolog1, or B7-H1) was of interest. This interaction (the PD pathway) was shown to be a potential immune escape mechanism for tumor cells [19, 98, 125]. In the last decade, a novel category of therapeutic agents, so called checkpoint inhibitors, has emerged. These immunotherapeutic drugs have provided new opportunities in treating a variety of cancers including lung cancer. Several of these agents have been developed and tested in clinical trials and some have resulted in a significant improvement in clinical outcome [3, 5, 13, 15, 18, 24, 39, 40, 42, 104, 126-128].

In this review, we provide a brief overview of immunotherapeutic drugs aimed at the PD pathway, which are currently available in the clinic or in various testing phases in clinical trials. We focus on the development and characteristics of pembrolizumab, one such checkpoint inhibitor, discussing its current uses and future perspectives in treatment of NSCLC.

Pembrolizumab (Keytruda®, lambrolizumab, MK-3475) is a humanized monoclonal antibody (class IgG4/kappa) which was granted accelerated approval for treatment of patients with metastatic NSCLC, whose tumors show expression of PD-L1 [41, 129]. Pembrolizumab binds with high affinity to the PD-1 receptor, blocking its association with PD-L1 and PD-L2. This prevents an inhibitory signal from cancer cells to cytotoxic CD8 T cell [92, 130, 131] (Figure 1). It is the second anti-PD-1 antibody to be approved for second-line treatment of metastatic NSCLC, and the first to be approved for first-line treatment, provided it is accompanied by a diagnostic test selecting a specific subgroup of patients [15, 41, 132]. The goal of this article is to review the available clinical data on treatment of NSCLC with pembrolizumab, discussing efficacy, safety, quality of life (QoL) and the role of PD-L1 testing.

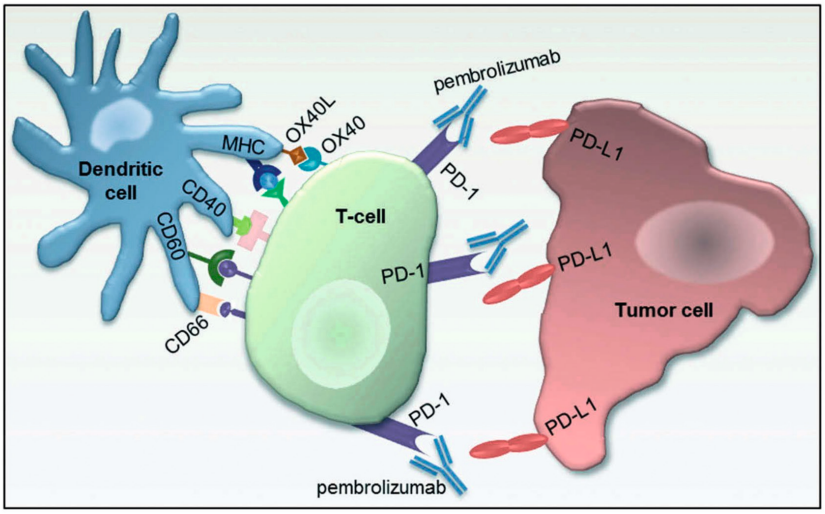

Figure 1 - Schematic of ligand-receptor pairs involved in inhibition and stimulation of T-cell antitumor activity.
Pembrolizumab blocks the PD-1/PD-L1 interaction, preventing immune escape by tumor cells.

1.1 Current needs, available therapies, competitor compounds

The class of checkpoint inhibitors receiving most attention and achieving best results in treatment of NSCLC in recent years are monoclonal antibodies (mAb) blocking the PD-1/PD- L1 (and PD-1/PD-L2) interaction. These can be divided into two groups: anti-PD-1 or anti-PD-L1 mAb.

The first anti-PD-1 mAb receiving approval for second-line treatment of NSCLC was nivolumab (Opdivo®, MDX-1106, BMS-936558, ONO-4538), showing objective responses in 17% of patients and an increased median overall survival (OS) of 14.9 months across all NSCLC patients at a dose of 3 mg/kg administered every other week [3, 5, 9, 132]. Pembrolizumab, an anti-PD-1 mAb, is registered for treatment of NSCLC in both first line (dependent on PD-L1 expression, discussed later in this article) and second line, and has shown increasing efficacy in the presence of PD-L1 expression on tumor cells [39, 40, 42, 127, 128, 133].

Other monoclonal antibodies aimed at PD-L1 have been developed as well. Atezolizumab (Tecentriq®, MPDL3280A) has recently been approved by the US FDA for treatment of NSCLC. Compared to docetaxel, it showed a significant improvement in OS of 4.2 months (13.8 months versus docetaxel, OS 9.6 months) in patients with previously treated NSCLC. This result was independent of both the histological subtype and level of PD-L1 expression. OS was longer in patient groups with higher levels of PD-L1 expression as was observed in the studies with pembrolizumab [104, 107, 134, 135]. The anti-PD-L1 mAb durvalumab (MEDI4736) is currently tested in phase III trials in multiple settings, including first, second and later lines of treatment, multiple stages of NSCLC, and in multiple combinations, including adjuvant after intended curation with chemoradiation therapy. Results of these trials are yet to be published [136-138]. Avelumab (MSB0010718C), a third anti-PD- L1 mAb tested in the large phase I 'Javelin'

trial, showed an objective response rate (ORR) of 22.4% of NSCLC patients not selected by histologic subtype of PD-L1 expression in first-line setting. The 24-week progression-free survival (PFS) rate was 37.2%. Further results are pending [139]. Last, the anti-PD-L1 mAb codenamed BMS-936559 has been tested in several solid tumors in a phase I trial and an objective response was seen in 5 out of 49 patients with NSCLC. This agent is not being further developed at this time [140, 141].

