Efficacy of PD-1/PD-L1 inhibitors in PD-L1 Negative Patients with Cancer: A Meta-analysis

Journal:	*BMJ*
Manuscript ID	BMJ.2018.044566
Article Type:	Research
BMJ Journal:	BMJ
Date Submitted by the Author:	11-Apr-2018
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Keywords:	PD-1, PD-L1, biomarker, meta analysis, cancer

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Abstract

Objective To evaluate the relative efficacy of PD-1/PD-L1 inhibitors vs conventional agents in PD-L1 negative subjects with cancer.

Design Systemic review and meta-analysis

Data sources Embase, PubMed, Cochrane database, and abstracts presented at American Society of Clinical Oncology and European Society of Medical Oncology from inception to Mach 2018.

Study selection Randomized clinical trials (RCTs) that compared PD-1/PD-L1 inhibitors (avelumab, atezolizumab, durvalumab, pembrolizumab, and nivolumab) with controls in PD-L1 negative patients. The definition of PD-L1 negativity was that PD-L1 stained cell accounted for less than 1% of tumor cells, immune cells, or both assessed by immunohistochemistry assay.

Data extraction and synthesis Two reviewers conducted study selection, data extraction, and quality assessment. Fixed-effects and random-effects models were applied to calculate the overall combined risk estimates.

Results A total of 1,964 PD-L1 negative patients from 9 RCTs were included in this study. Compared with conventional agents, PD-1/PD-L1 inhibitors were associated with significantly prolonged OS (HR, 0.80; 95% CI, 0.71-0.90; p=0.008). The relative benefits were observed consistently across interventional agent, cancer type, number of patients recruited, and follow-up duration. No significant differences were observed with respect to ORR (RR, 1.14; 95% CI, 0.71-1.82) and PFS (HR, 0.97; 95% CI, 0.68-1.40).

Conclusions For PD-L1 negative cancer patients, PD-1/PD-L1 blockade therapy is a preferable treatment option over conventional therapy. This finding does not support PD-L1 as a biomarker

for patient selection in PD-1/PD-L1 blockade therapy. Moreover, it may assist in the design and interpretation of clinical trials.

Introduction

The immune suppression and evasion of malignant cancer cell has been known as one of the hallmarks of cancer[1]. A series of co-inhibitory and co-stimulatory receptors and their ligand, known as immune checkpoints, control this process. Among them, The PD-1/PD-L1 axis stands out as a valuable therapeutic target because it not only plays a key role in physiological immune homeostasis, but also appears to be a means through which cancer cell evade the immune system[2]. The development and application of antibodies targeting PD-1 (nivolumab and pembrolizumab) and PD-L1 (atezolizumab, avelumab, and durvalumab) has been a major advance in the treatment of cancer[3]. Currently, these PD-1/PD-L1 inhibitors are being investigated in more than 1,000 clinical trials and has been approved for a variety of cancers including non-small cell lung cancer, melanoma, renal cell carcinoma, bladder cancer, Hodgkin's lymphoma, Merkel-cell carcinoma, head and neck squamous cell carcinoma, hepatocellular carcinoma, gastroesophageal junction cancer, and tumors of any organ with high microsatellite instability[2 3].

Although the introduction of PD-1/PD-L1 inhibitors into clinical practice has a revolutionary effect on cancer treatment, durable responses and favorable long term outcomes are not observed in all patients [4]. Accordingly, identifying the optimal molecular or clinical biomarkers that can predict the benefit of PD-1/PD-L1 inhibitors is essential in selecting the appropriate subjects for these therapies. Direct evaluation of PD-L1 expression on cancer cells is treated as a biologically plausible and the best available biomarker in predicting the tumor response and survival prognosis in the long term[5]. Numerous studies[6-15] have consistently demonstrated longer overall survival and better tolerability of PD-1/PD-L1 inhibitors compared with conventional therapy in PD-L1 positive patients. Moreover, evidence suggests that there is a linear relationship between the level

of PD-L1 expression and the extent of the benefit from checkpoint inhibitors treatment[7 9 14 15]. Considering the fundamental nature of these checkpoint inhibitors, it seems logical that PD-L1 expression should be correlated with clinical outcomes. However, a non-negligible number of exceptions is observed. Tumor responses have been reported in 0% - 17% of patients with low or undetectable PD-L1 expression[16]. In some studies[6 8 17], favorable long term outcomes can be achieved in PD-L1 negative patients. Accordingly, the predictive and prognostic role of PD-L1 status remains to be determined, and PD-L1 expression has not been approved in patient selection although complementary PD-L1 diagnostics have been accepted by FDA[18].

Currently, individual randomized trials have not been designed to indicate a treatment difference between PD-1/PD-L1 inhibitors and conventional agents in PD-L1 negative patients. Therefore, a pooled analysis of available trials restricted to PD-L1 negative patients may provide critical and clinically useful information with respect to anti-PD-1/anti-PD-L1 treatment. Here, with recently accumulating evidence, we conducted a meta-analysis of RCTs to evaluate the relative efficacy of PD-1/PD-L1 inhibitors in PD-L1 negative cancer patients.

Methods

The present study was conducted in compliance with the recommendations of the Cochrane Handbook for Systematic Reviews of Interventions and reported based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines[19].

