European Journal of Cancer 108 (2019) 120–128

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Original Research

Randomised phase 2 study of pembrolizumab plus CC-486 versus pembrolizumab plus placebo in patients with previously treated advanced non-small cell lung cancer

Benjamin P. Levy [a,*], Giuseppe Giaccone [b], Benjamin Besse [c], Enriqueta Felip [d], Marina Chiara Garassino [e], Manuel Domine Gomez [f], Pilar Garrido [g], Bilal Piperdi [h], Santiago Ponce-Aix [i], Daniel Menezes [j], Kyle J. MacBeth [j], Alberto Risueño [k], Ruta Slepetis [j], Xiaoling Wu [j], Abderrahim Fandi [j], Luis Paz-Ares [i,**]

[a] *Johns Hopkins Sidney Kimmel Cancer Center, Washington, DC, USA*
[b] *Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC, USA*
[c] *Department of Cancer Medicine, Gustave Roussy, Villejuif and Paris-Sud University, Orsay, France*
[d] *Hospital University Vall d'Hebron, Barcelona, Spain*
[e] *Istituto Nazionale dei Tumori, Milan, Italy*
[f] *Instituto de Investigacion Sanitaria-Fundación Jimenez Diaz (IIS- FJD), Madrid, Spain*
[g] *IRYCIS, Hospital Universitario Ramón y Cajal, Madrid, Spain*
[h] *Merck & Co., Inc., Kenilworth, NJ, USA*
[i] *Hospital Universitario 12 de Octubre, Universidad Complutense, CNIO and CiberOnc, Madrid, Spain*
[j] *Celgene Corporation, Summit, NJ, USA*
[k] *Celgene Institute for Translational Research Europe, Seville, Spain*

Received 12 September 2018; received in revised form 15 November 2018; accepted 23 November 2018
Available online 14 January 2019

KEYWORDS
Non-small cell lung cancer;
Pembrolizumab;
CC-486;
Epigenetics;
Azacitidine

Abstract ***Introduction:*** Preclinical and early clinical studies suggest that combining epigenetic agents with checkpoint inhibitors can potentially improve outcomes in patients with previously treated advanced non-small cell lung cancer (NSCLC). This phase 2 trial examined second-line pembrolizumab + CC-486 (oral azacitidine) in patients with advanced NSCLC. ***Methods:*** Patients with one prior line of platinum-containing therapy were randomised in a ratio of 1:1 to CC-486 or placebo, on days 1–14, in combination with pembrolizumab on day 1 of a 21-day cycle. The primary end-point was progression-free survival (PFS). Key secondary end-points included overall survival (OS), overall response rate (ORR) and safety.

* *Corresponding author.*
** *Corresponding author.*
E-mail addresses: blevy11@jhmi.edu (B.P. Levy), lpazaresr@seom.org (L. Paz-Ares).

https://doi.org/10.1016/j.ejca.2018.11.028
0959-8049/© 2018 Elsevier Ltd. All rights reserved.

Results: Among 100 patients randomised (pembrolizumab + CC-486: 51; pembrolizumab + placebo: 49), most were male (57.0%), were white (87.0%) and had Eastern Cooperative Oncology Group performance status 1 (68.0%). No significant difference in PFS was observed between the pembrolizumab + CC-486 and pembrolizumab + placebo arms (median, 2.9 and 4.0 months, respectively; hazard ratio [HR], 1.374; 90% confidence interval [CI], 0.926–2.038; $P = 0.1789$). Median OS was 11.9 months versus not estimable (HR, 1.375; 90% CI, 0.830–2.276; $P = 0.2968$); ORR was 20% versus 14%. Median treatment duration was shorter (15.0 versus 24.1 weeks), and the number of cycles was lower (5.0 versus 7.0) with pembrolizumab + CC-486 versus pembrolizumab + placebo. No new safety signals for CC-486 or pembrolizumab were detected. Treatment-emergent adverse events were more common in the pembrolizumab + CC-486 arm, particularly gastrointestinal, potentially impacting treatment feasibility.

Conclusions: No improvement in PFS was observed with pembrolizumab + CC-486 versus pembrolizumab + placebo. Decreased treatment exposure due to adverse events may have impacted efficacy with pembrolizumab + CC-486.

© 2018 Elsevier Ltd. All rights reserved.

1. Introduction

Patients with previously treated advanced non-small cell lung cancer (NSCLC) typically have a poor prognosis; real-world studies have shown that the median survival after initiation of second-line treatment is 5 to 13 months [1]. Although the introduction of immunotherapy agents has improved outcomes in this disease setting, most chemorefractory patients do not respond to single-agent programmed death 1 (PD-1)/programmed death-ligand 1 (PD-L1) inhibitor therapy (overall response rate [ORR] ≈ 20%), and long-term survivorship remains low [2–6]. Emerging evidence supports the critical role of epigenetic silencing in impairing tumour immunogenicity by suppressing antigen processing and presentation and tumour-associated antigen expression and has laid the scientific rationale for combining epigenetic agents with immunotherapy in NSCLC [7–12].

Pembrolizumab is an anti-PD-1 antibody approved by the United States Food and Drug Administration for advanced NSCLC as first-line or second-line monotherapy in patients with a PD-L1 tumour proportion score (TPS) ≥ 50% or ≥ 1%, respectively, and in combination with carboplatin and pemetrexed in treatment-naive patients with non-squamous histology irrespective of PD-L1 TPS [13]. Azacitidine (epigenetic agent) is a DNA methyltransferase inhibitor approved for the treatment of patients with myelodysplastic syndromes, acute myeloid leukaemia or chronic myelomonocytic leukaemia [14,15].