2. Pharmacology of pembrolizumab

2.1 Pharmacodynamics

Pembrolizumab is a highly selective antibody that blocks binding of PD-1 to PD-L1 and PD-L2 [142]. PD-1 was discovered in 1992 as member of the B7-CD28 superfamily. The immune regulatory role of PD-1 was shown in PD-1-deficient mice by inducing peripheral tolerance and antigen-specific immunity in infection and cancer. PD-1 is expressed on activated T cells (both CD8+ and CD4+), natural killer (NK) cells, antigen presenting cells (APC), monocytes, and B cells. Inflammatory cytokines produced by these cells induce expression of PD-L1 and PD-L2 on APCs. In this negative feedback loop, PD-1/PD-L1 and PD-1/PD-L2 interactions induce inhibitory signaling to T- and B cells, leading to decreased cytokine production and decreased antibody formation, and consequently to inhibition of antitumor immunity [143]. Thus, blocking interactions of the PD-1 receptor by pembrolizumab prevents inhibition of the immune response by tumor cells.

2.2 Pharmacokinetics

Ahamadi et al. pooled study data of 2195 patients who were treated for various types of cancer with pembrolizumab at intravenous doses of 1–10 mg per kilogram bodyweight (mg/kg) and in intervals of two or three weeks. Data were collected from three trials: KEYNOTE-001, KEYNOTE-002, and KEYNOTE-006 [144]. The pharmacokinetics of pembrolizumab were similar to other monoclonal antibodies. The clearance of pembrolizumab was low with 0.22 L/day. Central volume of distribution (V_c) was limited at 3.48 l and interindividual variability was low to moderate, with 38% for clearance and 21% for V_c. The elimination half-life was estimated at 14–27 days, with an apparently linear serum exposure with steady-state dosing in the range of 0.1–10 mg/kg [40, 133]. Steady state was reached after approximately 129 days and a modest accumulation of about 2.2-fold, based on AUC calculation at a three- weekly dosing schedule [144]. Effects of sex, baseline Eastern Cooperative Oncology Group Performance Status (ECOG-PS), ipilimumab treatment prior to pembrolizumab exposure, renal function, hepatic function, and tumor type are lacking in clinical significance [9, 144]. Recently presented data show that a flat dose of 200 mg results in equal serum exposure levels as compared to a three-weekly dose of 2 mg/kg, as tested in 152 individuals [145].

3. Clinical efficacy of pembrolizumab

3.1 Pembrolizumab monotherapy

The first study with pembrolizumab for use in treatment of metastatic NSCLC, the KEYNOTE-001 [40], was published in April 2015. In this phase I trial, with both pretreated and non-pretreated patients, 495 patients received at least one cycle of pembrolizumab,

at a dose of 2 mg/kg once every 3 weeks (Q3W, *n* = 6), 10 mg/kg Q3W (*n* = 287) or 10 mg/kg Q2W (*n* = 202). The ORR in all patients in this trial was 19.4% (95% CI:16.0–23.3%), PFS 3.7 months (median, range: 2.9–4.1 months) and OS 12.0 months (median, range: 9.3–14.7 months) (Table 1).

Table 1 - Overview of trials evaluation pembrolizumab in NSCLC

Name of study NCT number	**Phase Line**	**Disease setting Treatment**	**Patients (N)**	**ORR (percentage (95% CI)**	**PFS (months (95% CI))**	**OS (months)**
KEYNOTE 001 [40] NCT01295827	Phase I ≥ *1st line*	Treated and untreated A) Pembrolizumab 2 mg/kg Q3W B) Pembrolizumab 10 mg/kg Q3W C) Pembrolizumab 10 mg/kg Q2W	495 6 287 202	19.4% (16.0-23.2%)	3.7 months (2.9-4.1)	12.0 months (9.3-14.7)
KEYNOTE 001 [133] NCT01295827	Phase I ≥ *1st line*	Treated and untreated A) Pembrolizumab 2 mg/kg Q3W	52	15% (7-28%)	NA	NA
KEYNOTE 010 [39] NCT01905657	Phase II-III ≥ *2nd line*	PD-L1 positive ≥1% A) Pembrolizumab 2 mg/kg Q3W B) Pembrolizumab 10 mg/kg Q3W, C) Docetaxel 75 mg/m2 Q3W	1034 345 346 343	18% 18% 9% **P-values** A-C: P=0.0005 B-C: P=0.0002	3.9 months (3.1-4.1) 4.0 months (2.7-4.3) 4.0 months (3.1-4.2) **Hazard ratio** A-C: 0.88 (0.74-1.05, p=0.07) B-C: 0.79 (0.66-0.94, p=0.004)	10.4 (9.4-11.9) 12.7 (10.0-17.3) 8.5 (7.5-9.8) **Hazard Ratio** A-C: 0.71 (0.58-0.88, p=0.0008) B-C: 0.61 (0.49-0.75; p<0.0001)
KEYNOTE 021 [135] NCT02039674	Phase II *1st line*	Multicohort study A) Pembrolizumab + Chemotherapy B) Chemotherapy	123 59 62	55% (42-68%) 29% (18-41%)	13.0months (8.3-NR) 8.9 months (4.4-10.3) **Hazard ratio** 0.53 (0.31-0.91; p=0.010)	NR NR **Hazard ratio** 0.9 (0.42 – 1.91, P=0.39)
KEYNOTE 021 [146] Cohort A+B+C NCT02039674	Phase I ≥ *3rd line*	NSCLC Pembrolizumab + A) Carboplatin + Paclitaxel B) Carboplatin + Paclitaxel + Bevacizumab C) Carboplatin + Pemetrexed	74 25 25 24	57% (45-68%) 52% (31-72%) 48% (28-69%) 71% (49-87%)	10 months (6-NR) 10 (4-NR) NR (4.1-NR) 10 (6-15)	NR (17-NR) NR (11-NR) NR (NR-NR) NR (14-NR)
KEYNOTE 021 [147] Cohort D+H NCT02039674	Phase I ≥ *2nd line*	NSCLC A) Pembrolizumab + Ipilimumab	45	24% (13-40%)	6 months (1-17)	17 months (6-17)
KEYNOTE 024 [42] NCT02142738	Phase III *1st line*	PD-L1 Positive (≥50%) A) Pembrolizumab 200 mg Q3W B) Platinum-based chemotherapy	305 154 151	44.8% (36.8-53.0%) 27.8% (20.8-35.7%)	62.1% (53.8-69.4) 50.3% (41.9-58.2) **Hazard ratio:** 0.5 (0.37-0.68, P<0.001)	NR NR **Hazard ratio:** 0.6 (0.41-0.89; p=0.005)