Literature search and Study selection

A comprehensive systematic search of PubMed, Embase and Cochrane databases from inception to March 2018 was conducted with no language restrictions. Giving that recent studies might be unpublished, additional electronic searches were performed through two major international congresses' proceedings (American Society of Clinical Oncology Annual Meeting and European Society of Medical Oncology). The main keywords used were: avelumab, atezolizumab, durvalumab, pembrolizumab, nivolumab, checkpoint inhibitors, PD-1, PD-L1 and randomized controlled trial (Supplemental materials).

Both exclusion and inclusion criteria were pre-specified. To be eligible, randomized controlled trials had to meet the following criteria: (1) population: the PD-L1 status of the included patients (>18 years old) were examined. The definition of PD-L1 negativity was PD-L1 stained cell accounted for less than 1% of tumor cells, immune cells, or both assessed by immunohistochemistry assay; (2) intervention: treated with checkpoint inhibitors (avelumab, atezolizumab, durvalumab, pembrolizumab, and nivolumab) irrespective of dosage and duration; (3) main outcomes: the primary outcome was overall survival (OS) measured as hazard ratios (HRs). The secondary outcomes were objective response rate (ORR) and progression-free survival (PFS) expressed as relative risks (RRs) and HRs, respectively.

Studies were excluded if they were retrospective or prospective observational cohort studies.

In addition, Phase I and non-randomized phase II were excluded. Other publications on the topic, including review articles, basic science papers, commentaries, conference abstract, quality of life studies, editorials, cost effectiveness analyses, early versions of data later published, articles in which the effect of the drug could not be ascertained, such as when the control was a different dose of the same drug (Figure 1). Additionally, the reference lists of all trials fulfilling the eligibility criteria were also examined for any relevant studies missed by initial searches. When multiple articles of the same clinical trial appeared or if there was a case mix between different publications, only the most recent and/or most complete reporting study was included. Any discrepancies were settled by discussion and consensus. All the included RCTs represented unique studies.

Data extraction and risk of bias assessment

The key exposure variable was the intervention of anti-PD-1 and anti-PD-L1 agents. In most trials, conventional chemotherapy served as the control group. In two studies, KEYNOTE-006[11] and CheckMate 025[8], ipilimumab and everolimus served as the controls. We included all these RCTs for analysis and conducted a sensitivity analysis that only included trials with a control group defined as strictly chemotherapy.

Outcomes of interest in the present study included OS, ORR and PFS. Of all the eligible RCTs, tumor response was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) v1.1[20].

Data extraction was carried out using a standardized data-collection form. We extracted all the reported HRs for OS and PFS, and tumor response events for ORR calculation from eligible RCTs. The following clinicopathological characteristics for each study were also recorded: study

name, trial phase, disease type, experimental drug, age, the antibody clones and assay developers for PD-L1 detection, number of PD-L1 negative patients and median follow-up (Table 1).

The Cochrane risk-of-bias tool[21] was applied to evaluate the risk of bias in this study. We examined every trial and scored as high, low, or unclear risk of bias to the following criteria: random sequence generation; allocation concealment; blinding of participants and personnel to the study protocol; blinding of outcome assessment; incomplete outcome data and selective reporting. Two authors independently carried out the data extraction and quality assessment. Any disagreements were resolved by discussion and consensus.

Statistical Analysis

All analysis was carried out using Stata version 12.0 (StataCorp LP, USA). For OS and PFS, the HR and its 95% confidence interval (CI) were extracted from the research manuscript directly. As for ORR, the relative risks (RRs) were calculated using the data provided in each eligible trials. In addition, pre-defined subgroup analysis were conduct based on the following criteria: interventional agent, disease type, total number of PD-L1 negative patients included in each trial, and median follow-up duration. Both random-effects models and fixed-effects models were utilized to calculate pooled HRs, RRs, and their 95% CIs.

Statistical heterogeneity between different trials and subgroups was assessed by Cochrane's Q statistic. The I^2 statistic was calculated to assess the extent of inconsistency contributable to the heterogeneity across different studies[22]. The assumption of homogeneity was considered invalid for $I^2 > 25\%$ and $p<0.10$. Potential publication bias was assessed by visual inspection of a funnel plot, and also evaluated using the tests of Egger et al.[23] and Begg et al.[24]. Two-sided p values <0.05 were considered statistically significant.

Results

Literature search

A total of 6,461 related articles were identified by the initial search strategy. 2,764 studies were removed because of duplications. After eligibility screening of the titles and abstracts, 3,606 studies were excluded since they did not meet the inclusion criteria. When carefully reviewed the full texts of the remaining 91 potentially eligible papers, 9 RCTs were chosen for the final analysis[7-15] . A flow chart showing the study selection was showed in Figure 1. Data from all eligible RCTs were obtained from published manuscripts.

Study characteristics

A total of 1,964 subjects from nine RCTs were included in this study. The main characteristics of the eligible trials were presented in Table 1. All these studies were international multi-center RCTs funded by the pharmaceutical industry, and published between 2015 and 2017. The numbers of PD-L1 negative patients in these eligible trials ranged from 44 to 575. The immunohistochemistry assay developers and antibody clones were stated in all trials except CheckMate 025[8].

