Pembrolizumab KEYNOTE-001: an adaptive study leading to accelerated approval for two indications and a companion diagnostic

The landscape of cancer treatment has undergone a vast change over the past four decades [1, 2]. Discovery of the heterogeneous molecular features of tumors and the associated microenvironment has led to the development of novel classes of targeted therapeutics [3–5], the two main types of which are small-molecule inhibitors and monoclonal antibodies (mAbs) [1–3]. These targeted drugs have furthered the development of personalized therapeutic regimens in oncology.

Cancers exhibit numerous genetic and epigenetic alterations manifesting as a diverse population of antigens that the immune system should be able to use to differentiate between tumor tissues and their healthy counterparts [6]. However, many cancers can 'hide' from immune surveillance (i.e. immune evasion) [7, 8]. Maintenance of self-tolerance under normal physiologic conditions is regulated by immune checkpoints, and expression of immune-checkpoint proteins can be impaired in tumor tissue [6]. Early research into the manipulation of antitumor immunity focused on T cells, and specifically blockade of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) [9]. Clinical trials of ipilimumab, a fully humanized CTLA-4 mAb, demonstrated antitumor activity and a survival benefit in patients with unresectable or metastatic melanoma [10–12], resulting in its approval in 2011 by the United States Food and Drug Administration (FDA) for this patient population [6].

Programmed death 1 (PD-1) is another immune-checkpoint receptor; specifically, a negative costimulatory receptor that is expressed on the surface of activated T cells, B cells, natural killer T cells, and dendritic cells [6, 13–16]. Binding of PD-1 with its ligands, PD-L1 and PD-L2, inhibits the cytotoxic T-cell response [17]. The PD-1 pathway plays an important role in the induction and maintenance of immune tolerance, enabling the body to defend itself against a wide variety of pathogens while simultaneously protecting against self-reactivity (autoimmunity) [18]. In this way, expression of PD-L1 on endothelial cells may be, in part, responsible for maintaining tissue tolerance [18]. PD-L1 and PD-L2 are expressed on the surface of tumor cells in many cancer types; PD-L1 expression has been found both intracellularly and extracellularly in epithelial cancers, including melanoma and non-small cell lung cancer (NSCLC), and PD-L2 expression has been found in lymphoid malignancies such as mantle cell lymphoma [15, 19, 20], as well as in several solid tumors including head and neck squamous carcinoma (HNSCC), both with and without concomitant PD-L1 staining [21]. Furthermore, PD-1 expression is upregulated on tumor-infiltrating lymphocytes [15, 20]. While the presence of these immune-checkpoint receptors enables some tumors to escape destruction via the T-cell immune response, it also provides a promising target for antitumor therapy [6, 20].

Pembrolizumab (Keytruda; Merck & Co., Inc., Kenilworth, NJ, USA) is a humanized mAb that blocks the interaction between PD-1 and its ligands [22], thereby enabling an antitumor immune response (Figure 1). The unique clinical development of pembrolizumab began in 2010 with an investigational new drug application submitted to the FDA, followed by the initiation of a seminal phase 1 clinical trial in patients with advanced solid tumors—KEYNOTE-001 (ClinicalTrials.gov identifier: NCT01295827; Figure 2). Pembrolizumab was granted orphan drug designation for the treatment of advanced melanoma in late 2012 [23, 24] and was subsequently awarded breakthrough therapy designation for advanced melanoma in 2013 (Figure 2) [25]; this was the first FDA-granted breakthrough therapy designation for a cancer drug. Orphan drug designation is granted by the FDA for drugs intended to treat rare diseases [23]. Breakthrough therapy designation is granted by the FDA for drugs intended to treat a serious condition and for which preliminary clinical evidence has demonstrated a marked improvement in a clinically significant endpoint over existing therapies [26]. Breakthrough therapy designation enables expedited clinical development, which in the case of pembrolizumab ultimately led to its accelerated approval in the USA in 2014 for the treatment of patients with unresectable or metastatic melanoma and disease progression after ipilimumab and, if $BRAF^{V600}$ mutation positive, a BRAF inhibitor [22, 23, 27]. Accelerated approval is granted by the FDA for drugs that fill an unmet medical need for a serious or life-threatening disease or condition based on a surrogate endpoint that is reasonably likely to predict clinical benefit. It allows for earlier approval, enabling the drug to be provided to patients sooner than would otherwise be possible. Confirmation of benefit is required through confirmatory trials [26]. This milestone for pembrolizumab represented the first regulatory approval for an anti-PD-1 agent in the USA.

The clinical evaluation of pembrolizumab in the KEYNOTE-001 trial in patients with metastatic NSCLC led to breakthrough therapy designation in that indication in 2014 [28] and subsequent accelerated FDA approval in 2015 [29] for the treatment of patients with PD-L1-expressing metastatic NSCLC with disease progression on or after platinum-containing therapy. The approval for NSCLC was accompanied by approval of a companion diagnostic (PD-L1 immunohistochemistry [IHC] 22C3 pharmDx, Dako, Carpinteria, CA) for PD-L1 expression status [30, 31]. This review describes the unique design and evolution of the pembrolizumab KEYNOTE-001 study and the resulting unprecedented regulatory outcomes.