Abbreviations: n: number, ORR: objective response rate, PFS: progression free survival, OS: Overall Survival, Q2W: one course every two weeks, Q3W: One course every three weeks. NR: Not reached, NCT: Number at clinicaltrials.gov.

Treatment naive patients had a slightly higher ORR of 24.8% (95% CI: 16.7–34.3%) compared to pre-treated patients: 18.0% (14.4–22.2%). In addition, a significant difference between smokers (ORR 22.5% (18.3–27.1%)) and nonsmokers (ORR 10.3% (5.6–17.0%)) was found. As a result of another trial in melanoma with pembrolizumab, which showed no differences between 2 mg/kg and 10 mg/kg [148], an extra cohort of 55 patients was added who received 2 mg/kg Q3 W pembrolizumab. The ORR in this group of patients was 15% (95% CI: 7–28%). In October 2015, based on this trial, the FDA approved pembrolizumab for the treatment of patients with metastatic NSCLC who are progressive on or after platinum based chemotherapy.

The KEYNOTE-010 trial [39] studies the effect of two doses of pembrolizumab in pretreated patients with NSCLC who expressed PD-L1. It was a randomized controlled phase II/III trial, with a total of 1034 patients, equally randomized to either pembrolizumab (2 mg/kg Q3W or 10 mg/kg Q3W) or docetaxel (75 mg/m2 Q3W). The ORR was 18% in both pembrolizumab groups compared to 9% in the docetaxel group, which was significant in both cases. Furthermore, the PFS was significantly different between pembrolizumab 10 mg/kg and docetaxel (Hazard Ratio (HR) 0.79 (95% CI: 0.66–0.94, $p = 0.004$)), but not between pembrolizumab 2 mg/kg and docetaxel (HR 0.88 (0.74–1.05, $p = 0.07$)) for patients who had tumors with only ≥1% of cells expressing PD-L1. The updated OS data of this trial and duration of clinical benefit of patients who completed their treatment with pembrolizumab (total treatment time of 24 months), were presented at the World Conference on Lung Cancer (WCLC) in Vienna in 2016 [149]. Median follow-up was 2.1 years (median, range: 1.5–3.0 years). Median OS was 10.5 months (range: 9.6–12.4) in the 2 mg/kg and 13.4 months (median, range: 11.2–17.0) in the 10 mg/kg group. Compared to docetaxel (median 8.6 months, range: 7.0–9.8) this was significantly longer, with Hazard Ratios of 0.72 (95% CI: 0.60–0.86, $p = 0.00017$) and 0.60 (95% CI: 0.49–0.72, $p < 0.0001$), respectively. The 24 months OS was 30.1% (95% CI: 25.0–35.4%) and 37.5% (95% CI: 32.2–42.9%) in both pembrolizumab groups, significantly higher than in the docetaxel group (14.5% (95 CI: 10.5–19.2%)).

In the first-line setting, pembrolizumab monotherapy was compared to standard chemotherapy in the KEYNOTE-024 phase III trial [42]. Only patients with tumors expressing ≥50% PD-L1 were included. The treatment schedule was a three weekly flat dose of 200 mg pembrolizumab. Results were first published in October 2016. Of the 305 patients assigned to this trial, 154 received pembrolizumab and 151 received chemotherapy. The ORR for pembrolizumab was 44.8% (95% CI: 36.8–53.0%) compared to 27.8% (95% CI: 20.8–35.7%) for chemotherapy. The median duration of response was not reached (NR) for the pembrolizumab group, compared to 6.3 months (range: 2.1–12.6 months) for the chemotherapy group. Median PFS was 10.3 months (range: 6.7-NR) compared to a median 6.0 months (range: 4.2–6.2) in the chemotherapy arm. The HR for the occurrence of a PFS event was 0.5 (95% CI: 0.37–0.68). OS was not reached in both cohorts.