Here, we present results from a phase 2 trial that evaluated second-line pembrolizumab + CC-486 (investigational compound; oral formulation of azacitidine) versus pembrolizumab + placebo in patients with advanced NSCLC [16]. To our knowledge, this is the first report of a randomised, placebo-controlled trial evaluating an epigenetic agent plus immunotherapy in this setting.

2. Materials and methods

2.1. Patients

Patients in this phase 2, multicenter, international (US and Europe), randomised, placebo-controlled, double-blind study were aged ≥ 18 years, with histologically or cytologically confirmed locally advanced or metastatic NSCLC and measurable disease per Response Evaluation Criteria in Solid Tumors (RECIST) 1.1. Prior treatment with one platinum-based systemic therapy was required; more than one prior line of systemic therapy was not allowed. Key eligibility requirements included Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤ 1, adequate organ and bone marrow function and no prior treatment with a hypomethylating agent or immunotherapy. Patients with uncontrolled or symptomatic central nervous system metastases were excluded. Patients with non-squamous histology who had a sensitising epithelial growth factor receptor (*EGFR*) mutation and/or positive anaplastic lymphoma kinase (*ALK*) fusion or whose mutation/fusion status was unknown were excluded. Those with squamous histology who had unknown *EGFR* mutation and *ALK* fusion status were allowed to enrol.

2.2. Treatment and end-points

Randomisation was by centralised interactive response technology, with stratification by histology (non-squamous versus squamous) and using a permuted block method. Patients were randomised in a ratio of 1:1 to receive either 300 mg of CC-486 (oral) on days 1–14 and 200 mg of pembrolizumab (30-min infusion) on day 1 or

placebo (oral) on days 1–14 and 200 mg of pembrolizumab (30-min infusion) on day 1 of a 21-day cycle. CC-486 and matching placebo were supplied in blister packs and administered in a double-blind manner; pembrolizumab was administered open label. Patients were enrolled by the study investigators; treatment assignment was performed centrally by Endpoint Headquarters. CC-486 or pembrolizumab dose/schedule modification due to toxicity was allowed in both arms according to protocol-specified guidelines; however, pembrolizumab dose reduction was not allowed (Supplemental Table 1). The first patient's first visit was on November 16, 2015; by April 12, 2017, all patients had discontinued from CC-486 or placebo.

The primary end-point was progression-free survival (PFS). Key secondary end-points included overall survival (OS), ORR, disease control rate (DCR) and safety. PFS was defined as the time from randomisation to progression (investigator assessment according to RECIST 1.1) or death from any cause. OS was defined as the time from randomisation to death from any cause. ORR was defined as the percentage of patients with a confirmed complete response (CR) or partial response (PR), and DCR was defined as the percentage of patients with stable disease for $\geq$ 18 weeks, CR or PR, as assessed by the investigator (RECIST 1.1 guidelines). Efficacy analyses were performed on the intention-to-treat (ITT) population, defined as all randomised patients.

Safety assessments included adverse events (AEs), laboratory abnormalities and incidence of AEs leading to dose reductions, interruptions and/or premature discontinuation. AEs were coded according to the Medical Dictionary for Regulatory Activities and summarised by system organ class and preferred term, frequency and severity grade based on the Common Terminology Criteria for Adverse Events, version 4.0. Safety analyses were performed on the safety population, defined as patients who received $\geq$1 dose of study treatment.

Tissue biopsies were mandatory at screening and optional on cycle 2, day 14. Fresh tumour biopsies were preferred; if not feasible, archival formalin-fixed, paraffin-embedded tissue could be used for PD-L1 analysis using IVD PD-L1 IHC 22C3 pharmDx assay (Agilent; Carpinteria, CA, USA).

2.3. DNA methylation profiling

Blood for DNA methylation analysis was collected from all patients on day 1 of cycles 1 and 2 before treatment administration. After study unblinding, whole-blood genomic DNA methylation profiling was performed in patients in the pembrolizumab + CC-486 arm using the Infinium Methylation EPIC BeadChip as described by the manufacturer (Illumina; San Diego, CA, USA). Probes with single-nucleotide polymorphisms at CpG, detection P value > 0.01 in more than 10% of samples or location on sex chromosomes were filtered out. Samples with detection P value > 0.01 in more than 1% of the probes were excluded. Colour bias adjustment and intensity normalisation were performed using the quantile method. Beta values were calculated as the ratio of methylated signal to the combined locus signal using an offset of 100 and were subsequently corrected using beta mixture quantile dilation to mitigate bias between Infinium I and II probe types. Global DNA methylation scores (GDMS) were derived for each sample by calculating the percentage of methylated loci with beta > 0.7.

2.4. Statistical analysis

Planned enrolment was approximately 90 total patients. Analysis of the primary end-point was conducted after 70 PFS events. The primary goal of this study was to estimate the difference in efficacy and safety between pembrolizumab + CC-486 and pembrolizumab + placebo. Power calculations showed 82.4% power (with 2-sided $\alpha = 0.10$) to detect a 2.5-month difference in median PFS, assuming the median PFS with pembrolizumab alone is 3.0 months, and time to PFS events in both arms followed exponential distributions. Median PFS and OS were calculated based on Kaplan–Meier estimates; hazard ratios (HRs) and 2-sided 90% confidence intervals (CIs) were estimated using the Cox proportional hazard model, with stratification factors as model covariates. Two-sided 90% CIs of the difference in ORR and DCR between the two arms were calculated.

This study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines of the International Conference on Harmonization. The trial protocol was reviewed and approved by the participating institutions' review board/ethics committee. Written informed consent was obtained from all patients before study entry. Trial registration: ClinicalTrials.gov (NCT02546986).