Five studies were conducted in patients with lung cancer[7 9 12 14 15], and one each in renal cancer[8], head and neck cancer[10], melanoma[11], and urothelial cancer[13]. Subjects in the intervention arm received nivolumab in four studies[7-10], pembrolizumab in three studies[11-13], and atezolizumab in two studies[14 15]. One trial evaluated the combination of pembrolizumab and chemotherapy compared with chemotherapy alone[12]. For all but one study, the primary endpoint was overall survival; in KEYNOTE-021, the primary endpoint was ORR[12]. Among the nine trials included here, eight reported OS[7-11 13-15], four reported ORR[9 10 12 15], and three reported PFS[7 9 15]. All except

two trials were phase 3 RCTs, KEYNOTE-021 and POPLAR were phase 2 trials[12 15]. These checkpoint inhibitors were used as first line treatment in KEYNOTE-021[12] and part of patients in KEYNOTE-006[11]; and as second line or later treatment in the rest of eligible trials.

The methodological quality of the included trials was generally moderate to good (Supplemental Table 1). The main issue affecting quality was lack of blinding because all the eligible RCTs were open-labeled.

Efficacy of PD-1/PD-L1 inhibitors in PD-L1 negative patients

For OS analysis, eight trials with a total of 1,920 patient were included. Overall, the pooled model showed PD-L1 negative patients treatment with PD-1/PD-L1 inhibitors was associated with a better OS compared with controls (HR, 0.80; 95% CI, 0.71-0.90; p=0.008) (Figure 2). No substantial heterogeneity was observed (p=0.61, I^2 = 0.0%). It is noted that chemotherapy served as control in all but two trials, namely KEYNOTE-006 and CheckMate 025[8 11]. Exclusion of these two studies yielded similar result (HR, 0.81; 95% CI, 0.71-0.93).

In the four pre-defined subgroup analysis (Figure 3), the treatment effects were similar. It suggested that greater benefit achieved from treatment with PD-1/PD-L1 inhibitors over control was independent of checkpoint antibody, cancer type, number of patients, and follow-up duration.

Four trials with 456 subjects were included in ORR analysis, and three trials with 407 patients in PFS analysis. The pooled models revealed that compared with conventional chemotherapy, PD-L1 negative patients did not benefit from treatment with PD-1/PD-L1 antibodies in term of ORR (RR, 1.14; 95% CI, 0.71-1.82; p>0.05) and PFS (HR, 0.97; 95% CI, 0.68-1.40; p>0.05) (Figure 4). Additionally, significant heterogeneities were observed in both ORR and PFS analysis.

Publication bias

Visual inspection of the Begg funnel plot did not identify substantial asymmetry (Supplemental Figure 1). The Begg rank correlation test and Egger linear regression test also indicated no evidence of publication bias.

Discussion

To our knowledge, this study is the first meta-analysis with a focus on examining the association between PD-1/PD-L1 inhibitors vs conventional agents and survival outcomes in PD-L1 negative patients with cancer. With approximate 2,000 PD-L1 negative patients from 9 RCTs, the pooled analysis revealed that the risk of death decreased by 20% with PD-1/PD-L1 inhibitors treatment, as compared with conventional therapy. Moreover, the survival benefit was consistent across all the pre-specified subgroup. However, ORR and PFS did not differ between the PD-1/PD-L1 inhibitors group and conventional agents group. The apparent discrepancy between OS and ORR/PFS could be partly explained by the limited number of trials included in ORR and PFS analysis. Therefore, our study suggests that, even for PD-L1 negative cancer patients, PD-1/PD-L1 inhibitors therapy could be a preferable treatment option over conventional therapy. Moreover, PD-L1 expression alone was not an adequate biomarker for routine clinical practice in determining which patients should be offered PD-1/PD-L1 blockade therapy.

Most of the previous studies focused on the role of PD-L1 expression as predictive biomarker rather than a prognostic biomarker[5 16], which might partly because only limited information regarding overall survival in PD-L1 negative patients is available. Even as a predictive biomarker for tumor responses, the role of PD-L1 expression remained controversial due to the existence of various antibody clones, positivity/negativity cut-offs, and sometimes scoring system. It was reported that clone SP142 bound to the cytoplasmic domain of PD-L1, while the clones 22C3, 28-8 and SP263 bound to the extracellular domain of PD-L1[25]. Consequently, the positive rate using SP142 was lower than that obtained using clones 22C3, 28-8 and SP263 according to the report by Blueprint Working Group[26]. Additionally, in pembrolizumab trials, extensive biomarker

research had been conducted in KEYNOTE-001 resulting the identification of the receiver operating characteristic curve for PD-L1 expression > 50%[27]. However, 1%, 5%, and 10% were usually applied as the cut-off values for the definition of PD-L1 positivity. Therefore, the strategy in choosing the optimal cut-offs for PD-L1 positivity/negativity were different among various trials. In this study, to minimize these technique issues, only high quality RCTs conducted at multi-centers were eligible for analysis. Furthermore, a very strict cut-off value (i.e. <1%) was set in the definition of PD-L1 negativity. This high-level threshold meant that the expression of PD-L1 was almost undetectable in tumor samples and it satisfied all the requirement in the definition of PD-L1 negativity.