3.2 Combination therapy

The combination of pembrolizumab with chemotherapy was examined in a small phase II trial [135]. This phase II trial, KEYNOTE-021, was part of a multicohort trial and compared the combination of pembrolizumab and chemotherapy ($n = 59$) to chemotherapy alone

(*n* = 62) in the first line. Response evaluation was done using RECIST version 1.1 assessed by blinded, independent central review. The ORR for combination therapy was 55% (95% CI: 42–68%) compared to 29% (95% CI: 18–41%). The benefit in the HR of PFS 0.53 (95% CI: 0.3–0.91; *p* = 0.010) was observed in favor of the combination therapy, with a median PFS in the combination group of 13.0 months (range: 8.3-NR), compared to a median 8.9 months (range: 4.4–10.3) in the chemotherapy alone group. For both groups, the median OS was not reached.

Data of five cohorts in the phase I part of the KEYNOTE-021 trial are not published yet. However, some preliminary data were presented at the annual meeting of the American Society of Clinical Oncology (ASCO) in 2016. Cohorts A, B, and C compared three combination therapies: pembrolizumab with (A) carboplatin + paclitaxel (*n* = 25), (B) carboplatin, paclitaxel and bevacizumab (*n* = 25), or (C) carboplatin + pemetrexed (*n* = 24). ORR for all patients was 57% (95% CI: 45–68%). So far, the combination with carboplatin and pemetrexed seems the most promising with an ORR of 71% (49–87%). Both PFS and OS data are incomplete and results are expected in June 2019 [146]. Cohorts D and H explore the combination therapy of pembrolizumab together with ipilimumab. The results of 45 enrolled patients showed an ORR of 24% (95% CI: 13–40%), median PFS of 6 months (range: 1–17), and median OS of 17 months (range: 6–17 months) [147].

KEYNOTE-189 and KEYNOTE-407 evaluated the combination of chemotherapy and pembrolizumab in first line in two randomized, placebo controlled trials. Results are expected in March 2019 (Figure 2). An overview of the most important other ongoing trials are listed in Table 2, including one trial exploring pembrolizumab after adjuvant therapy (KEYNOTE-091).

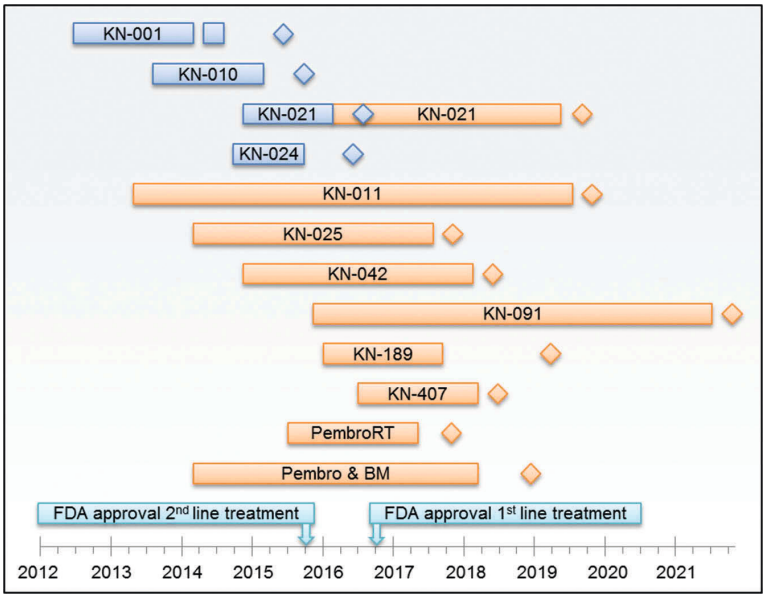

Figure 2 - Overview of published (blue) and current (orange) trials.
Diamonds represent (expected) results. Trials are corresponding with Table 1 (published trials) and 2 (ongoing trials).

Table 2 - Most important ongoing trials with pembrolizumab

Name of study NCT number	Phase Line	Disease setting Treatment	Estimated enrollment of patients (n)
KEYNOTE 011 NCT01840579	Phase I *2nd line*	Cohort B + C: Confirmed NSCLC B) Pembrolizumab + carboplatin + pemetrexed C) Pembrolizumab + carboplatin + (nab-) paclitaxel	48
KEYNOTE 025 NCT02007070	Phase I *2nd line*	PD-L1 positive (≥1%) A) Pembrolizumab 10 mg/kg Q3W	38
KEYNOTE 042 NCT02220894	Phase III *1st line*	PD-L1 positive A) Pembrolizumab 200 mg Q3W B) Chemotherapy (investigator's choice)	1240
KEYNOTE 091 (PEARLS) NCT02504372	Phase III *Post adjuvant treatment*	Stage IB, II - IIIA A) Pembrolizumab 200 mg Q3W B) Placebo	570
KEYNOTE 189 NCT02578680	Phase III *1st line*	Non-Squamous A) Pembrolizumab + chemotherapy B) Placebo (saline) + chemotherapy	570
KEYNOTE 407 NCT02775435	Phase III *1st line*	Squamous A) Pembrolizumab + chemotherapy B) Placebo (saline) + chemotherapy	560
Pembrolizumab and brain metastases NCT02085070	Phase II	Brain metastases + NSCLC A) Pembrolizumab	64
PEMBRO-RT NCT02492568	Phase II *2nd line*	Advanced NSCLC A) Pembrolizumab after SBRT B) Pembrolizumab alone	74

Abbreviations: *NCT: Number at clinicaltrials.gov. N: Number, Q2W: one course every two weeks, Q3W: one course every three weeks.*