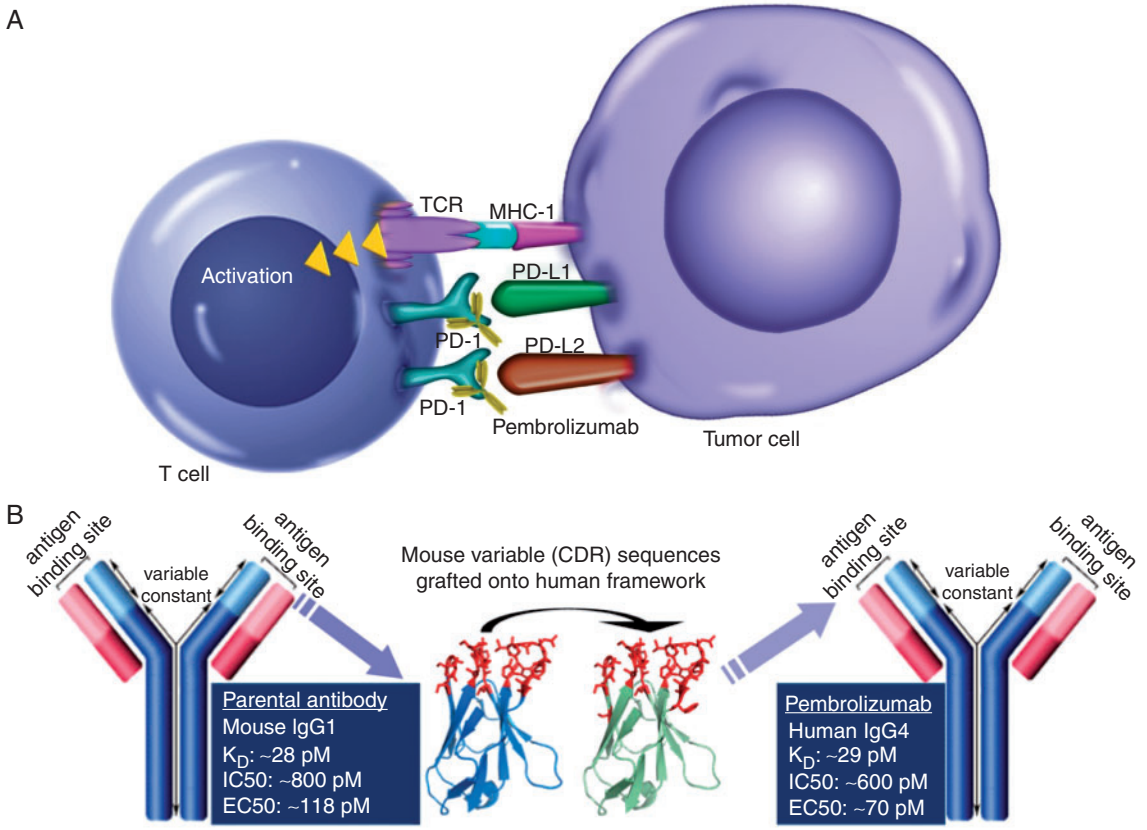

Figure 1. (A) Engagement between programmed death 1 (PD-1) and its ligands, PD-L1 and PD-L2, can enable some tumors to evade T-cell immune surveillance. PD-1 inhibitors such as pembrolizumab can 'unmask' PD-L1-expressing cells from the antitumor immune response. (B) Design of the pembrolizumab monoclonal antibody. CDR, complementarity determining region; EC50, half-maximal effective concentration; IC_{50}, half-maximal inhibitory concentration; K_D, dissociation constant; MHC-1, major histocompatibility complex 1; PD-1, programmed death 1; PD-L1, programmed death ligand 1; PD-L2, programmed death ligand 2; TCR, T-cell receptor.

Design and evolution of the KEYNOTE-001 study

Clinical development

At the time of the pembrolizumab investigational new drug application, there was a substantial unmet need for new treatments in both melanoma and NSCLC [32, 33]. It was hypothesized that with its novel mechanism of action, pembrolizumab might be of clinical benefit in patients with these tumor types. This hypothesis was supported by the finding of PD-L1 expression in a proportion of both melanoma and NSCLC tumors [15, 19, 20] and the reported correlation between PD-L1 expression, poor prognosis, and high invasiveness in NSCLC [19, 34–37]. Preclinical studies suggesting that pembrolizumab had antitumor properties in multiple cancers [38, 39] led to initiation of the phase 1 KEYNOTE-001 first-in-human study in January 2011 (Figure 2). Initially designed as a dose-finding study, the primary objective of KEYNOTE-001 was to explore the safety and tolerability of pembrolizumab and to determine whether it conferred antitumor activity in patients with advanced solid tumors. Primary efficacy endpoints were objective response rate (ORR) per Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1) and disease control rate (DCR) per RECIST v1.1; progression-free survival (PFS) and overall survival (OS) were included as secondary efficacy objectives. Through its unique evolution based on interim findings and the addition of melanoma- and NSCLC-specific expansion cohorts that included three dose-finding, randomized experiments with pre-specified statistical analyses, this study eventually culminated in the recruitment and treatment of 1235 patients; enrollment was complete in July 2014 (Figure 3) [40].

Pembrolizumab in advanced solid tumors. The initial aims of the first cohorts in KEYNOTE-001 were to define dose-limiting toxicities (DLTs), to characterize the pharmacokinetic properties, and to establish a recommended phase 2 dose (RP2D) for pembrolizumab in patients with advanced solid tumors. Therefore, the first part of the study was composed of a 3 + 3 dose-escalation design (cohort A), the main aim of which was to establish the safety and tolerability of pembrolizumab and to identify the RP2D, with doses ranging up to a maximum of 10 mg/kg every 2 weeks (Q2W) [41]. Confirmed ORR as assessed by investigator review and duration of response (DOR) were also evaluated. Based on the findings of cohort A, additional patients were enrolled in two additional cohorts: one to be treated at the maximum-tolerated dose (MTD) or the maximum administered dose (MAD) defined in cohort A (cohort A1, $n = 7$) and the second (cohort A2, $n = 13$) in which patients were randomly