3. Results

3.1. Patients

Of 135 patients screened for inclusion into the study, 100 patients (ITT population) were randomised to treatment: 51 to pembrolizumab + CC-486 and 49 to pembrolizumab + placebo (Fig. 1). The median age was 65 years. Most patients were male (57.0%) and white (87.0%) and had an ECOG PS of 1 (68.0%), stage IV disease at baseline (97.0%) and non-squamous histology (82.0%; Table 1). A greater percentage of patients had a PD-L1 TPS $<$ 1% (39.0%) than TPS $\geq$ 1 to $\leq$ 49% (19.0%) or TPS $\geq$ 50% (18.0%). Baseline characteristics and demographics were generally well matched between arms.

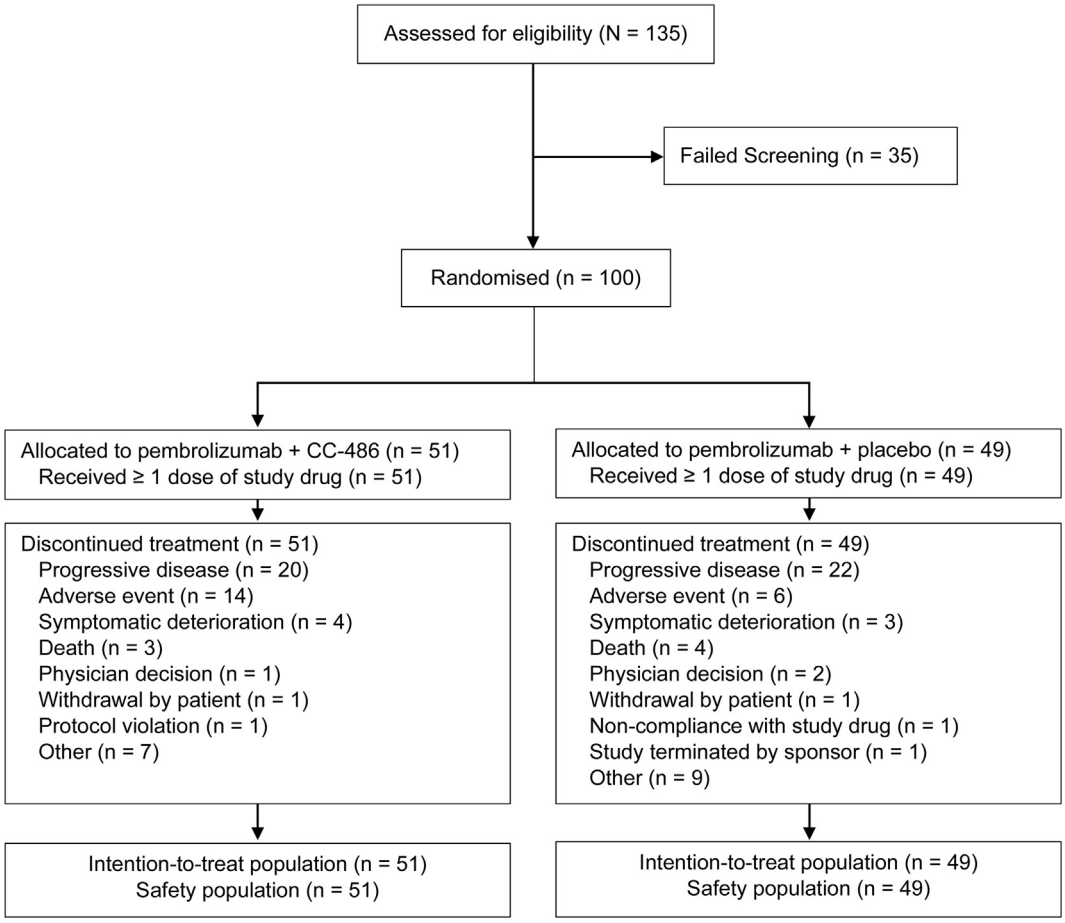


Fig. 1. CONSORT diagram. CONSORT, consolidated standards of reporting trials.

3.2. Treatment exposure

Patients in the pembrolizumab + CC-486 arm had shorter duration of treatment (median, 15.0 versus 24.1 weeks) and lower number of treatment cycles (median, 5.0 versus 7.0 cycles; Table 2) than those in the pembrolizumab + placebo arm. The relative dose intensity of pembrolizumab (median, 96.84% versus 98.07%) and the proportion with ≥ 90% relative dose intensity (56.9% versus 77.6%) were both lower with pembrolizumab + CC-486.

A greater percentage of patients in the pembrolizumab + CC-486 arm versus the pembrolizumab + placebo arm had a CC-486 or placebo dose reduction (25.5% versus 6.1%) or interruption (80.4% versus 61.2%). Similarly, a greater percentage of patients in the pembrolizumab + CC-486 arm had a pembrolizumab dose interruption (54.9% versus 34.7%).

3.3. Efficacy

The median PFS in patients treated with pembrolizumab + CC-486 versus pembrolizumab + placebo was 2.9 versus 4.0 months (HR, 1.374 [90% CI, 0.926–2.038]; $P = 0.1789$), respectively (Fig. 2A). At 12 months, PFS rates were 8% and 29% in the treatment and placebo arms, respectively. Based on these results, the independent data-monitoring committee recommended that the study be unblinded, and investigators were advised to discontinue CC-486 immediately. Pembrolizumab could be continued in both arms as per physician assessment.

After median follow-up of 11.3 and 12.2 months, the median OS in patients treated with pembrolizumab + CC-486 versus pembrolizumab + placebo was 11.9 months versus not estimable (HR, 1.375 [90% CI, 0.830–2.276]; $P = 0.2968$), respectively (Fig. 2B). At 12 months, OS rates were 46% and 60%. ORR was 19.6% versus 14.3% (90% CI, −11.5% to 21.3%), and DCR was 25.5% versus 38.8% (90% CI, −29.0% to 3.5%; Table 3).