3.3 PD-L1 expression and efficacy.

In the KEYNOTE-001 study, PD-L1 expression was assessed in tumor samples using two assays. First, the prototype assay which was developed and validated for use in a single accredited laboratory. Second, the clinical trial assay which was developed by DAKO, sponsored by MSD. Both assays are similar and the latter is now commercially available in an investigational kit. This immunohistochemistry (IHC) assay with anti-PD-L1 antibody clone 22C3 was tested by pathologists at DAKO and LabCorp facilities for accuracy and intra- pathologist reproducibility. The cut point was defined after a receiver operating characteristic (ROC) analysis, comparing multiple IHC scoring methods related to investigator-assessed immune-related response criteria (irRC) [40, 106]. As no major difference between scoring methods was observed, an operating point on the curve representing (simple) proportion score (PS, the proportion of viable cells expressing PD-L1 on their membrane on any level of intensity) was selected, which correlated with a Youden Index of 0.45–0.50. This operating point corresponded to a cutoff value of 50% (PS). The predictive value of the assay did not improve by including PD-L1 expression on T cells [40].

In the clinical setting, PD-L1 assessment is performed by scoring the percentage of viable tumor cells showing membranous staining of PD-L1 (tumor proportion score, or TPS). In the KEYNOTE-001 study, PD-L1 positivity was defined as a TPS of ≥1%. For defining the PD-L1 cut-of a training cohort was formed with 182 patients, of whom 129 had measurable disease according to RECIST 1.1 criteria.

Table 3 - ORR, PFS and OS on pembrolizumab, stratified by PD-L1 expression

Name of study NCT number	Treatment	Cut-Off	Patients (n)	ORR (percentage (95% CI))	PFS in months (median (range))	OS in months (median (range))
KEYNOTE 001 [40] NCT01295827	Pembrolizumab All regimes: 1) 2 mg/kg Q3W 2) 10 mg/kg Q3W 3) 10 mg/kg Q2W	Training: ≥50% 1-49% <1% Validation ≥50% 1-49% <1%	38 43 40 73 103 28	34.2% 9.3% 10.0% 45.2% 16.5% 10.7%	4.5 (1.9-12.5) 2.1 (2.0-2.9) 2.1 (1.8-2.5) 6.4 (4.2-NR) 4.1 (2.3-4.4) 4.0 (2.1-6.2)	13.7 (6.9-NR) 5.9 (4.2-8.2) 6.7 (3.9-10.0) NR (NR-NR) 10.6 (7.3-NR) 10.4 (5.8-NR)
KEYNOTE 001 [133] NCT01295827	Pembrolizumab: 1) 2 mg/kg Q3W 2) 10 mg/kg Q3W 3) 10 mg/kg Q2W	≥50% 1-49% <1% ≥50% 1-49% <1% ≥50% 1-49% <1%	23 23 4 42 49 18 31 43 9	30%(13-53%) 0% (0-15%) 25% (<1-81%) 48 (32-64%) 14 (6-27%) 6 (<1-27%) 39%(22-58%) 14% (5-28%) 11% (<1-48%)		
KEYNOTE 010 [39] NCT01905657	Pembrolizumab 1) 2 mg/kg Q3W 2) 10 mg/kg Q3W	≥50% ≥50%	139 151	30% 29%	5.0 (4.0-6.5) 5.2 (4.1-8.1)	14.9 (10.4-NR) 17.3 (11.8-NR)
KEYNOTE 021 [135] NCT02039674	Pembrolizumab + chemotherapy	≥50% 1-49% <1%	20 19 22	80% (56-94%) 26% (9-51%) 57% (34-78%)		
KEYNOTE 021 [146] Cohort A+B+C NCT02039674	Pembrolizumab + chemotherapy	≥50% 1-49% <1%	25 26 22	54% (33-73%) 55% (32-76%) 62% (45-78%)	15 (6-15) 14 (6-NR) 6 (4-NR)	17 (15-NR) NR (14-NR) 11 (7-NR)
KEYNOTE 021 [147] Cohort D+H NCT02039674	Pembrolizumab + ipilimumab	≥50% 1-49% <1%	6 18 21	17% 33% 19%	1 (0.2-6) NR (1-NR) 6 (1-17)	2 (0.3-NR) NR (3-NR) 17 (6-17)
KEYNOTE 024 [42] NCT02142738	Pembrolizumab 200 mg Q3W	≥50%	154	44.8% (36.8-53.0)	10.3 (6.7-NR)	NR

Abbreviations: *n: number, ORR: objective response rate, PFS: progression free survival, OS: Overall Survival, Q2W: one course every two weeks, Q3W: One course every three weeks. NR: Not reached. NCT: Number at clinicaltrials.gov.*

After stratifying results for PD-L1 expression, the cut-off for maximum efficacy was set at TPS ≥50%. In this training cohort, 38 patients had TPS ≥50%, with an ORR of 34.2% (95% CI: 19.6–51.4%) according to RECIST 1.1 (Table 3). 313 patients were included in the validation cohort, in which 73 patients had TPS ≥50%. Here, ORR was 45.2% (95% CI: 33.5–57.3%), median PFS in this cohort was 6.4 months (range: 4.2–NR), and median OS was not reached (range: NR–NR). For this analysis, only patients treated with pembrolizumab (mostly 10 mg/kg Q2W or Q3W) were evaluated [40]. In the added cohort

of the KEYNOTE-001 study, 23 out of 55 patients receiving 2 mg/kg Q3W pembrolizumab had TPS ≥50%. Here, ORR was 30% (95% CI: 13–53%) [133].