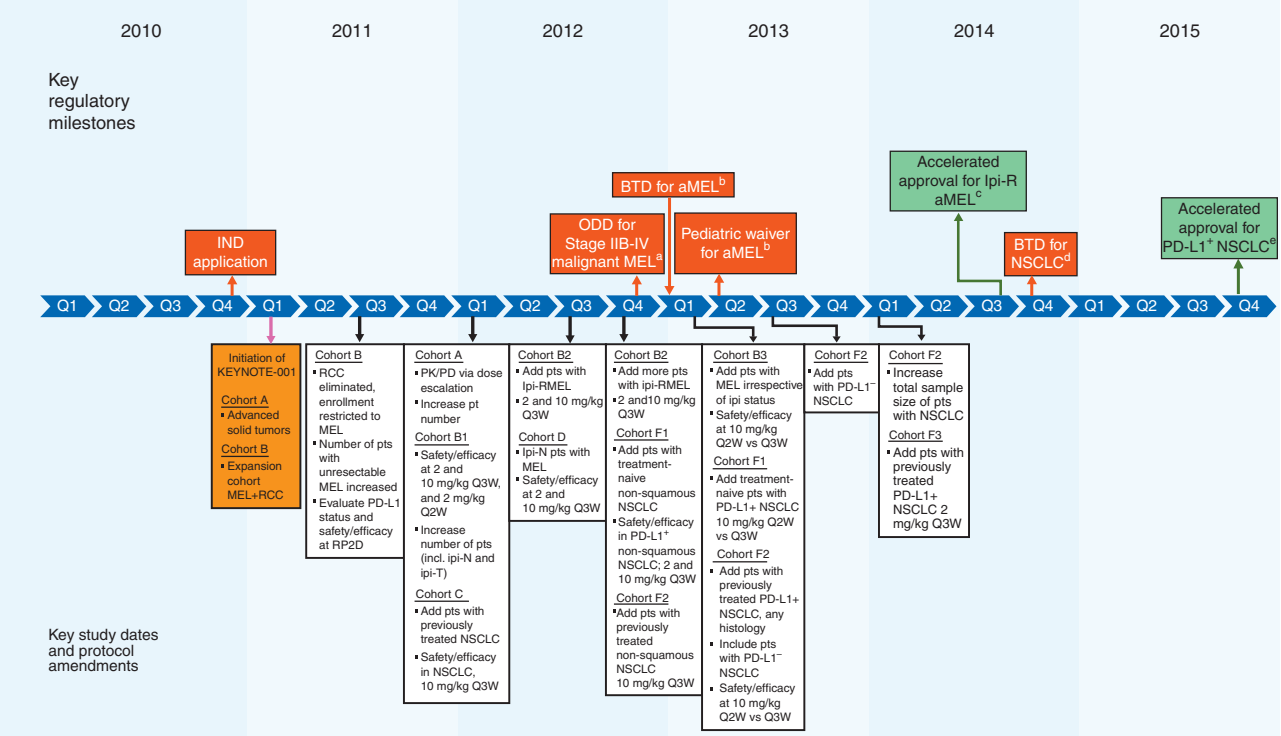

Figure 2. KEYNOTE-001: timeline of key study design elements and US FDA regulatory milestones. ALK, anaplastic lymphoma kinase; aMEL, metastatic melanoma; BTD, breakthrough therapy designation; chemo, chemotherapy; EGFR, epidermal growth factor receptor; incl., including; IND, investigational new drug; Ipi(-R, -T, -N), ipilimumab (-refractory, -treated, -naive); MEL, melanoma; NSCLC, non-small cell lung cancer; ODD, orphan drug designation; OL, open label; PD, pharmacodynamics; PD-L1^{+}, positive for expression of programmed death ligand 1; PD-L1^{-}, negative for expression of programmed death ligand 1; PK, pharmacokinetic; pts, patients; Q2W, every 2 weeks; Q3W, every 3 weeks; RCC, renal cell carcinoma; RP2D, recommended phase 2 dose; US FDA, United States Food and Drug Administration. [a]FDA granted ODD for stage IIB-IV malignant melanoma [24]. [b]FDA granted BTD for advanced melanoma and a pediatric waiver based on ODD status [25]. [c]FDA granted accelerated approval for unresectable or metastatic melanoma and disease progression after ipilimumab and, if *BRAF*^{V600} mutation positive, a BRAF inhibitor; approved dose 2 mg/kg Q3W [23]. [d]FDA granted BTD for treatment of *EGFR* mutation negative and *ALK* rearrangement-negative NSCLC with disease progression on or after platinum-based chemotherapy [28]. [e]FDA granted accelerated approval for metastatic NSCLC with tumors expressing PD-L1 (as determined by an FDA-approved test) and with disease progression on or after platinum-containing chemotherapy (*EGFR* or *ALK* genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations before receiving pembrolizumab); approved dose 2 mg/kg Q3W [29].

assigned to one of the three parallel intra-patient dose-escalation schedules (starting at 0.005, 0.02, and 0.06 mg/kg titrating 8 days into the first cycle), followed by treatment with either 2 or 10 mg/kg every 3 weeks (Q3W), to further define the pharmacokinetic and pharmacodynamic properties of the drug [41].

Pembrolizumab was generally well tolerated, with no DLTs reported in these cohorts. There was no MTD, and the MAD was 10 mg/kg Q2W. Treatment-related AEs were observed in 70% of all patients in cohorts A, A1, and A2, but none were grade 3 or 4; however, the death of one patient because of disseminated cryptococcal infection was considered to be indirectly related to treatment because it was probably caused by prolonged use of corticosteroids to treat grade 2 gastritis, which was itself considered related to treatment. Three patients discontinued therapy because of treatment-related AEs (grade 2 fatigue, pneumonitis, and decreased weight, $n = 1$ each) [41]. Although these cohorts were not powered for efficacy, substantial antitumor activity was observed. Across all doses and schedules, two patients had complete response (CR; melanoma and Merkel cell carcinoma, $n = 1$ each), 3 had partial response (PR; all melanoma), and 15 had stable disease (10 solid tumor types, including melanoma and NSCLC) [41].