Tissue biopsies were available for 99 patients (99.0%; fresh biopsy tissue, 9 [9.0%]; archival tissue, 90 [90.0%]). Among these, 76 were evaluable for PD-L1 status (18 not evaluable, 5 missing). Patients in either arm with baseline PD-L1 TPS ≥ 50% generally had numerically longer median PFS and higher ORR than those with TPS ≥ 1 to ≤ 49% or those with TPS < 1% (Supplemental Table 2). There was no significant difference in PFS between the two arms in patients with baseline PD-L1 TPS ≥ 50% (HR, 1.776 [90% CI,

Table 1
Patient baseline characteristics.

Characteristic	Pembrolizumab + CC-486 (n = 51)	Pembrolizumab + placebo (n = 49)	Overall (N = 100)
Median age (range), y	65 (39–82)	66 (39–82)	65 (39–82)
Male, n (%)	26 (51.0)	31 (63.3)	57 (57.0)
Race, n (%)			
White	42 (82.4)	45 (91.8)	87 (87.0)
Black or African American	3 (5.9)	2 (4.1)	5 (5.0)
Asian	0	1 (2.0)	1 (1.0)
Data missing	6 (11.8)	1 (2.0)	7 (7.0)
ECOG PS, n (%)			
0	15 (29.4)	17 (34.7)	32 (32.0)
1	36 (70.6)	32 (65.3)	68 (68.0)
Stage at baseline, n (%)			
IIIB	2 (3.9)	1 (2.0)	3 (3.0)
IV	49 (96.1)	48 (98.0)	97 (97.0)
Histology, n (%)			
Squamous	10 (19.6)	8 (16.3)	18 (18.0)
Adenocarcinoma	38 (74.5)	36 (73.5)	74 (74.0)
Other	3 (5.9)	5 (10.2)	8 (8.0)
Metastatic sites, n (%)			
Lung/thoracic	46 (90.2)	43 (87.8)	89 (89.0)
Lymph nodes	38 (74.5)	35 (71.4)	73 (73.0)
Bone	19 (37.3)	11 (22.4)	30 (30.0)
Central nervous system/brain	11 (21.6)	8 (16.3)	19 (19.0)
Number of metastatic sites, median (mean)	3.0 (3.1)	2.0 (2.8)	3.0 (3.0)
PD-L1 level at baseline, n (%)			
TPS $\geq$ 50%	9 (17.6)	9 (18.4)	18 (18.0)
TPS $\geq$ 1 to $\leq$ 49%	11 (21.6)	8 (16.3)	19 (19.0)
TPS < 1%	19 (37.3)	20 (40.8)	39 (39.0)
Not evaluable/missing	12 (23.5)	12 (24.5)	24 (24.0)

ECOG PS, Eastern Cooperative Oncology Group performance status; PD-L1, programmed death-ligand 1; TPS, tumour proportion score.

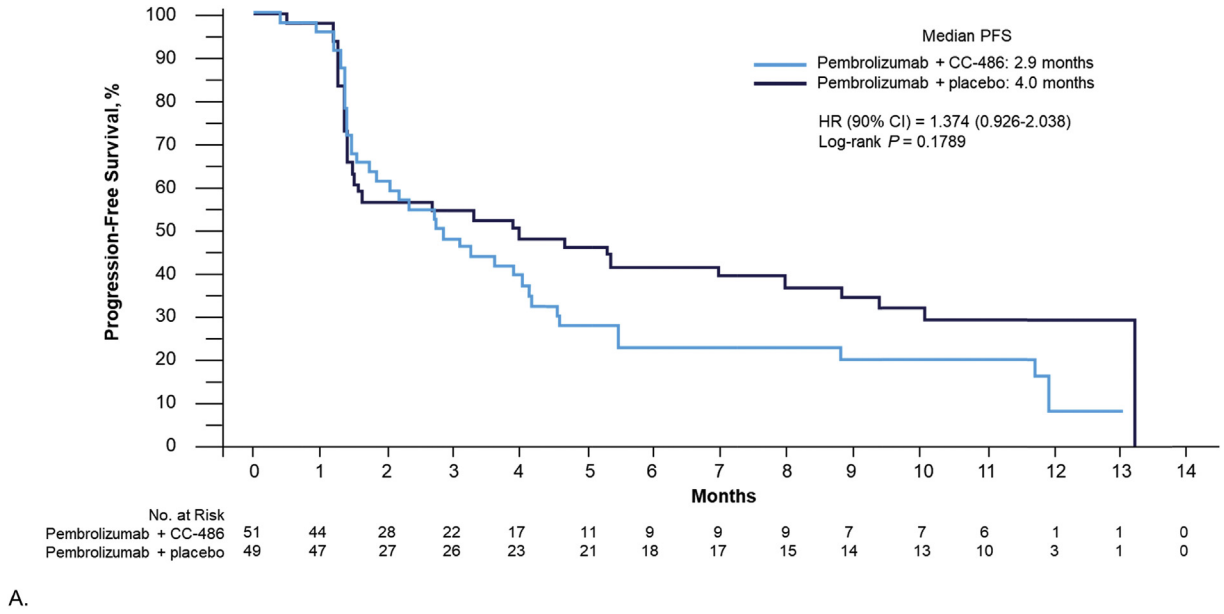
Table 2
Treatment exposure and dose modification.