In the KEYNOTE-010 study [39], only patients with PD-L1 expression of at least 1% were included. In this study, 2222 screened patients were evaluable for their PD-L1 status, of whom 747 were PD-L1 negative (TPS <1%). For further PD-L1 positivity scoring in this trial, the cut-off was again set at TPS ≥50%. The number of patients with TPS ≥50% was similar in the three groups, namely *n* = 139 patients in the 2 mg/kg group, *n* = 151 in the 10 mg/kg group and *n* = 152 in the docetaxel group. ORR was 30% vs. 29% vs. 8%, respectively, which was higher compared to the total group (ORR: 18%, 18%, and 9%, respectively). PFS in both pembrolizumab groups was significantly superior over the docetaxel group with HR 0.59 (95% CI: 0.44–0.78, $p = 0.0001$) and HR 0.59 (95% CI: 0.45–0.78, $p < 0.0001$), respectively. Hazard ratios for OS were significantly lower in the pembrolizumab groups compared to the docetaxel group as well: 0.54 (95% CI: 0.38–0.77, $p = 0.0002$) and 0.50 (95% CI: 0.36–0.70, $p < 0.0001$), with a median OS of 14.9 months (range: 10.4–NR) and 17.3 months in the pembrolizumab groups, versus 8.2 months in the docetaxel group. In this trial, analysis of the PD-L1 expression was performed on both archival and fresh tumor samples [39]. At the ASCO Annual Meeting 2016, a poster was presented summarizing a comparative study on archival versus fresh tumor samples [150]. 44% of the patients had archival material and 56% had fresh samples, with a median of 250 days between sample collection and PD-L1 assessment (range: 2–2510 days) and 11 days (range: 1–371 days), respectively. For both groups, OS and PFS were significantly better compared to docetaxel, independent of age of the tumor samples. These data suggest archival tissue is sufficient for assessment of PD-L1 status, potentially saving patients the risks associated with obtaining tumor biopsies.

In the recently published KEYNOTE-021 trial [135], the combination of pembrolizumab with chemotherapy is compared to chemotherapy alone. Patients were stratified based on their PD-L1 status, classified in PD-L1 negative (<1%), or PD-L1 positive (1–49% or ≥50%). Patients who had a TPS ≥50% (*n* = 20) had a higher ORR (80% (95% CI: 56–94%)), compared to the TPS 1–49% group (ORR 26%, *n* = 19) and PD-L1 negative tumors (ORR 57%, *n* = 22), and compared to chemotherapy alone (ORR 35%, *n* = 17).

Finally, in the KEYNOTE-024 trial [14], patients were only included if they had TPS ≥50%. The group which received pembrolizumab (*n* = 154) had an ORR of 44.8% (95% CI: 36.8–53.0%), compared to 27.8% for chemotherapy. Based on this trial, the FDA approved pembrolizumab for first-line treatment of patients with metastasized NSCLC who are ≥50% PD- L1 positive, using the validated IHC assay with the 22C3 antibody.

Most studies show a relationship between the efficacy of pembrolizumab and level of PD-L1 expression on tumor cells. Expression is assessed by a validated assay using the 22C3 antibody on tumor biopsies, which are typically small in lung cancer. There have been several reports mentioning a heterogeneity of PD-L1 expression between tumor and metastatic sites, both lymph nodes and (distant) metastases. The rate of discordance between resections and matched biopsies may be up to 48%, suggesting that scoring of PD-L1 TPS on a single biopsy may be unreliable. Further research on this subject is needed, but being aware of the possible variability in PD-L1 expression within the same tumor is imperative [151-153].

4. Safety and tolerability

4.1 Pembrolizumab as monotherapy

Pembrolizumab blocks the PD-1 checkpoint, which is involved in preventing autoimmunity. Blocking this pathway may induce autoimmunity with unregulated activation of T cells directed at normal tissue. Therefore, toxicity profiles differ from chemotherapy or targeted therapy by inducing immune-mediated advance events (imAEs) [154].

The KEYNOTE-001 trial reported modest toxicity to pembrolizumab and the agent seems well tolerated. Treatment related adverse events of any grade were reported in 70.9% of patients. Grade 3 or higher toxicities were reported in 9.5% of treated patients. Less severe treatment-related side effect were low-grade diarrhea, fatigue and skin reactions. Most reported imAEs were hypothyroidism (6.9%), pneumonitis (3.6%), and infusion-related reaction (3.0%). Particularly, pneumonitis is an imAE of relevance in patients with NSCLC, as it may be life-threatening. During KEYNOTE-001, one patient died from this serious immunotherapy-related toxicity [40]. Chatterjee et al. reported an additional cohort of KEYNOTE-001 (pembrolizumab 2 mg/kg Q3W). In this cohort of 55 patients, 26 (47%) experienced a treatment-related adverse event, five (9%) of these were grade 3 or above. Data from the KEYNOTE-001 trial showed that the dose of pembrolizumab was not correlated with hazard for the occurrence of imAEs. However, the duration of treatment was positively correlated with the probability of experiencing an immune mediated adverse event [133].

