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Systematic or Meta-analysis Studies

PD-L1 expression and clinical outcomes in patients with advanced urothelial carcinoma treated with checkpoint inhibitors: A meta-analysis

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ABSTRACT

Context: Five checkpoint inhibitors have been approved as 1st line (cisplatin-ineligible) or 2nd line therapies for patients with metastatic urothelial carcinoma of the bladder. As only about 30% of patients respond, the need for a biomarker for patient selection exists.

Objective: To determine if PD-L1 expression is a prognostic factor of objective response rate (ORR) and overall survival (OS) in patients with urothelial carcinoma being treated with checkpoint inhibitors.

Evidence acquisition: A search of PubMed and major conference proceedings identified trials of PD-L1 inhibitors as first- or second-line therapies for metastatic bladder cancer. Odds ratios (OR) for ORR and OS compared PD-L1 positive and PD-L1 negative patients. Data were weighted and pooled in a meta-analysis, and subgroup analyses compared PD-L1 status cut-offs.

Evidence synthesis: Ten studies comprising 2755 patients were identified, of which 2030 patients (74%) received immune checkpoint inhibitors. Eight studies were eligible for ORR analysis (1530 patients) and five studies for OS (829 patients). PD-L1 patients had a significantly higher ORR than PD-L1 negative patients (1.82, 95%CI 1.18–2.77; p = 0.007). Weighted mean OS was 11.5 months (range 8.7–15.9 months). PD-L1 status was not prognostic for 12 month OS (OR = 0.81, 95%CI 0.47–1.40; p = 0.45).

Conclusion: In patients treated with PD-L1 inhibitors for metastatic urothelial carcinoma, PD-L1 status is prognostic for ORR but not OS. Our findings warrant additional investigation.

Patient summary: Five immunotherapy drugs are approved for bladder cancer therapy. PD-L1 expression predicts higher ORR but not OS. More data is needed to identify the patient population most benefitted by immunotherapy.

Introduction

Bladder cancer is one of the most lethal malignancies worldwide [1]. Patients with advanced disease or those who relapse after radical cystectomy have very poor outcomes. For two decades, cisplatin-based combination therapy has been the standard of care for first-line treatment of metastatic urothelial cancers [1,2]. While these combinations have high response rates, almost all patients will ultimately progress and die from their disease. Several cytotoxic agents have modest clinical activity in the second-line setting (vinflunine, paclitaxel, docetaxel, pemetrexed) with overall response rates (ORR) ranging from 8 to 30% and overall survival (OS) between 7 and 10 months; however, none of these agents have been approved for use in North America [3–5]. Advanced age, poor performance status, comorbidities and rapidly progressive disease has made accrual into second-line chemotherapy trials difficult.

In recent years immunotherapy has become an increasingly attractive strategy in many solid tumors. Multiple new monoclonal antibodies targeting the CTLA-4 and PD-1/PD-L1 immune checkpoint pathways have been granted accelerated approvals for cancers of the lymphoma type [6]. In lung cancer, pembrolizumab has demonstrated significant improvement in survival for patients who express PD-L1 in ≥50% of cells [7]. High levels of PD-L1 expression have been shown to correlate with more advanced and aggressive bladder cancer and poorer survival

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outcomes [8–11]. Additionally, PD-L1 expression is also associated with resistance to BCG therapy which is thought to be related to immune suppression [9–11]. Checkpoint inhibitors have demonstrated a higher benefit in cancer with heavy mutational burden, such as bladder cancer, due to greater T cell mediated antitumor immune response elicited by these tumors [10,11].

Atezolizumab was the first PD-L1 inhibitor to be granted accelerated approval by the US Food and Drug Administration (FDA) in May 2016 on the basis of a phase II trial documenting improved response rates compared to historical controls [12]. Since then, nivolumab, pembrozilumab, durvalumab and avelumab have all shown activity in bladder cancer, and have received accelerated approval by the FDA [13–20]. To date, two randomized phase III studies (pembrozilumab and atezolizumab) with an active comparator chemotherapy arm (docetaxel, paclitaxel or vinflunine) have been published [16,21]. While superiority was confirmed with pembrolizumab showing an improvement in median survival from 7.4 months to 10.3 months (HR = 0.73, 95%CI 0.59–0.91; p = 0.002), the atezolizumab trial did not reach its primary endpoint, reporting a median OS (mOS) of 11.1 months versus 10.6 months in the chemotherapy arm (HR = 0.87, 95%CI 0.63–1.21; p = 0.41). Adding to the complexity, all agents tested have evaluated companion diagnostics focusing on PD-L1 expression using different platforms and cut-offs to define PD-L1 positivity.

Immuno-oncology agents although effective in bladder cancer, only a small proportion of patient will find benefit, and high proportion will be exposed to potentially significant side effects with no improvement neither in quality of life or survival; no biomarker at this point has been associated with response. Here we report a meta-analysis evaluating the association between PD-L1 expression, treatment with immune checkpoint inhibitors, and patient outcomes, in patients with metastatic urothelial carcinoma of the bladder. We hypothesized that PD-L1 positive patients would have improved outcomes compared to PD-L1 negative patients.