Parameter	Pembrolizumab + CC-486 (n = 51)	Pembrolizumab + placebo (n = 49)	Overall (N = 100)
Median duration (range), weeks	15.0 (1–61)	24.1 (3–60)	17.9 (1–61)
Median number of cycles (range)	5.0 (1–20)	7.0 (1–20)	6.0 (1–20)
Median relative dose intensity of pembrolizumab, %[a]	96.84	98.07	97.67
$\geq$ 90% dose, n (%)	29 (56.9)	38 (77.6)	67 (67.0)
CC-486/placebo			
Patients with dose reduction, n (%)	13 (25.5)	3 (6.1)	16 (16.0)
Patients with dose interruption, n (%)	41 (80.4)	30 (61.2)	71 (71.0)
Pembrolizumab			
Patients with dose reduction, n (%)	–	–	0
Patients with dose interruption, n (%)	28 (54.9)	17 (34.7)	45 (45.0)

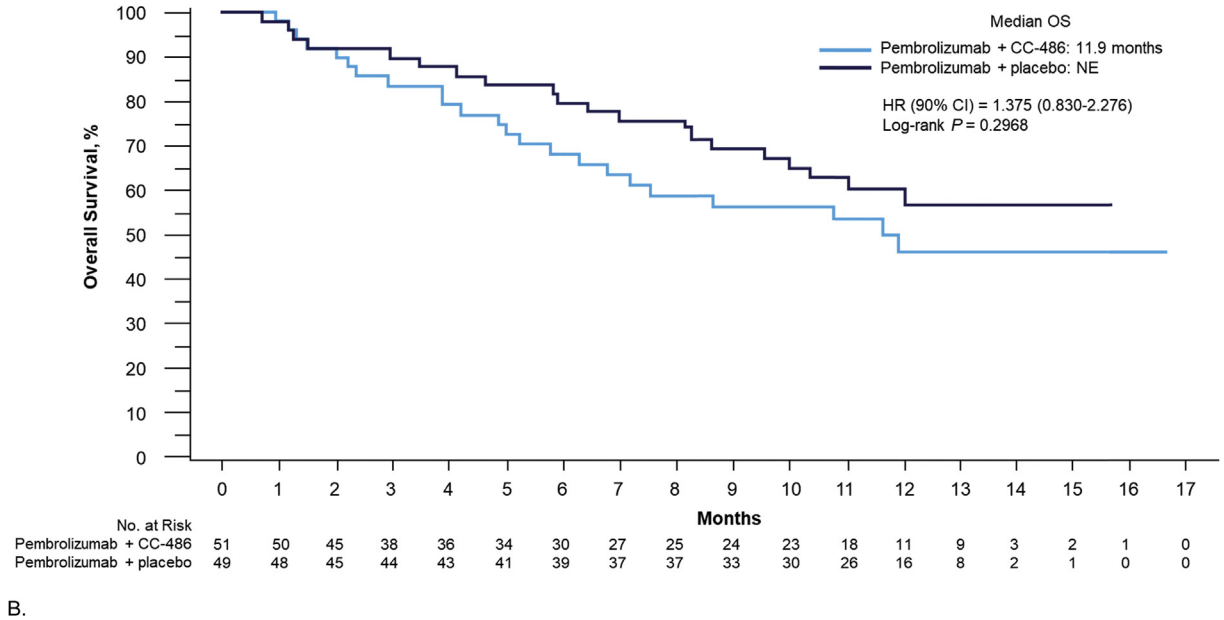
[a] Defined as cumulative dose per week/protocol weekly dose × 100%. The original protocol weekly dose for pembrolizumab was 66.67 mg/week.

0.544–5.798]; $P = 0.418$) or in patients with TPS $\geq$ 1% to $\leq$ 49% (HR, 1.038 [90% CI, 0.459–2.351]; $P = 0.871$). Among patients with PD-L1 TPS < 1%, those treated with pembrolizumab + CC-486 had a significantly shorter PFS (HR, 2.222 [90% CI, 1.146–4.308]; $P = 0.04$).

DNA methylation analysis was conducted on 37 evaluable samples (38 samples available, 1 excluded due to quality control) from patients treated with pembrolizumab + CC-486. Overall, median GDMS was significantly lower after one cycle of treatment ($P < 0.0001$; Fig. 3A). Analysis of DNA methylation in individual patient samples revealed that GDMS decreased from cycle 1 to 2 in 36 of 37 patients; 18 patients had > 10% reduction (Fig. 3B and C). No significant association was observed between change in GDMS from cycles 1 to 2 and PFS or OS (Fig. 3D and E). When patients were grouped by GDMS decrease (> 10% versus < 10%), no significant association with OS was observed ($P = 0.35$); however, a trend towards



A.



B.

Fig. 2. Kaplan–Meier (KM) plot of (A) progression-free survival (PFS) and (B) overall survival (OS). CI, confidence interval; HR, hazard ratio; NE, not estimable.

increased OS benefit after 4 months of treatment was observed in those with a > 10% decrease in GDMS (Fig. 3F).

3.4. Safety

In both arms, all patients had at least one treatment-emergent AE (TEAE); 78.4% and 55.1% of patients in the pembrolizumab + CC-486 and pembrolizumab + placebo arms had a grade 3/4 TEAE, respectively. Serious TEAEs were reported in 60.8% and 55.1%, respectively. Common (≥ 10.0% in any arm) TEAEs of any grade and grade 3/4 were generally higher in the pembrolizumab + CC-486 arm (Table 4). In particular, a higher proportion of patients treated with pembrolizumab + CC-486 had gastrointestinal-related TEAEs of nausea, vomiting and diarrhoea (all grades and grade 3/4). No differences in immunologic AEs were observed between the groups.

A greater percentage of patients in the pembrolizumab + CC-486 versus pembrolizumab + placebo arm had ≥ 1 TEAE that led to discontinuation (39.2% versus 16.3%). The most common TEAEs leading to a discontinuation were gastrointestinal with

Table 3
Best response to treatment.