The KEYNOTE-010 trial reported a comparable safety profile. 81% of the patients in the docetaxel group experienced an adverse event, which was higher than both pembrolizumab groups (63% in the 2 mg/kg group and 66% in the 10 mg/kg group). As was shown previously in KEYNOTE-001, fatigue and diarrhea were frequently reported. Grade 3–5 adverse events were reported in 13% and 16% of patients in the 2 mg/kg and 10 mg/kg groups, respectively, and in 35% of patients in the docetaxel group. The incidence of imAEs was similar in both pembrolizumab groups: 20% in the 2 mg/kg group and 19% in the 10 mg/kg group. Of 682 treated patients, six (<1%) died of pembrolizumab-related toxicities. Three deaths were due to pneumonitis, two due to pneumonia, and one due to myocardial infarction [16]. In the KEYNOTE-024 trial, pembrolizumab was compared to chemotherapy and diarrhea was the most reported adverse event in both treatment groups (14.3% and 13.3%, respectively, Table 4). Pneumonitis was reported in 5.8% of patients treated with pembrolizumab [42].

Table 4 - Toxicities compared with other agents

Study	First line treatment						Second line treatment					
Agent	**Pembrolizumab**		**Chemotherapy**		**Nivolumab**		**Pembrolizumab**		**Docetaxel**		**Nivolumab**	
Dose	**200 mg flat dose**				**3 mg/kg**		**2 mg/kg**		**75 mg/m2**		**3 mg/kg**	
	KEYNOTE-024 [42]		**KEYNOTE-024 [42]**		**CHECKMATE-012 [36]**		**KEYNOTE-010 [39]**		**KEYNOTE-010 [39]**		**CHECKMATE-057 [5]**	
Patients (N)	**N=145**		**N=150**		**N=52**		**N=339**		**N=309**		**N=287**	
	Any grade	**Grade 3-5**	**Any grade**	**Grade 3-5**	**Any grade**	**Grade 3-5**	**Any grade**	**Grade 3-5**	**Any grade**	**Grade 3-5**	**Any grade**	**Grade 3-5**
Any adverse event	73.4%	26.6%	90.0%	53.3%	71%	19%	63%	13%	81%	35%	69%	10%
Pneumonitis	5.8%	2.6%	0.7%	0.7%	6%	2%	4.1%	1.8%	1%	0.3%	3%	1%
Diarrhea	14.3%	3.9%	13.3%	1.3%	12%	2%	7.1%	0.6%	18.1%	2.3%	8%	1%
Hypothyroidism	9.1%	None	1.3%	None	6%	None	7.4%	None	0.3%	None	7%	None
Skin toxicity (rash, any)	3.9%	3.9%	None	None	19%	4%	10,70%	0.6%	5.1%	None	13%	0.3%
Renal toxicity (creat. increase)	1.9%	None	10.0%	0.7%	NR	NR	1.8%	None	None	None	1.7%	None
Fatigue	10.4%	1.3%	28.7 %	3.3%	29%	None	13.6%	1.2%	24.6%	3.6%	3%	25%

Abbreviations: *N: number, mg/kg: milligrams per kilogram bodyweight, NR: Not Reported, creat: creatinine*

4.2 Pembrolizumab in combination therapy

In the KEYNOTE-021 study, chemotherapy plus pembrolizumab was compared to chemotherapy alone. Adverse events of any grade were reported in 90% and 93%, respectively. Grade 3–5 toxicities were reported in 39% and 26% of treated patients. The incidence of imAE was higher in the pembrolizumab combination arm (22% versus 11%, respectively). Pneumonitis of any grade was reported in 3% of cases in the combination cohort and in none of the patients treated with chemotherapy alone. Other treatment groups of the KEYNOTE-021 included cohort A (pembrolizumab 2 or 10 mg/kg Q3W + carboplatin AUC 6 + paclitaxel 200 mg/m^2) and cohort C (pembrolizumab 2 or 10 mg/kg Q3W + carboplatin AUC 5 + pemetrexed 500 mg/m^2). In these cohorts, the rate of grade 3 or 4 treatment-related adverse events was 15% in cohort A, and 38% in cohort C. Toxicities included reversible transaminase elevation (*n* = 3 in cohort C), anemia (*n* = 1 in A, *n* = 2 in C), rash (*n* = 1 in A, *n* = 1 in C), and colitis (*n* = 2 in C). No treatment-related deaths occurred [43].

4.3 Quality of Life

The toxicity profile and derived therefrom the quality of life showed good results, as presented by Dr. Brahmer at the WCLC in Vienna 2016 [155]. The incidence of any grade of adverse event was 73% for pembrolizumab, compared to 90% for docetaxel, with incidences of grade 3–5 events of 27% and 53%, respectively. QoL was assessed, at baseline, at cycles 1, 2, and 3, and every 9 weeks thereafter, with two different questionnaires. The general QoL (QLQ-C30) showed a difference between the least square means of 7.82 (95% CI: 2.85–12.79, $p = 0.002$) in favor of pembrolizumab. The deterioration of typical lung cancer symptoms was assessed with the QLQ-LC13. Patients receiving pembrolizumab reported less deterioration compared to docetaxel (30% versus 39%). The hazard ratio (0.66, 95% CI: 0.44–0.97, $p = 0.029$) for time to deterioration was in favor of pembrolizumab as well.