Non-compartmental analysis of the data from cohorts A and A1 revealed a pembrolizumab half-life of 14–22 days, and the findings of *ex vivo* experiments suggested that complete peripheral target engagement commenced at 1 mg/kg across doses and was durable for at least 21 days. Further analysis of data from cohort A2, with intra-patient dose escalation from 0.005 to 10 mg/kg over a 3-week period demonstrated a linear serum exposure to pembrolizumab over the range of 0.1–10.0 mg/kg; lower doses were associated with a non-linear clearance component. Translational modeling predicted robust responses at doses $\geq$2 mg/kg Q3W, with no (or limited) activity at $<$1.0 mg/kg Q3W. These findings provided the rationale for studying a dose range of 2 mg/kg Q3W to 10 mg/kg Q2W in subsequent KEYNOTE-001 cohorts and clinical trials [41].

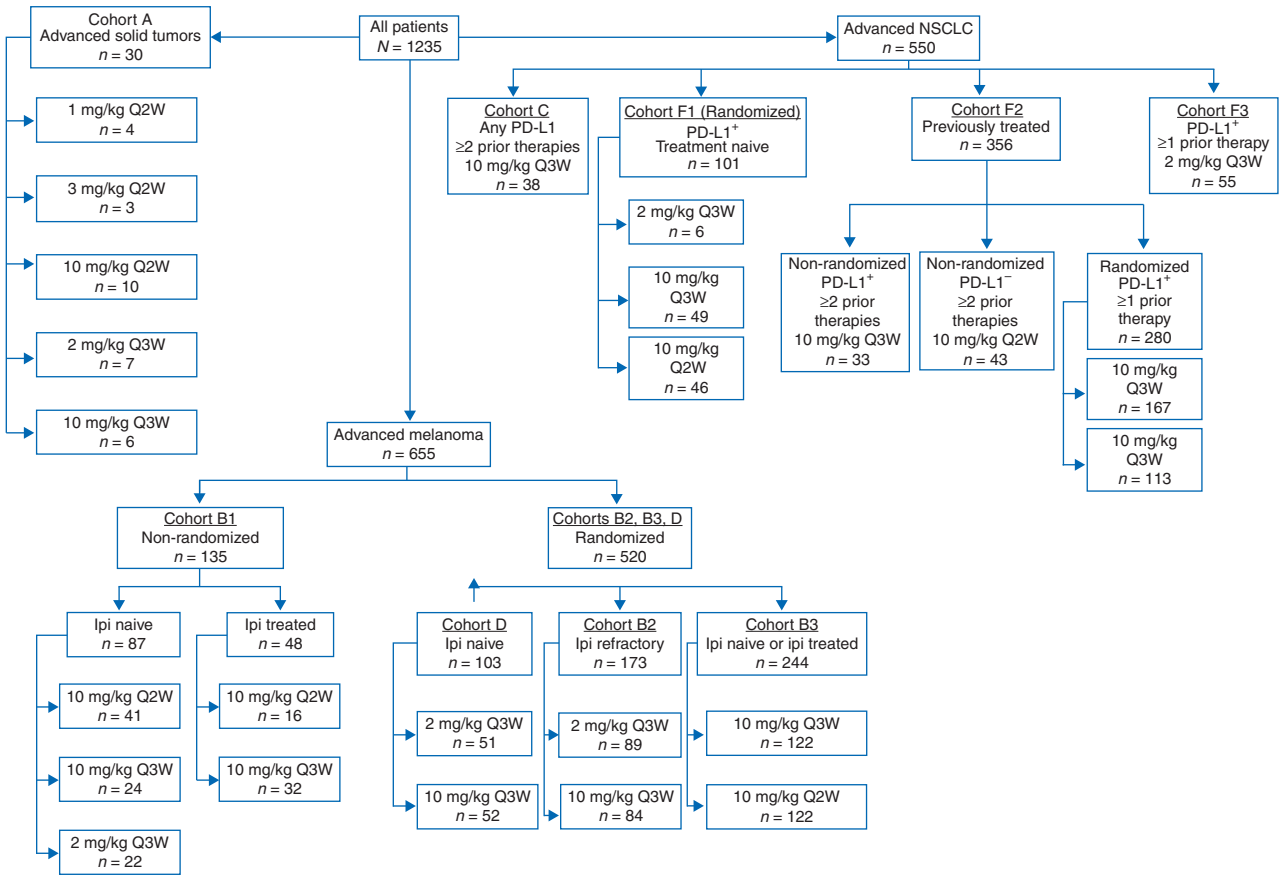

Figure 3. KEYNOTE-001 treatment cohorts. Ipi, ipilimumab; PD-L1, programmed dealth ligand 1; Q2W, every 2 weeks; Q3W, every 3 weeks. Figure adapted with permission from Khoja et al. [40].

Pembrolizumab in melanoma. Several melanoma expansion cohorts were initiated based on the findings from cohort A. The first was a non-randomized cohort in which 135 ipilimumab-naive (ipi-N; $n = 87$) or ipilimumab-treated (ipi-T; $n = 48$) patients were enrolled and administered pembrolizumab at doses of 10 mg/kg every Q3W or Q2W, or 2 mg/kg Q3W (cohort B1; Table 1). The aim was to more fully characterize the safety and tolerability of pembrolizumab at different doses and schedules and to assess preliminary antitumor activity in both ipi-N and ipi-T patients [42]. A sample size of 61 for ipi-N patients provided >99% power to detect an overall ORR of 30% or DCR of 55% in ipi-N patients versus an ORR of 10% and DCR of 30% (one-sided $P = 0.05$; based on the Hochberg procedure). The confirmed ORR across all doses was 38% and was not different between ipi-N and ipi-T patients; the highest confirmed ORR (52%) was observed with the 10 mg/kg Q2W dose regimen. Responses seemed to be durable in the majority of patients, with DORs in the range of 2–11 and 3–8 months for ipi-N and ipi-T patients, respectively. Treatment was ongoing for 81% of patients who had a response at the time of the analysis in March 2013 (median follow-up time, 11 months) [42].