Response	Pembrolizumab + CC-486 (n = 51), n (%)	Pembrolizumab + placebo (n = 49), n (%)
Overall response rate	10 (19.6)	7 (14.3)
Complete response	0	0
Partial response	10 (19.6)	7 (14.3)
Stable disease	3 (5.9)	12 (24.5)
Progressive disease	31 (60.8)	25 (51.0)
Unevaluable/missing	7 (13.7)	5 (10.2)

pembrolizumab + CC-486 (13.7%) and respiratory with pembrolizumab + placebo (6.1%). A greater percentage of patients receiving pembrolizumab + CC-486 had ≥ 1 TEAE that led to a reduction (19.6%) or interruption (62.7%) compared with those receiving pembrolizumab + placebo (reduction, 2.0%; interruption, 40.8%). The most common TEAEs leading to CC-486 reduction or interruption were gastrointestinal (7.8% and 25.5%, respectively).

4. Discussion

Although DNA methylation analysis in the pembrolizumab + CC-486 arm suggested pharmacodynamic responses indicative of CC-486 activity, there was no significant difference in PFS and OS between the two arms. Median PFS and OS were numerically shorter with pembrolizumab + CC-486. Several factors may have contributed to these outcomes. Patients receiving pembrolizumab + CC-486 had lower treatment exposure and more dose interruptions versus pembrolizumab + placebo, which may have impacted efficacy. Although no new safety signals were observed for CC-486 or pembrolizumab, the incidence of gastrointestinal TEAEs (known to be associated with CC-486) was higher in the pembrolizumab + CC-486 arm; optimisation of CC-486 dose and schedule may be needed to fully realise the clinical benefits of this combination [17,18]. There also remains the possibility that combination of pembrolizumab + CC-486 is antagonistic in this patient population. For example, treatment with azacitidine could have upregulated immune checkpoints in the tumour microenvironment, negatively regulating T-cell activity [19].

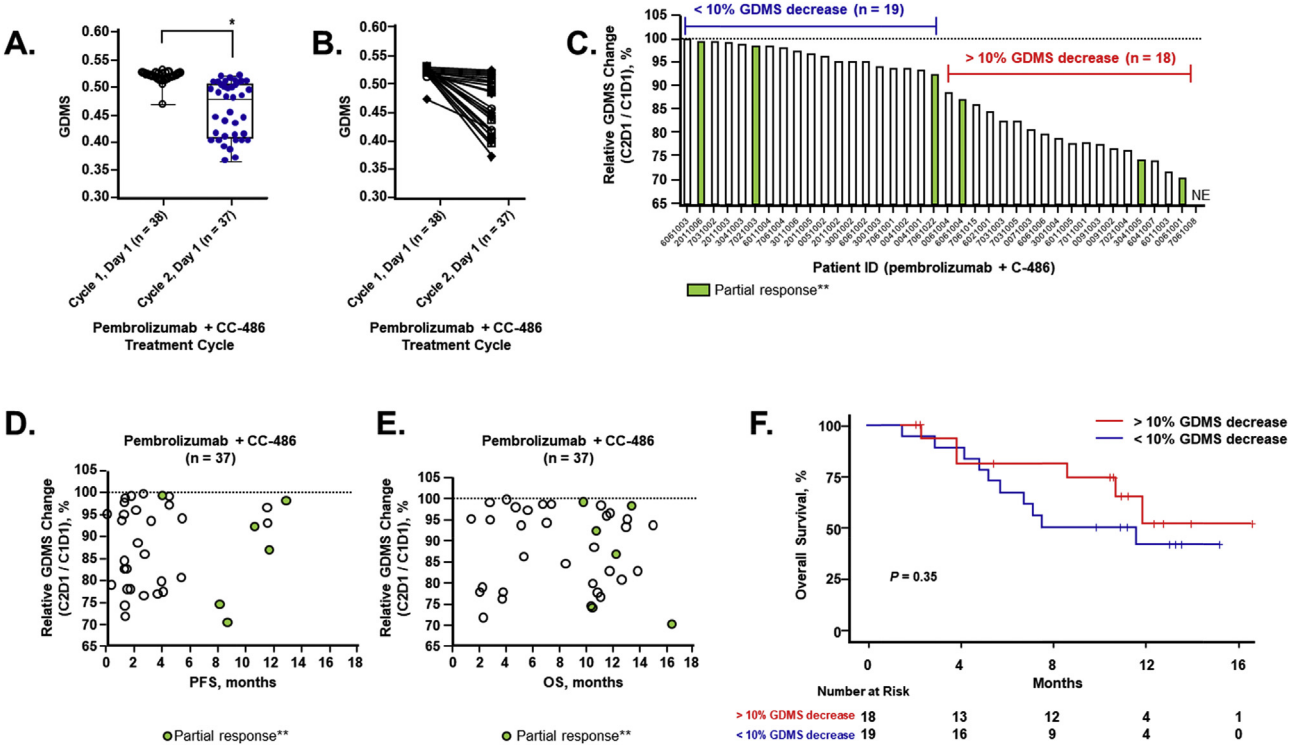


Fig. 3. Changes in DNA methylation from cycle 1 to 2 in patients in the pembrolizumab + CC-486 arm. (A) Median global DNA methylation scores (GDMS) in cycles 1 and 2. (B) Change in GDMS values from cycle 1 to 2. (C) Percent change in patients' GDMS from cycle 1 to 2. (D) Progression-free survival (PFS) and (E) overall survival (OS) by percent change in GDMS from cycle 1 to 2. (F) Kaplan–Meier OS curve for patients with > 10% decrease in GDMS and those with < 10% decrease. ID, identification; NE, not evaluable. * Wilcoxon matched-pairs signed-rank test $P < 0.0001$. ** Data available for 6 of 10 patients with partial response.