5. Regulatory affairs

In October 2015, the FDA approved pembrolizumab for the treatment of patients with metastatic NSCLC who are progressive on or after platinum-containing chemotherapy. This approval was based on the KEYNOTE-001 trial. In October 2016, the FDA expanded the approval of pembrolizumab to first-line treatment of patients with NSCLC strongly positive for PD-L1 (TPS ≥50%, as assessed by IHC using the 22C3 anti- body). This was based on the KEYNOTE-010 and KEYNOTE-024 trials. The EMA stated in December 2016: ‘Keytruda as mono- therapy is indicated for the first-line treatment of metastatic non-small cell lung carcinoma (NSCLC) in adults whose tumors express PD-L1 with a ≥ 50% tumor proportion score (TPS) with no EGFR or ALK-positive tumor mutations. Keytruda as mono- therapy is indicated for the treatment of locally advanced or metastatic NSCLC in adults whose tumors express PD-L1 with a≥1% TPS and who have received at least one prior che- motherapy regimen. Patients with EGFR or ALK-positive tumor mutations should also have received targeted therapy before receiving Keytruda’ [156].

6. Expert commentary

Multiple therapies are currently available for patients who are diagnosed with stage IV lung cancer. Since the first FDA approval of pembrolizumab in October 2015, more data have become available from the KEYNOTE studies on pembrolizumab (Figure 2), including data concerning the role of the PD-L1 biomarker. Most important are the data of the KEYNOTE-024 trial, published in October 2016, a first-line phase III trial which only included patients with a TPS ≥50%. With an ORR of 44.8% in the pembrolizumab group, compared to 27.8% in the chemotherapy group, there seems to be some evidence PD-L1 is a good marker for clinical benefit, which lead to the second FDA approval. However, although the expression of PD-L1 with a TPS ≥50% was associated with a significantly improved clinical benefit compared to TPS <50%, there was no difference between >1% (PD-L1 positive) and <1% (PD-L1 negative) in all dose regimes of pembrolizumab (Table 3). This implies that even in PD-L1-negative patients, responses can be as high as in the intermediate group (TPS 1–50%). A difference between TPS <50% and TPS ≥50% groups is seen in the KEYNOTE-010 trial, where only patients who were PD-L1 positive, were included. Here, ORR improved from 18% to 29–30%, respectively. Noticeable, although the KEYNOTE-006 trial [42] for melanoma showed no difference between 2 and 10 mg/kg pembrolizumab, a difference in OS is seen between these two groups. The hazard ratio for 2 mg/kg was 0.71, improving to 0.61 with the use of 10 mg/kg, suggesting10 mg/kg might be an option for patients who are <50% positive for PD-L1. Nevertheless, the predictive value of PD- L1 staining remains suboptimal and its use in clinical practice is questionable, considering the relatively high false-positive and false-negative rates. Therefore, the search for better predictive biomarkers for response to pembrolizumab and other immunotherapeutic drugs should continue. An interesting theory, recently published by Blank et al. [62], introduced the paradigm 'The Immunogram', in which simple blood tests (e.g. CRP and LDH) predict the response to immunotherapy. Another suggestion, not yet published, is that patients whose tumor show rapid growth may not be suitable candidates for immune-oncologic treatment. Both theories need further research. A phenomenon seen in immunotherapy is pseudo progression. Due to infiltration by activated T cells, an increased tumor volume can be seen on CT imaging. The patient might then be wrongly classified as progressive and treatment might be discontinued. This phenomenon complicates response evaluation by CT imaging, although recent data from our institute show less pseudo progression than expected, with a prevalence of only 2% [24, 157]. An early response marker would be helpful and more research is needed.

7. 5 year view

The first trial with pembrolizumab included patients in 2012 and at this moment most clinical trials of monotherapy with pembrolizumab are published [39, 40, 42, 133], which proved itself by leading to two approvals by the FDA for patients with advanced NSCLC. However, the first approved immune therapy for NSCLC was nivolumab, which is now widely used in many trials and seen as first-choice anti-PD-L1 therapy. The use of nivolumab does not require PD-L1 testing, which makes it the biggest competitor of pembrolizumab. However, the treatment schedule of pembrolizumab is less intensive

(Q2W for nivolumab versus Q3W for pembrolizumab), with comparable survival and quality of life (mostly compared to chemotherapy). Pembrolizumab is a welcome addition to the arsenal of anticancer drugs and has the potential to become first choice of treatment for many NSCLC patients in the near future. Results of clinical trials on other checkpoint inhibitors, such as durvalumab, atezolizumab, and avelumab are promising and should be awaited as well. Most promising for the nearest future are the results of the combination therapies. Data from the first trial, KEYNOTE-021 [135], were recently published in The Lancet. The combination of chemotherapy with pembrolizumab was compared to chemotherapy alone. The ORR was 55%, higher than 44.8% among patients with TPS ≥50% group in the KEYNOTE-024 trial, which is promising for other combination therapies. However, two other cohorts in the KEYNOTE-021 trial, evaluating the efficacy of the combination of pembrolizumab with ipilimumab, showed disappointing results, with an ORR of 24% (all 45 treated patients) and 17% (TPS ≥50%, 6 patients). In Table 2, other ongoing trials are shown. The most important are KEYNOTE-189 and KEYNOTE-407, both evaluating the combination of pembrolizumab with chemotherapy in a randomized controlled trial. Results are expected in March 2019 and hopefully in 5 years' time a successful combination is found. One important question for the nearest future concerning current immunotherapy is the duration of the therapy itself.

Currently, the golden standard is to treat patients for 2 years with pembrolizumab. No maximal treatment time is defined for nivolumab. Based on the theory of stimulating and 'learning' of the immune system, 12, 6, or even three months of treatment may be sufficient to reach and maintain a durable response. Shorter treatment times are found with other monoclonal antibodies. Standard treatment with ipilimumab for example, consists of only four cycles. Currently, the longest follow-up time is 3 years [149], so data on this subject is scarce. In 5-years' time, this question will be answered.