This preliminary evidence of activity in both ipi-N and ipi-T patients [42] led to breakthrough therapy designation, and a cohort of patients with ipilimumab-refractory (ipi-R) melanoma was added to evaluate the safety and tolerability of pembrolizumab in a strictly defined population who had unequivocal or confirmed disease progression per immune-related response criteria after at least two ipilimumab doses (cohort B2; randomized 1:1 to receive either 2 or 10 mg/kg Q3W until disease progression, intolerable toxicity, or withdrawal of consent) [43, 44]. Pembrolizumab was similarly well tolerated between the two dose groups; grade 3 fatigue was the only grade 3 or 4 treatment-related AE reported in more than one patient, and there were no drug-related deaths. There was no difference in ORR between the two dose groups (26% in both; $P = 0.96$), and 73% and 68% of the 2 and 10 mg/kg groups, respectively, experienced a reduction from baseline in target lesion size. In terms of secondary end points, median PFS was 22 weeks [95% confidence interval (CI) 12–36] for the 2 mg/kg group and 14 weeks (95% CI 12–24) for the 10 mg/kg group [hazard ratio (HR) 0.84; 95% CI 0.57–1.23], and the 1-year OS rate (analysis date May 2014) was 58% (95% CI 47–68) and 63% (95% CI 51–72), respectively [43].

Along with the B2 cohort of patients with refractory melanoma, additional randomized dose cohorts were evaluated for ipi-N and ipi-T patients: 10 mg/kg Q2W and Q3W in ipi-T and ipi-N patients (cohort B3, $n = 244$), and 2 and 10 mg/kg Q3W in ipi-N patients (cohort D, $n = 103$) (Table 1) [44, 45]. As with previous KEYNOTE-001 cohorts, pembrolizumab was well tolerated and demonstrated efficacy among both ipi-T and ipi-N patients [44, 45]. The ORR was not significantly different between the 2 and 10 mg/kg ipi-N arms in cohort D (33% and 40%, respectively; $P = 0.48$) [44]. There were no significant differences between

Table 1. Primary efficacy data for the non-randomized ($n = 135$) and randomized melanoma cohorts ($n = 520$) from KEYNOTE-001 [41–43, 45, 46]

Cohort	*n*	ORR % (95% CI)
B1 (non-randomized)[a]		
Ipi-N		
10 mg/kg Q2W	39	49 (32–65)
10 mg/kg Q3W	19	26 (9–51)
2 mg/kg Q3W	20	25 (9–49)
Ipi-T		
10 mg/kg Q2W	13	62 (32–86)
10 mg/kg Q3W	26	27 (12–48)
B2 (randomized)[b]		
Ipi-R		
2 mg/kg Q3W	81	26 (17–37)
10 mg/kg Q3W	76	26 (17–38)
D (randomized)[b]		
Ipi-N		
2 mg/kg Q3W	51	33 (20–49)
10 mg/kg Q3W	52	40 (26–56)
B3 (randomized)[c]		
Ipi-N		
10 mg/kg Q3W	57	35 (23–49)
10 mg/kg Q2W	56	38 (25–52)
Ipi-T		
10 mg/kg Q3W	50	26 (15–40)
10 mg/kg Q2W	61	33 (21–46)
Pooled analysis of cohorts B1, B2, D, and B3 ($N = 655$)[d]	581	33 (30–37)
Ipi-N	277	39 (33–45)
Ipi-T	304	29 (24–34)
Treatment-naive	133	45 (36–54)

[a]Data cutoff, March, 2013.
[b]Data cutoff, October 18, 2013.
[c]Data cutoff, April 18, 2014.
[d]Data cutoff, October 18, 2014.
CI, confidence interval; Ipi-N, ipilimumab naive; Ipi-R, ipilimumab refractory; Ipi-T, ipilimumab treated; ORR, overall response rate; Q2W, every 2 weeks; Q3W, every 3 weeks.

schedules for either ORR (31% for Q3W and 35% for Q2W; $P = 0.51$) or DCR (48% for Q3W and 51% for treated with pembrolizumab 10 mg/kg Q3W or Q2W; $P = 0.59$) among ipi-T + ipi-N patients in cohort B3, and results were similar between the ipi-N and ipi-T arms [46]. Responses were durable in both patient cohorts, from 6+ weeks to 39+ weeks for cohort D (all ipi-N and 47% of ipi-T patients had ≥36 weeks of follow-up, with 90% of responses ongoing at the analysis cutoff date of 18 October 2013), and from 6+ to 36+ weeks for cohort B3 (with 91% of responses ongoing at the analysis cutoff date; median duration of follow-up, 42.3 weeks) [44, 46]. Preliminary survival findings demonstrated a 24-week PFS rate of 43% and 47% for the Q3W and Q2W dosing schedules, respectively, in cohort B3 [not significant (NS); $P < 0.3$], [45] and 51% and 48% for the 2 and 10 mg/kg schedules, respectively, in cohort D [44]. Taken together, the results from cohorts B2, D, and B3 led to the conclusion that the pembrolizumab RP2D should be 2 mg/kg Q3W [46].

Pooled analysis of the data from the entire melanoma population of KEYNOTE-001 (cohorts B1 + B2 + B3 + D; $N = 655$), regardless of ipilimumab treatment history or pembrolizumab dose or schedule, revealed an ORR of 33% (Table 1), a 12-month PFS rate of 35%, and a median OS of 23 months (median duration of follow-up, 21 months). Response lasted >1 year in 44% of responders, and the estimated median DOR was 28 months. Pooled sub-analysis of ipi-N patients yielded an ORR of 45% and a median OS of 31 months [47]. These data suggested that a substantial proportion of patients with advanced melanoma treated with pembrolizumab will achieve a durable objective response and supported accelerated approval of the 2 mg/kg Q3W dose [23].