One of the most important unanswered questions in checkpoint blockade therapy is whether PD-L1 expression is a prognostic biomarker for overall survival, which is the gold standard for therapeutic intervention. In fact, several previous studies revealed that favorable long term outcomes can be achieved in PD-L1 negative patients[6 8 17]. However, because it was generally believed that the negative expression of PD-L1 was associated with weak or no pre-existing anticancer immunity, some clinicians and scientists tended to explain it by technical arguments including (1) limited PD-L1 negative tissues available; (2) the expression of PD-L1 was evaluated in archival tissues, which might not reflect the PD-L1 status at the time of treatment; (3) PD-L1 expression in tumors is not uniform, and sampling location may affect the results of PD-L1 staining; and (4) different molecular mechanisms may involve in PD-L1 expression in different tumor histology. In the present study, with 9 randomized controlled trials including 1,964 PD-L1 negative patients, we conducted a comprehensive analysis to overcome the problem of inadequate power of individual trials, and to attenuate the potential impact of the dynamic expression of

PD-L1. Our results revealed that PD-1/PD-L1 inhibitors were associated with significantly prolonged overall survival in PD-L1 negative patients. Furthermore, this survival benefit was so stable that it did not alter much in all the pre-defined subgroup analysis. Therefore, we think the lack of an association between survival efficacy and PD-L1 expression was because of the biological function of PD-1/PD-L1 pathway itself and the complicated interaction between cancer and immune system.

The molecular mechanisms that control PD-L1 expression are not fully understood currently[2] [3]. However, it is believed that PD-L1 was regulated at the transcriptional, post-transcriptional, and protein levels. In clinical practice, conventional treatments such as chemotherapy and radiotherapy were also considered as potential regulators of PD-L1 expression[28]. In addition, PD-1 interacts with two ligands, PD-L1 and PD-L2. Although PD-L1 is the dominant ligand for PD-1, PD-L2 can compete with PD-L1 with two- to six-fold higher affinity to PD-1 than PD-L1[29]. However, the role of PD-L2 expression as a predictive or prognostic marker has not been evaluated. Considering the objective response rate with PD-1/PD-L1 blockade mono-therapy is only approximate 20%[4], and immune-related adverse events can be observed during treatment[30], the establishment of prognostic biomarkers for PD-1/PD-L1 blockade therapy is therefore of utmost important to maximize the long term outcomes. Interestingly, Lee et al. discovered that in advanced non-small cell lung cancer (NSCLC), PD-1/PD-L1 inhibitors could prolonged OS in the *EGFR* wild-type patients, but not in the *EGFR* mutant patients; in the *KRAS* mutant subgroup but not in the *KRAS* wild-type subgroup[31]. Larkin et al. tried to establish the association between *BRAF* status and efficacy in advanced melanoma but failed[32]. Other potential biomarkers such as tumor-infiltrating lymphocytes[33], T-cell receptor clonality[34], mutational or neo-antigen burden[35 36], combinations of

immune markers[37] were still under investigation. Hence further development of an effective biomarker for checkpoint inhibitor-based immunotherapy was needed.

Our results also have several important clinical and research implications. First, our findings reveals that PD-1/PD-L1 blockade therapy could be a preferable treatment option over conventional therapy even for PD-L1 negative cancer patients. Second, since the survival benefit that cancer patients derived from PD-1/PD-L1 inhibitors was independent of PD-L1 expression, PD-L1 testing seems to be unnecessary. Furthermore, PD-L1 expression should not be treated as a stratification factor in future clinical trials.

This study has some limitations. First, an optimal biomarker need to maximize the benefit as well as minimize the risk of toxicities. Toxicity profile is another important factor in choosing therapy options. However, it was impossible to conduct such an analysis to deal with this issue here because all the adverse events from PD-L1 negative patient in all the eligible trials were unavailable. Second, because of the strict threshold we set in the definition of PD-L1 negativity, only a few trials were eligible for ORR and PFS analysis. Therefore, our results regarding ORR and PFS cannot be conclusive and should be interpreted cautiously. Third, we carried out the present study at the trial level, no clinicopathological characteristics at the individual level could be examined. This might reduce our ability to test for associations between variables in specific subgroups and limit our ability to assess for sources of heterogeneity. Fourth, all the included trials were open-labelled, this could lead to potential bias. Despite these limitation, to our knowledge this study is the largest meta-analysis that incorporates results from 9 RCTs with approximate 2000 PD-L1 negative patients.

Conclusions

PD-1/PD-L1 inhibitors, compared with conventional agents, significantly prolonged overall survival in PD-L1 negative patients with cancer. This finding does not support PD-L1 expression as a biomarker for patient selection in PD-1/PD-L1 blockade therapy. Moreover, it may also assist in the design and interpretation of clinical trials.

Contributors: All authors had full access to the data in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis. BZ is the guarantor.

Funding: None.

Competing interests: All authors have completed the ICMJE form disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Ethical approval: Not required.

Data sharing: No additional data are available.

Transparency: All authors affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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Figure legend

Figure 1. Flow-chart diagram of selected trials included in this meta-analysis.

Figure 2. Forest plot of overall survival in PD-L1 negative patients. The treatment effect was calculated with fixed-effects model.

PD-L1, Programmed Death-Ligand 1; HR, hazard ratio; CI, confidence interval; NR, not reported.