Table 4
Common (≥10.0% any grade) treatment-emergent adverse events.

Common TEAEs, n (%)	Pembrolizumab + CC-486 (n = 51)		Pembrolizumab + placebo (n = 49)		Overall (N = 100)	
	Any grade	Grade 3/4	Any grade	Grade 3/4	Any grade	Grade 3/4
Nausea	37 (72.5)	7 (13.7)	13 (26.5)	1 (2.0)	50 (50.0)	8 (8.0)
Vomiting	35 (68.6)	6 (11.8)	9 (18.4)	0	44 (44.0)	6 (6.0)
Asthenia	22 (43.1)	5 (9.8)	17 (34.7)	4 (8.2)	39 (39.0)	9 (9.0)
Diarrhoea	20 (39.2)	5 (9.8)	12 (24.5)	3 (6.1)	32 (32.0)	8 (8.0)
Decreased appetite	19 (37.3)	0	20 (40.8)	1 (2.0)	39 (39.0)	1 (1.0)
Constipation	18 (35.3)	1 (2.0)	9 (18.4)	1 (2.0)	27 (27.0)	2 (2.0)
Pyrexia	18 (35.3)	0	14 (28.6)	2 (4.1)	32 (32.0)	2 (2.0)
Cough	12 (23.5)	0	17 (34.7)	0	29 (29.0)	0
Anaemia	12 (23.5)	5 (9.8)	8 (16.3)	1 (2.0)	20 (20.0)	6 (6.0)
Decreased weight	11 (21.6)	1 (2.0)	3 (6.1)	0	14 (14.0)	1 (1.0)
Dyspnoea	11 (21.6)	4 (7.8)	12 (24.5)	4 (8.2)	23 (23.0)	8 (8.0)
Fatigue	10 (19.6)	2 (3.9)	3 (6.1)	0	13 (13.0)	2 (2.0)
Back pain	9 (17.6)	2 (3.9)	3 (6.1)	0	12 (12.0)	2 (2.0)
Abdominal pain	8 (15.7)	1 (2.0)	5 (10.2)	0	13 (13.0)	1 (1.0)
Neutropenia	8 (15.7)	3 (5.9)	1 (2.0)	1 (2.0)	9 (9.0)	4 (4.0)
Hypokalaemia	6 (11.8)	1 (2.0)	1 (2.0)	0	7 (7.0)	1 (1.0)
Hypomagnesaemia	6 (11.8)	0	0	0	6 (6.0)	0
Hypertension	6 (11.8)	2 (3.9)	1 (2.0)	0	7 (7.0)	2 (2.0)
Peripheral oedema	5 (9.8)	1 (2.0)	8 (16.3)	1 (2.0)	13 (13.0)	2 (2.0)
Musculoskeletal pain	4 (7.8)	0	5 (10.2)	1 (2.0)	9 (9.0)	1 (1.0)
Cancer pain	4 (7.8)	1 (2.0)	8 (16.3)	2 (4.1)	12 (12.0)	3 (3.0)
Haemoptysis	3 (5.9)	0	5 (10.2)	2 (4.1)	8 (8.0)	2 (2.0)
Pain in extremity	3 (5.9)	0	5 (10.2)	0	8 (8.0)	0
Bronchitis	1 (2.0)	1 (2.0)	6 (12.2)	1 (2.0)	7 (7.0)	2 (2.0)
Myalgia	0	0	5 (10.2)	0	5 (5.0)	0

TEAE, treatment-emergent adverse event.

This remains speculative and needs to be tested in future studies.

Despite the negative results of this study, strong scientific rationale remains for continued exploration of strategies, combining epigenetic agents with immunotherapy in advanced NSCLC [7,8,11,12]. Further efforts evaluating these combinations would be aided by the identification of appropriate biomarkers rooted in scientific rationale. Recent preclinical data suggest that deficiencies in *LKB1*, a tumour suppressor gene frequently mutated in lung adenocarcinoma, lead to enhanced DNA methylation and sensitise tumours to DNA methylation inhibitors via reactivation of silenced retrotransposons [20]. Given the complexity of epigenetic modulation on the regulation of tumour-associated antigen presentation and the subsequent interplay with the immune system, future enrichment strategies may require biomarkers that characterise both the tumour and immune status.

There are some limitations to our study. At the time this study was designed, pembrolizumab was not yet approved for patients with advanced NSCLC and PD-L1 TPS $\geq$ 1%; therefore, our study did not contain any specific requirement for PD-L1 expression, nor was there stratification by expression, which could have impacted the results. Since then, pembrolizumab has been approved in this population. Future study designs combining pembrolizumab with an epigenetic agent should take the indicated patient population into account. DNA methylation analysis samples were collected but not tested in the placebo arm, precluding study of the prognostic impact of baseline methylation levels in that cohort and preventing comparisons of relative changes between the two arms. Although DNA methylation changes were most likely due to CC-486, we cannot definitively conclude this without a direct comparison with the placebo arm. In addition, survival comparisons between patient subgroups may have resulted in relatively small patient numbers; these analyses should be considered with appropriate caution.

No improvement in PFS was observed with the addition of CC-486 to pembrolizumab; this may have been caused by decreased treatment exposure due to gastrointestinal TEAEs known to be associated with CC-486. No new safety signals for CC-486 or pembrolizumab were detected. There is significant scientific interest in combining epigenetic and immunotherapy agents, but there is a relative lack of clinical data for these combinations in patients with NSCLC. This study contributes to the existing body of knowledge and may aid in designing future experiments for these combinations.

Funding

This study was funded by Celgene Corporation and Merck Sharp & Dohme, Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA. Editorial assistance

was provided by William Ho, PhD, MediTech Media, Ltd, funded by Celgene Corporation.