Pembrolizumab in NSCLC. A cohort of 38 previously treated patients with NSCLC (cohort C) was included in KEYNOTE-001 based on the observation that four of seven patients with NSCLC enrolled in cohort A (i.e. cohorts A, A1 and A2) experienced stable disease [41, 48]. Pembrolizumab had an acceptable and manageable toxicity profile in this group of patients, and antitumor activity was demonstrated in patients who had received two previous NSCLC treatment regimens, with an ORR of 21% per RECIST v1.1 by independent review. Preliminary data suggested that the level of PD-L1 expression was associated with increased antitumor activity of pembrolizumab [48].

Three cohorts in NSCLC ($n = 512$), which included previously treated and treatment-naive patients (cohorts F1–F3), were added to further investigate dose using a mainly randomized approach. Breakthrough designation for pembrolizumab in NSCLC was based on the findings from treated patients in cohorts F1, F2, and C irrespective of their line of therapy who had tumors evaluable for PD-L1 expression ($n = 146$). The findings from cohorts F1 ($n = 101$, treatment-naive, initially 2 mg/kg Q3W versus 10 mg/kg Q3W and then 10 mg/kg Q3W versus 10 mg/kg Q2W), F2 ($n = 356$, previously treated, 10 mg/kg Q3W versus Q2W), and F3 ($n = 55$, previously treated, 2 mg/kg Q3W) contributed to the approval of pembrolizumab in NSCLC (Figure 3).

Randomized comparisons between the 10 mg/kg Q2W and Q3W dosing schedules and between the 10 mg/kg Q3W and 2 mg/kg Q3W dosing schedules in patients with PD-L1-positive NSCLC (from cohorts F1 and F2) revealed similar ORRs and safety profiles [49, 50]. Furthermore, a pooled analysis demonstrated that pembrolizumab was well tolerated and conferred durable antitumor activity across all patient cohorts (i.e. cohorts C, F1, and F2; $n = 495$) regardless of prior treatment status, with no significant difference in either efficacy or safety among the three doses/schedules tested: ORR was 19.4% (95% CI 16.0–23.2), median PFS was 3.7 months (95% CI 2.9–4.1), and median OS was 12.0 months (95% CI 9.3–14.7). ORR was 18.0% (95% CI 14.4–22.2) in previously treated patients ($n = 394$; cohorts C and F2), and 24.8% (95% CI 16.7–34.3) in treatment-naive patients ($n = 101$; cohort F1) (Table 2) [51].

These cohorts also provided pre-specified training and validation sets that were instrumental in the development of the first companion diagnostic for an immunotherapy and ultimately resulted in FDA approval of the Dako IHC 22C3 pharmDx PD-L1 expression assay (to identify PD-L1-positive tumors) in patients with NSCLC [31]. A tumor proportion score (TPS) cut point of ≥50% (i.e. PD-L1 expression on ≥50% of tumor cells)

Table 2. Primary efficacy data for the NSCLC cohorts ($N = 495$) from KEYNOTE-001 [50]

Cohort	ORR % (95% CI)
All ($N = 495$)[a]	19.4 (16.0–23.2)
Previously treated ($n = 394$)[b]	18.0 (14.4–22.2)
Previously untreated ($n = 101$)[c]	24.8 (16.7–34.3)
PD-L1$^+$ TPS ≥50% ($N = 73$)[d]	45.2 (33.5–57.3)*
Previously treated ($n = 57$)	43.9 (30.7–57.6)
Previously untreated ($n = 16$)	50.0 (24.7–75.3)
PD-L1$^+$ TPS 1–49% ($n = 103$)	16.5 (9.9–25.1)
PD-L1$^+$ TPS <1% ($n = 28$)	10.7 (2.3–28.2)

[a]Cohorts C, F1, and F2.
[b]Cohorts C and F2.
[c]Cohort F1.
[d]Data for the validation set.
*Significantly greater than PD-L1$^+$ TPS 1–49% ($P < 0.001$) and PD-L1$^+$ TPS <1% ($P = 0.01$).
NSCLC, non-small cell lung cancer; PD-L1$^+$, positive for expression of programmed death ligand 1; TPS, tumor proportion score (percentage of PD-L1$^+$ tumor cells).

was selected by receiver operating characteristic curve analysis (and identified using the Youden index) for this assay based on clinical data from the NSCLC training set, which included patients from cohorts C ($n = 27$), F1 ($n = 9$), and F2 ($n = 110$) with at least one measurable lesion, adequate organ function, an Eastern Cooperative Oncology Group performance status of 0 or 1, and a tumor biopsy collected within 60 days of the first dose of pembrolizumab [51]. In patients with a tumor PD-L1 TPS ≥50%, the ORR for the validation set was 45.2% (95% CI 33.5–57.3), with a median PFS of 6.4 months (95% CI 4.2–not reached; median OS was not reached). ORR (Table 2) and median PFS for patients in the validation set with TPS 1%–49% were 16.5% (95% CI 9.9–25.1) and 4.1 months (95% CI 2.3–4.4), respectively, and were 10.7% (95% CI 2.3–28.2) and 4.0 months (95% CI 2.1–6.2), respectively, for those in the validation set with TPS <1% [51]. For the validation set, in patients with TPS ≥50%, the ORRs for all patients, those who were previously treated, and those who were treatment-naive were 45.2% (95% CI 33.5–57.3), 43.9% (95% CI 30.7–57.6), and 50.0% (95% CI 24.7–75.3), respectively, and the median PFS was 6.3 months (95% CI 2.9–12.5), 6.1 months (95% CI 2.1–12.5), and 12.5 months (95% CI 2.4–12.5), respectively. Median OS among those with TPS ≥50% was not reached for either previously treated or untreated patients [51].