Figure 3. Subgroup analyses for overall survival in PD-L1 negative patients with cancer.

Figure 4. Forest plots of objective response rate (A) and progression-free survival (B) in PD-L1 negative patients. The treatment effects were calculated with random-effects model.

HR, hazard ratio; RR, relative risk; CI, confidence interval.

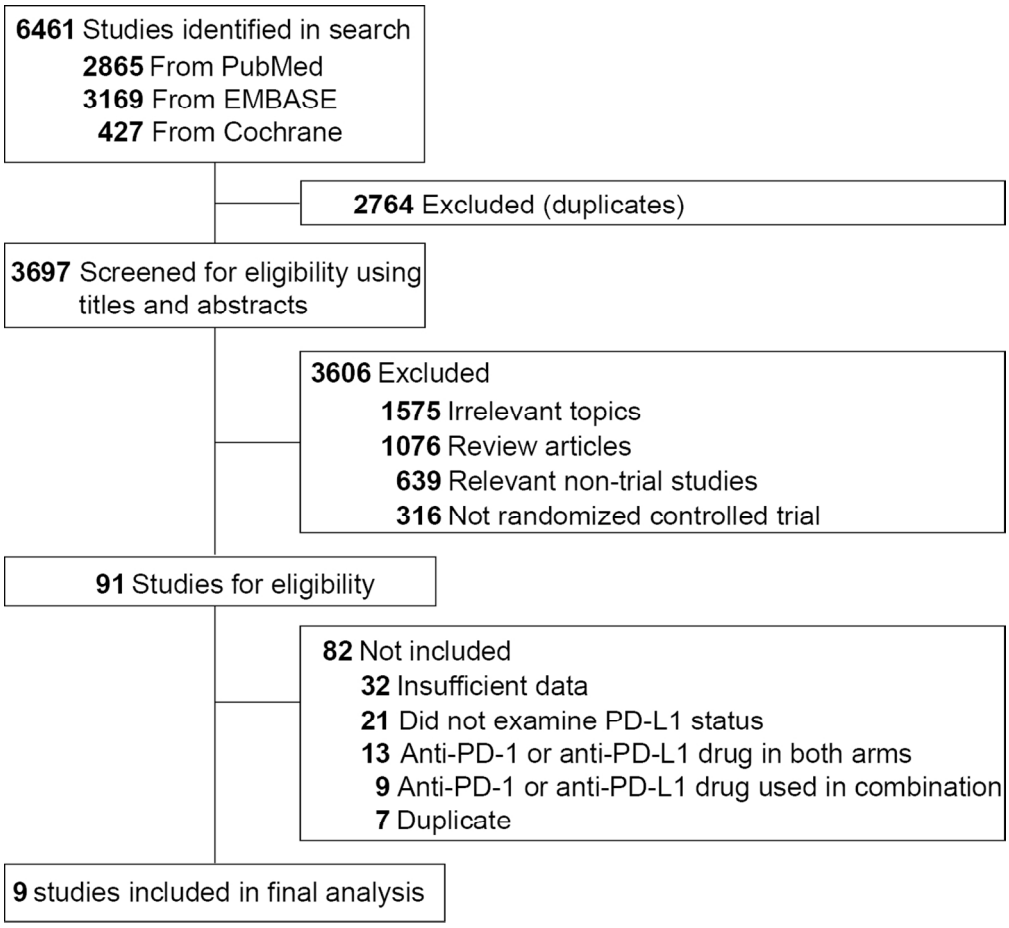
Table 1. Characteristics of the included randomized controlled trials

Study	Trial phase	Disease	Experimental drugs	Age, median (range), year	Antibody clone	Assay developer	No. of patients				median follow-up, month
							intervention		control		
							PD-L1 negative*	Total	PD-L1 negative*	Total	
CheckMate 017[6 7]	3	Lung cancer	Nivolumab vs docetaxel	63(39-85)	28-8	Dako	54	135	52	137	>24
CheckMate 025[8]	3	Renal cancer	Nivolumab vs everolimus	62(18-88)	NR	Dako	276	410	299	386	14
CheckMate 057[6 9]	3	Lung cancer	Nivolumab vs docetaxel	62(21-85)	28-8	Dako	108	292	101	290	>24
CheckMate 141[10]	3	Head and neck cancer	Nivolumab vs chemotherapy	60(28-83)	28-8	Dako	73	240	38	121	5.1
KEYNOTE-006[11]	3	Melanoma	Pembrolizumab vs ipilimumab	62(18-89)	22C3	Merck	103	556	47	278	22.9
KEYNOTE-021[12]	2	Lung cancer	Pembrolizumab + chemotherapy vs chemotherapy	63(54-70)	22C3	Dako	21	60	23	63	10.6
KEYNOTE-045[13]	3	Urothelial cancer	Pembrolizumab vs chemotherapy	66(26-88)	22C3	Dako	NR#	270	NR#	272	14.1
OAK[14]	3	Lung cancer	Atezolizumab vs docetaxel	64(33-85)	SP142	VENTANA	180	425	199	425	21
POPLAR[15]	2	Lung cancer	Atezolizumab vs docetaxel	62(36-84)	SP142	VENTANA	51	144	41	143	14.8

* The definition of PD-L1 negativity is that PD-L1 stained cell accounted for less than 1% of tumor cells, immune cells, or both assessed by immunohistochemistry assay.