Conflict of interest statement

BPL has received personal fees for medical advisory boards from Amgen, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Clovis Oncology, Lilly, Merck Sharp & Dohme, Novartis, Pfizer and Roche. GG has nothing to disclose. BB has received grants/research support from Celgene Corporation. EF received consultant fees for medical advisory boards from AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Celgene, Eli Lilly, Guardant Health, Merck Sharp & Dohme, Novartis, Pfizer, Roche, Takeda, AbbVie and Merck. MCG received consultant fees for medical advisory boards and was part of the Speakers' Bureaus for AstraZeneca, Bristol-Myers Squibb, Merck Sharp & Dohme and Roche. MDG has nothing to disclose. PG received honoraria or consultation fees from AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Merck Sharp & Dohme, Novartis, Pfizer and Roche and was part of the Speakers' Bureaus for AbbVie, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Guardant, Merck Sharp & Dohme, Novartis, Pfizer and Roche. BP is an employee of and has stock ownership with Merck Sharp & Dohme. SP-A has nothing to disclose. DM, KJM, AR, RS, XW and AF are employees of Celgene, the study sponsor and have stock ownership. KJM has a patent licenced (US9693987B2). LP-A has received honoraria or consultation fees from Roche, Lilly, Merck Sharp & Dohme, Bristol-Myers Squibb, Novartis, Boehringer Ingelheim, AstraZeneca, Amgen and Takeda.

Acknowledgements

Medical writing assistance was provided by William Ho, PhD, MediTech Media, Ltd, funded by Celgene Corporation. All listed authors were fully responsible for all content and editorial decisions for this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2018.11.028.

References

[1] Davies J, Patel M, Gridelli C, de Marinis F, Waterkamp D, McCusker ME. Real-world treatment patterns for patients receiving second-line and third-line treatment for advanced non-small cell lung cancer: a systematic review of recently published studies. PLoS One 2017;12. e0175679.

[2] Schvartsman G, Ferrarotto R, Massarelli E. Checkpoint inhibitors in lung cancer: latest developments and clinical potential. Therpeut Adv Med Oncol 2016;8:460–73.

[3] Horn L, Spigel DR, Vokes EE, Holgado E, Ready N, Steins M, 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;35:3924–33.

[4] Herbst RS, Baas P, Kim D-W, Felip E, Pérez-Gracia JL, Han J-Y, 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(10027):1540–50.

[5] Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus docetaxel in advanced non-squamous non-small-cell lung cancer. N Engl J Med 2015;373:1627–39.

[6] Brahmer J, Reckamp KL, Baas P, Crinò L, Eberhardt WE, Poddubskaya E, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med 2015;373:123–35.

[7] Juergens RA, Wrangle J, Vendetti FP, Murphy SC, Zhao M, Coleman B, et al. Combination epigenetic therapy has efficacy in patients with refractory advanced non-small cell lung cancer. Cancer Discov 2011;1:598–607.

[8] Wrangle J, Wang W, Koch A, Easwaran H, Mohammad HP, Vendetti F, et al. Alterations of immune response of non-small cell lung cancer with azacytidine. Oncotarget 2013;4:2067–79.

[9] Ansari J, Shackelford RE, El-Osta H. Epigenetics in non-small cell lung cancer: from basics to therapeutics. Transl Lung Cancer Res 2016;5:155–71.

[10] Chiappinelli KB, Zahnow CA, Ahuja N, Baylin SB. Combining epigenetic and immunotherapy to combat cancer. Cancer Res 2016;76:1683–9.

[11] Peng D, Kryczek I, Nagarsheth N, Zhao L, Wei S, Wang W, et al. Epigenetic silencing of TH1-type chemokines shapes tumour immunity and immunotherapy. Nature 2015;527:249–53.

[12] Stone ML, Chiappinelli KB, Li H, Murphy LM, Travers ME, Topper MJ, et al. Epigenetic therapy activates type I interferon signaling in murine ovarian cancer to reduce immunosuppression and tumor burden. Proc Natl Acad Sci U S A 2017;114:E10981–90.

[13] Keytruda (pembrolizumab) for injection, for intravenous use [package insert]. Whitehouse Station, NJ: Merck & Co., Inc; 2017.

[14] Vidaza (azacitidine) [package insert]. Summit, NJ: Celgene Corporation; 2016.

[15] Vidaza (azacitidine) [summary of product characteristics]. Uxbridge, United Kingdom: Celgene Europe Ltd; 2017.

[16] Laille E, Shi T, Garcia-Manero G, Cogle CR, Gore SD, Hetzer J, et al. Pharmacokinetics and pharmacodynamics with extended dosing of CC-486 in patients with hematologic malignancies. PLoS One 2015;10. e0135520.

[17] Cogle CR, Scott BL, Boyd T, Garcia-Manero G. Oral azacitidine (CC-486) for the treatment of myelodysplastic syndromes and acute myeloid leukemia. Oncologist 2015;20:1404–12.

[18] Von Hoff DD, Rasco DW, Heath EI, Munster PN, Schellens JHM, Isambert N, et al. Phase I study of CC-486 alone and in combination with carboplatin or nab-paclitaxel in patients with relapsed or refractory solid tumors. Clin Cancer Res 2018;24:4072–80.

[19] Ørskov AD, Treppendahl MB, Skovbo A, Holm MS, Friis LS, Hokland M, et al. Hypomethylation and up-regulation of PD-1 in T cells by azacytidine in MDS/AML patients: a rationale for combined targeting of PD-1 and DNA methylation. Oncotarget 2015;6:9612–26.

[20] Kottakis F, Nicolay BN, Roumane A, Karnik R, Gu H, Nagle JM, et al. LKB1 loss links serine metabolism to DNA methylation and tumorigenesis. Nature 2016;539:390–5.