The final cohort, F3, enrolled 55 patients with NSCLC who had at least one prior systemic therapy and whose tumors were PD-L1–positive (i.e. TPS ≥1%). Patients were administered pembrolizumab 2 mg/kg Q3W to further characterize the antitumor activity and safety profile of pembrolizumab monotherapy, and to evaluate the dose and antitumor activity in NSCLC. Together with cohorts F1 and F2, cohort F3 also evaluated the relationship between the degree of biomarker positivity and tumor response. The results were consistent with those of a subpopulation analysis of patients in cohort F2 with previously treated NSCLC and a TPS of ≥50%, and who were treated with pembrolizumab at 10 mg/kg Q2W or Q3W [52]. These findings provided supportive data for 2 mg/kg Q3W as the RP2D for NSCLC, and confirmation of that dose as the RP2D for melanoma. The overall safety profile of pembrolizumab in patients with NSCLC was similar, particularly with respect to immune- and drug-related AEs, to that observed in the melanoma cohorts [42, 43, 52].

The efficacy of pembrolizumab in patients with NSCLC enrolled in KEYNOTE-001 paved the way for further development in this indication and introduced the potential for prospective testing of PD-L1 expression to focus treatment on those patients most likely to benefit from pembrolizumab. These data supported accelerated approval of pembrolizumab 2 mg/kg Q3W for patients with metastatic NSCLC whose tumors express PD-L1 (TPS ≥50%) as determined by an FDA-approved test, and who have progressed on or after platinum-containing treatment, or for patients with PD-L1-expressing NSCLC tumors with genomic epidermal growth factor receptor (EGFR) or anaplastic lymphoma kinase (ALK) aberrations, and disease progression after FDA-approved therapy for those aberrations [29].

Study design and regulatory considerations

Drug development

Unlike the classic drug-development pathway of progressing from phase 1 (MTD, DLTs, safety, and tolerability) to phase 2 (dose-finding, efficacy assessment), and then to phase 3 (randomized controlled trials designed to demonstrate efficacy and safety in support of regulatory approval) for a single indication, KEYNOTE-001 was composed of nested phase-2-like studies in two oncologic indications—melanoma and NSCLC—with six randomized dose/schedule-comparison sub-studies involving both patient populations, as well as training and validation sets for the development of the PD-L1 IHC companion diagnostic assay. The use of multiple expansion cohorts conferred certain advantages. First, they allowed multiple hypotheses to be addressed (pertaining to populations, doses, and biomarker development) with appropriate type 1 error (false-positive) control and allowed for the simultaneous evaluation of multiple tumor types. In addition, they enabled the expedited development and approval of a drug that was considered transformative based on early and strong efficacy signals, with sufficient rigor to support regulatory filings, while being aligned with the single-arm trial design as one of the accepted approaches for seeking accelerated approval. The time from investigational new drug designation for pembrolizumab to first FDA approval in melanoma was approximately 4 years (Figure 2). Using the traditional development approach, this process would likely have taken more than twice as long, with the time from first testing of a potential therapeutic agent to regulatory approval generally being 10–15 years [53, 54].

However, there can also be inherent issues with this type of design. The rapid patient accrual in multiple separate cohorts, inclusion of additional tumor types, and multiple protocol amendments (nine in total) led to a high level of protocol complexity, which carries with it the potential to cause adherence issues at both the site and the patient levels. Testing of multiple hypotheses simultaneously rather than sequentially also increases the complexity of data analysis and interpretation. For example,

Table 3. Selection of ongoing phase 2/3 trials of pembrolizumab in melanoma and NSCLC

Trial	Phase	Indication	Estimated enrollment	Intervention/arms	Status
Melanoma					
NCT02362594 KEYNOTE-054	3	Stage III, high-risk melanoma after complete resection	900	(i) Pembrolizumab (ii) Placebo	Currently recruiting
NCT02752074 KEYNOTE-252/ECHO-301	3	Unresectable or metastatic melanoma	600	(i) Pembrolizumab + epacadostat (ii) Pembrolizumab + placebo	Currently recruiting
NCT02263508 MasterKey-265	3	Unresected melanoma	660	(i) Pembrolizumab + talimogene laherparepvec (ii) Pembrolizumab + placebo	Currently recruiting
NCT02506153	3	Stage III-IV, high-risk melanoma that has been removed by surgery	1378	(i) Chemotherapy (IFN-α2b) or ipi (ii) Pembrolizumab	Currently recruiting
NSCLC					
NCT01905657 KEYNOTE-010[a]	2/3	NSCLC with disease progression after platinum-containing therapy	1034	(i) Pembrolizumab (low dose) (ii) Pembrolizumab (high dose) (iii) Docetaxel	Active, not recruiting
NCT02142738 KEYNOTE-024	3	Previously untreated, PD-L1^{+}-strong NSCLC	305	(i) Pembrolizumab (ii) Platinum-based chemotherapy[b]	Active, not recruiting
NCT02039674 KEYNOTE-021	1/2	Previously untreated, metastatic NSCLC	308	(i) Pembrolizumab + paclitaxel + carboplatin (ii) Pembrolizumab + paclitaxel + carboplatin + bevacizumab (iii) Pembrolizumab + pemetrexed + carboplatin (iv) Pembrolizumab + ipilimumab (v) Pembrolizumab + erlotinib (vi) Pembrolizumab + gefitinib	Currently recruiting
NCT02578680 KEYNOTE-189	3	Previously untreated non-squamous, metastatic NSCLC	570	(i) [Pembrolizumab + pemetrexed (with folic acid) + cisplatin] or [carboplatin + pembrolizumab + pemetrexed] (ii) [Placebo + pemetrexed (with folic acid) + cisplatin] or [carboplatin + placebo + pembrolizumab]	Currently recruiting
NCT02220894 KEYNOTE-042	3	Previously untreated, PD-L1^{+} NSCLC	1240	(i) Pembrolizumab (ii) [Carboplatin + paclitaxel] or carboplatin + pemetrexed (SOC)	Currently recruiting

Continued

Table 3. *Continued*

Trial	Phase	Indication	Estimated enrollment	Intervention/arms	Status
NCT02775435 KEYNOTE-407	3	First-line, metastatic, squamous NSCLC	560	(i) Pembrolizumab + [paclitaxel or nab-paclitaxel] + carboplatin (ii) Placebo + [paclitaxel or nab-paclitaxel] + carboplatin	Currently recruiting
NCT02504372 KEYNOTE-091	3	NSCLC after resection	1380	(i) Pembrolizumab (ii) Placebo	Currently recruiting
Melanoma or NSCLC					
NCT02085070	2	Melanoma + NSCLC with at least 2 untreated brain metastases	64	Pembrolizumab (OL)	Currently recruiting