The total number of patients in experiment group and control group is 298.

NR, not reported



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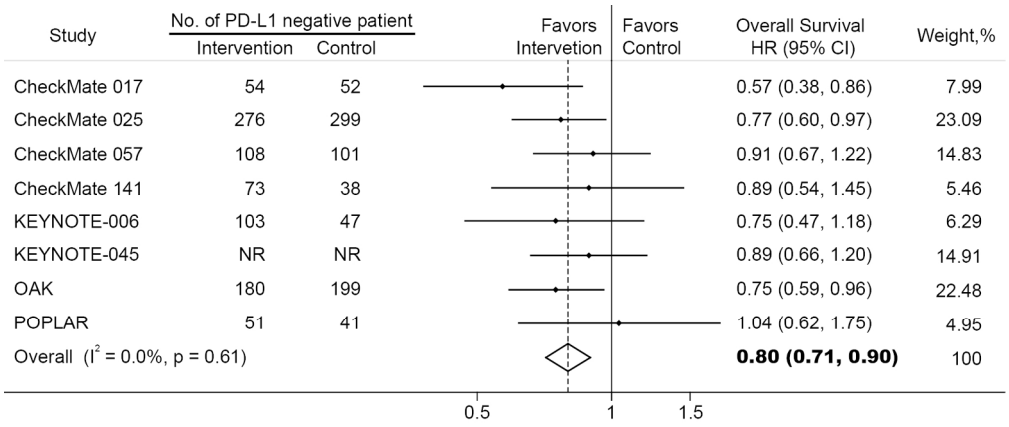

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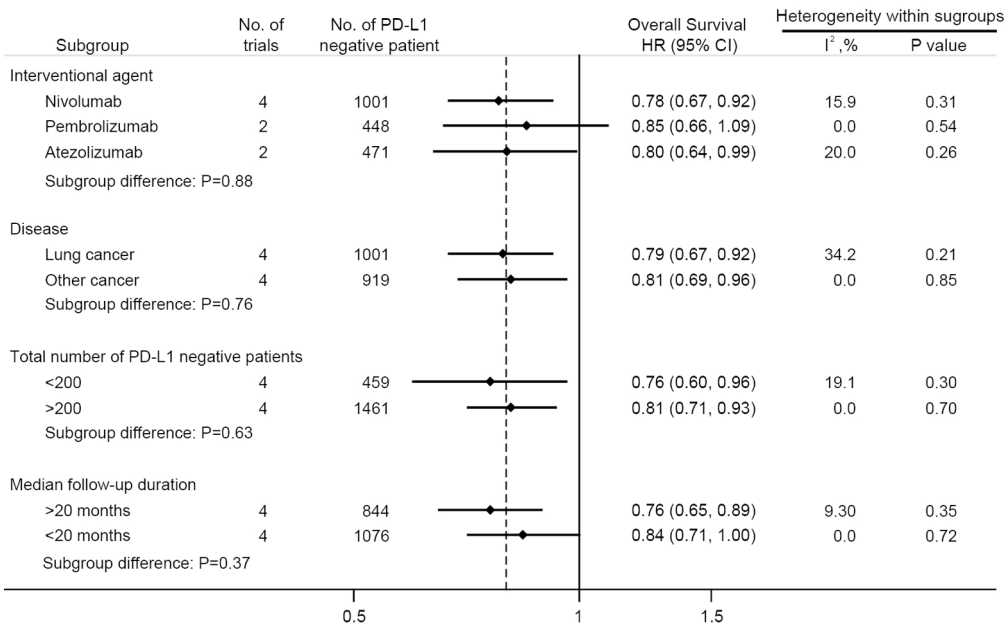

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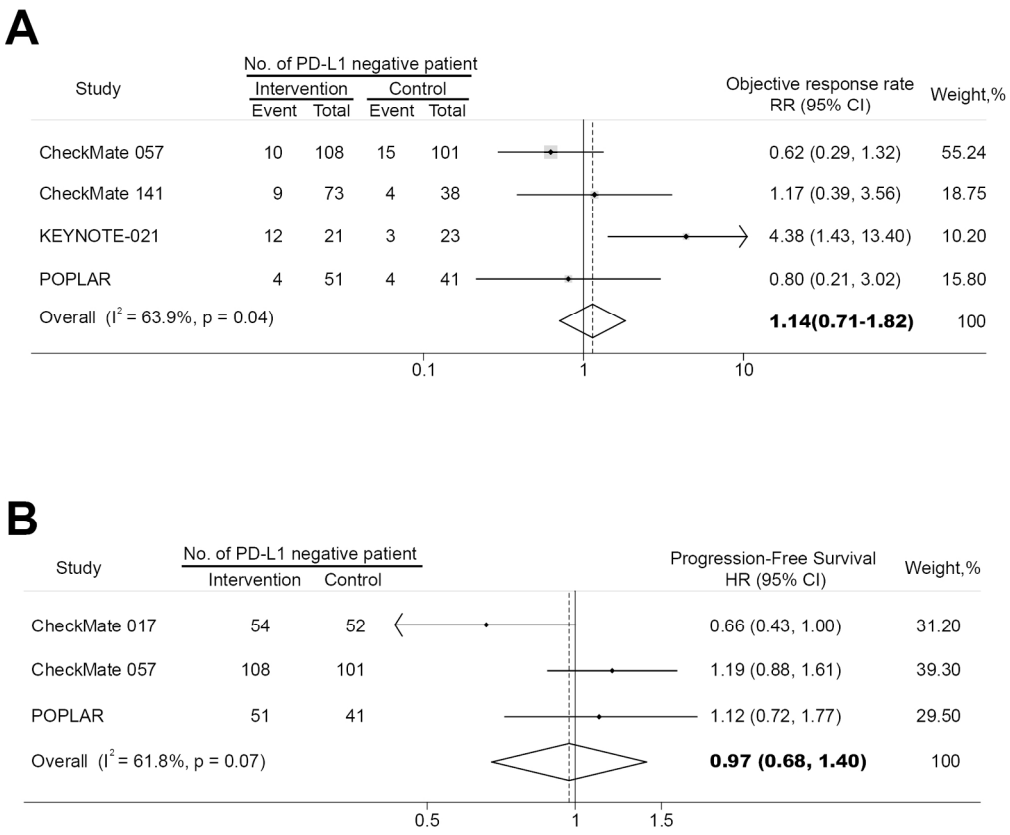

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Search strategies for PubMed and EMBASE

PubMed: 2,865 results

(("Nivolumab"[Substance] OR "Nivolumab "[All Fields] OR "Opdivo"[All Fields] OR "ONO-4538"[All Fields] OR "MDX-1106"[All Fields] OR "BMS-936558"[All Fields] OR "Nivo"[All Fields]) OR ("Pembrolizumab"[Substance] OR "Pembrolizumab "[All Fields] OR "lambrolizumab"[All Fields] OR "keytruda"[All Fields] OR "SCH 900475"[All Fields]OR "MK-3475"[All Fields])) OR ("Atezolizumab"[Substance] OR "Atezolizumab "[All Fields] OR "MSB0010718C"[All Fields] OR "Tecentriq"[All Fields] OR "RO5541267"[All Fields] OR "RG7446"[All Fields] OR "MPDL3280A"[All Fields])) OR ("Durvalumab"[Substance] OR " Durvalumab "[All Fields] OR "MEDI-4736"[All Fields] OR "MEDI4736"[All Fields])) OR ("checkpoint inhibitor"[All Field] OR " PD-1 "[All Fields] OR "PD-L1"[All Fields]))AND ("carcinoma"[Mesh] OR ("cancer"[All Fields] OR "tumor"[Title/Abstract]))) AND ((("random allocation"[MeSH Terms] OR "randomized"[All Fields]) AND ("clinical trials as topic"[MeSH Terms] OR "trial"[All Fields])) OR randomized controlled trial[All Field]) AND ("humans"[MeSH Terms])

EMBASE: 3,169 results

No. 1: ' Nivolumab ' OR ' Opdivo ' OR ' ONO-4538 ' OR ' MDX-1106 ' OR ' BMS-936558 ' OR ' Nivo ' OR ' Pembrolizumab ' OR ' lambrolizumab ' OR ' keytruda ' OR ' SCH 900475 ' OR ' MK-3475' OR ' Atezolizumab ' OR ' MSB0010718C ' OR ' Tecentriq ' OR ' RO5541267 ' OR ' RG7446 ' OR ' MPDL3280A ' OR ' Durvalumab ' OR ' MEDI-4736 ' OR ' MEDI4736 ' OR ' checkpoint inhibitor ' OR ' PD-1 ' OR ' PD-L1 ' AND (' carcinoma ' OR ' cancer' OR ' tumor '): 6,372 results