[a]Some study results have now been published [64].
[b]Investigator's choice of: paclitaxel + carboplatin, pemetrexed + carboplatin, pemetrexed + cisplatin, gemcitabine + carboplatin, gemcitabine + cisplatin.
IFN-α2b, interferon alpha 2b; ipi, ipilimumab; NSCLC, non-small cell lung cancer; OL, open label; SOC, standard of care.

dose hypotheses were evaluated in melanoma and NSCLC simultaneously, rather than waiting for the melanoma dose data to become available to inform the NSCLC studies. Finally, with complex protocols such as this, it is difficult to isolate single cohorts for submission purposes because multiple database locks are required during an ongoing study.

Regulatory considerations

For pembrolizumab, the approvals for metastatic melanoma and NSCLC arose from a single, first-in-human protocol. This was made possible by a solid scientific base together with close and frequent interactions, facilitated by the breakthrough designations, between the FDA and Merck & Co., Inc., which helped to rapidly resolve any issues and ensured alignment on study design and data analyses. The FDA provided the regulatory oversight necessary to ensure patient protection, reviewed and responded to the data regularly, and ensured appropriate statistical rigor. The achievement of breakthrough therapy designations, which involved multiple disciplines of the FDA, enabled a proactive approach to the availability of pembrolizumab to patients in the clinic within a relatively short timeframe. In fact, it has been proposed that breakthrough therapy designation should be used as a method of distinguishing drugs for which early efficacy has been demonstrated that is sufficient to justify precisely this type of drug development program [55]. Thus, the clinical development of pembrolizumab, beginning with KEYNOTE-001, has set a new precedent for the way in which such drugs can be more swiftly brought to patients in need [55].

Further clinical development of pembrolizumab

In December 2015 pembrolizumab received full approval for the treatment of patients with unresectable or metastatic melanoma and an expanded indication to include first-line treatment of this patient population [56], based on data from the large phase 2 KEYNOTE-002 (NCT01704287) and phase 3 KEYNOTE-006 (NCT01866319) studies [57, 58]. The durability of response to pembrolizumab has also been demonstrated by longitudinal data from KEYNOTE-001 in advanced melanoma [59, 60]. In August 2016 pembrolizumab received accelerated approval from the FDA for the treatment of HNSCC with progression on or after platinum-containing therapy [61], based on data from the KEYNOTE-012 trial (NCT01848834) [62, 63]. Based on the recently published results of KEYNOTE-010 (NCT01905657) [64], pembrolizumab received full approval in October 2016 for the treatment of patients with metastatic NSCLC whose tumors express PD-L1 (TPS $\geq$1%) as determined by an FDA-approved test, and who have progressed on or after platinum-containing treatment, or for patients with PD-L1-expressing NSCLC tumors with genomic EGFR or ALK aberrations and disease progression after FDA-approved therapy for those aberrations [65]. An expanded indication was also granted for the first-line treatment of patients with metastatic NSCLC whose tumors have high PD-L1 expression (TPS $\geq$50%) as determined by an FDA-approved test, and no *EGFR* or *ALK* aberrations [65]. The FDA has also approved the PD-L1 IHC 22C3 pharmDX assay as a companion diagnostic for the identification of PD-L1-positive tumors with a cutoff TPS of $\geq$1% (with high PD-L1 expression being defined as TPS $\geq$50%) [66]. In March 2017, pembrolizumab received accelerated approval from the FDA for the treatment of adult and pediatric patients who have refractory classical Hodgkin lymphoma, or who have relapsed after 3 or more prior lines of therapy [67]. In May 2017, pembrolizumab in combination with pemetrexed and carboplatin received accelerated approval from the FDA as first-line treatment of patients with metastatic non-squamous NSCLC based on results of the KEYNOTE-021 trial (NCT02039674) [68].

Pembrolizumab continues to be explored for the treatment of specific melanoma and NSCLC subpopulations (e.g. patients with asymptomatic brain metastases) and different stages of disease

(e.g. adjuvant and neoadjuvant for melanoma and first-line for NSCLC), both as a monotherapy and in combination with other therapies (Table 3). This immune-checkpoint inhibitor has also demonstrated efficacy in several other advanced solid tumors and hematologic cancers [67–72] and is currently under investigation (also as a monotherapy and in combination with other cancer therapies) for the treatment of more than 30 cancers across more than 320 clinical trials [73]. In fact, breakthrough therapy designation for pembrolizumab has recently been granted for classic Hodgkin's lymphoma, microsatellite-instability-high metastatic colorectal cancer, and unresectable or metastatic microsatellite-instability-high non-colorectal cancer [74–76].

Conclusions

The pembrolizumab KEYNOTE-001 study is an innovative and groundbreaking study that has led to multiple regulatory achievements, including orphan drug designation, breakthrough therapy designations, accelerated approvals for the treatment of melanoma and NSCLC, and approval for a companion diagnostic for PD-L1 tumor expression in NSCLC. This was a phase 1 first in-human study with a single-arm, non-randomized design that was subsequently adapted on the basis of interim analyses and, through numerous amendments and addition of multiple expansion cohorts with carefully pre-specified statistical analysis plans, ultimately enrolled 1235 patients to address an unmet clinical need in melanoma and NSCLC. Its success is attributable to a strong scientific base, partnership with the FDA through frequent interactions, academic partners who quickly mobilized resources and efforts to facilitate execution of the study, and commitment from all parties involved to expedite the delivery of pembrolizumab to patients in need. This regulatory outcome is unprecedented and has undoubtedly altered the way in which clinical development of oncology therapeutics can be achieved.

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