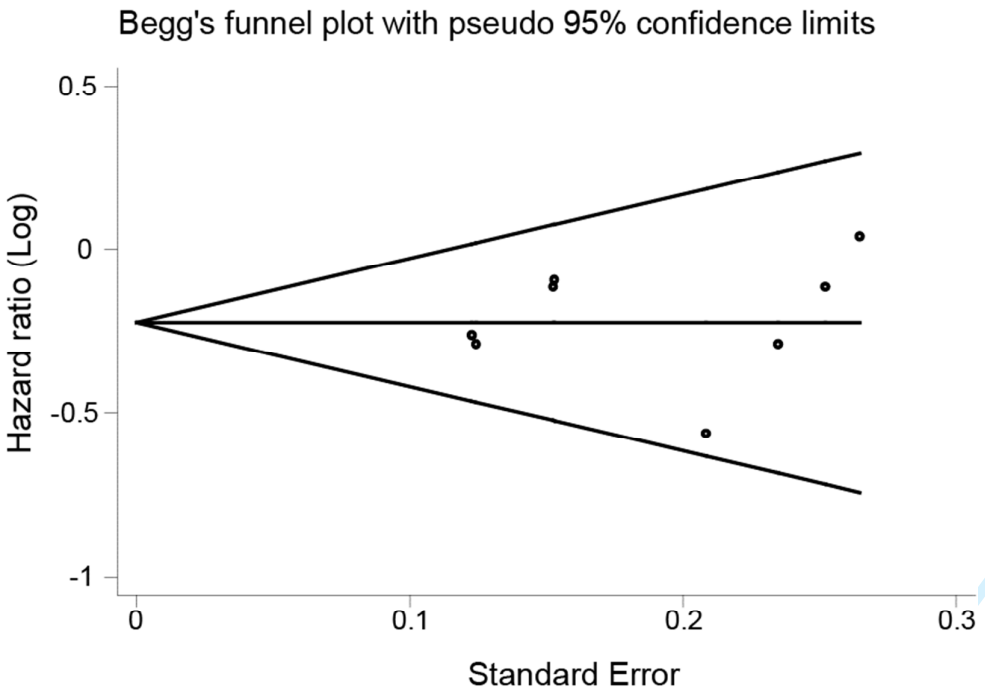
No. 2: AND 'human'/de: 4,984 results

No. 3: AND 'clinical trial'/de: 4,169 results

Cochrane database: 427 results

#1: ' Nivolumab ' OR ' Opdivo ' OR ' ONO-4538 ' OR ' MDX-1106 ' OR ' BMS-936558 ' OR ' Nivo ' OR ' Pembrolizumab ' OR ' lambrolizumab ' OR ' keytruda ' OR ' SCH 900475 ' OR ' MK-3475' OR ' Atezolizumab ' OR ' MSB0010718C ' OR ' Tecentriq ' OR ' RO5541267 ' OR ' RG7446 ' OR ' MPDL3280A ' OR ' Durvalumab ' OR ' MEDI-4736 ' OR ' MEDI4736 ' OR ' checkpoint inhibitor ' OR ' PD-1 ' OR ' PD-L1 ' AND (' carcinoma ' OR ' cancer' OR ' tumor '): 695 results

#2: trials: 427 results

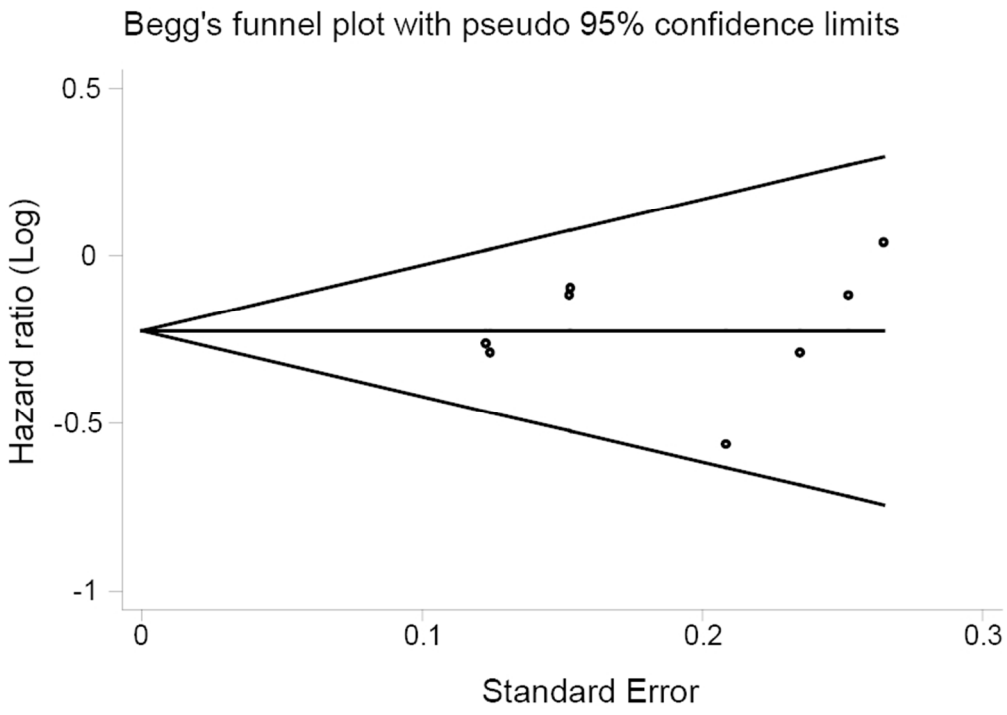


Supplemental Figure 1. Begg's funnel plot for publication bias test. Each circle represents a separate study for indicated association. Horizontal line, mean effect size.

Supplemental Table 1. Risk of bias of the included trials

Study	Randomization	Allocation concealment	Blinding of participants and staff	Blinding of outcome assessors	Incomplete outcome data*	Selective outcome reporting*	Other sources of bias
CheckMate 017	Low	Low	High	Low	High	Low	Low
CheckMate 025	Low	Low	High	Low	High	High	Low
CheckMate 057	Low	Low	High	Low	Low	Low	Low
CheckMate 141	Low	Low	High	Low	Low	Low	Low
KEYNOTE-006	Low	Low	High	Low	High	High	Low
KEYNOTE-021	Low	Low	High	Low	High	High	Low
KEYNOTE-045	Low	Unclear	High	Low	High	High	Low
OAK	Low	Low	High	Low	High	High	Low
POPLAR	Low	Low	High	Low	Low	Low	Low

*applies to PD-L1 negative patients.



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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3-4
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	6
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	7
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	7
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	7-8
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	7
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	8
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	8-9
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	9
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	9
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	9

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	9
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	9
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	10
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	10
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	10-11
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	10
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	11
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	11
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	11
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	12
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	14-15
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	16
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	17

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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