Evidence acquisition

Search strategy

An electronic search of Medline (host: Pubmed), clinicaltrials.gov and major conference proceedings from 1946 to November 2017 was undertaken. The search terms included urothelial cancer, bladder cancer, atezolizumab, avelumab, durvalumab, nivolumab and pembrolizumab. This analysis was conducted in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines [22].

Study eligibility

Inclusion criteria were studies of patients with urothelial carcinomas treated with a PD-1 or PD-L1 checkpoint inhibitor that reported PD-L1 expression status, with availability of objective response rate (ORR), overall survival (OS) or progression free survival (PFS) data. There was no restriction based on study methodology. Exclusion criteria were studies not reporting any of the above-mentioned endpoints. For the primary analysis, studies not reporting ORR by PD-L1 expression were not included.

Two analyses were performed: The main analysis was undertaken in studies reporting response rates for each treatment intervention on PD-L1 negative and PD-L1 positive populations. The secondary analysis was undertaken in studies for which the median OS and PFS were reported by PD-L1 status.

Statistical analysis

Data were extracted using a pre-designed electronic form. The following information was collected by two reviewers (KG and FEVB): tested drug, dose, study phase, sample size, line of therapy, median follow up (months), control arm, PD-L1 assay, PD-L1 cut-off used to define positive status, proportion of patients determined to be PD-L1 positive, primary outcome, ORR, OS, PFS, response rate on PD-L1 positive and negative and median time to response.

Data are presented descriptively as weighted means, medians, or proportions. ORR for PD-L1 positive and PD-L1 negative populations were weighted and pooled using the generic inverse variance and random effect model. Analyses were performed using RevMan 5.3 analysis software (Cochrane Collaboration, Copenhagen, Denmark). Statistical heterogeneity was assessed using the Cochran's Q and I^2 statistics. Meta-regression was performed to explore the influence of PD-L1 expression level on OS and ORR. Meta-regression was performed using SPSS version 21 (IBM Corp, Armonk, NY, USA) using the weighted least squares (mixed effect) function. All statistical tests were two sided, and statistical significance was defined as $p \leq 0.05$. No correction was applied for multiple statistical testing.

Evidence synthesis

Patient and treatments

Ten studies comprising 2766 patients were identified, of which 2030 patients (73%) received immune checkpoint inhibitors. All studies which reported ORR by PD-L1 expression were included in the analysis; for trials that not include this analysis, the sponsor of the study was contacted to retrieve more information. Characteristics of included studies are reported in Tables 1 and 2. Balar and colleagues was the only study designed to exclusively enroll patients in the first-line setting for platinum ineligible patients [15]. Eight out of ten studies were single arm trials, and two studies were randomized trials with standard of care control arm. Two studies of the ten initially identified were not included for analysis as ORR and OS by PD-L1 expression were not reported [18,21].

Eight studies reporting outcomes on 1468 patients were eligible for ORR analysis and 5 studies comprising 691 patients were evaluable for OS analysis. The median follow up was 11.56 months (range 4.3–17.2 months). The median time to response was 1.88 months (range 1.5–2.1 months). Forty-two percent (1165/2766) of treated patients were PD-L1 positive. Of the patients eligible for ORR analysis, forty-five percent (659/1468) were PD-L1 positive. Of the patients evaluable for OS analysis, forty-two percent (292/691) were PD-L1 positive.

Response rates

Eight studies reported ORR according to PD-L1 expression. Weighted mean response rates in the PD-L1 positive and PD-L1 negative group were 31% (range 18–50%) and 17% (range 0–26%) respectively. There was an association between PD-L1 status and ORR in favour of PD-L1 positive patients (OR = 1.82, 95%CI 1.18–2.77; p = 0.007). When all 8 studies were included in the model, there was significant heterogeneity (I^2 = 50%, p = 0.05). Although heterogeneity was driven independently by the two studies with the smallest sample size and by the study with the largest sample size, statistical significance of the difference was always maintained [13,16,17] (Fig. 1). Importantly, when the studies were subdivided into groups based on their drug mechanism of action (PD-1 Inhibitors and PD-L1 Inhibitors), there was no difference reported between the subgroups (X^2 = 1.94, p = 0.16), while the overall effect in each subgroup was maintained (Fig. 2). Meta-regression showed that there was no difference in the prognostic effect for ORR of PD-L1 status based on the cut-off used (Beta coefficient −0.036, p = 0.93) (See Table 3).

Table 1
Study characteristics.

Study Name	Study Type	Line	Drug Name	Doses	Control	Sample Size	Median Follow up (months)	Male/Female (%)
Apolo 2016	Phase Ib	Second	Avelumab	10 mg/kg Q2 wks	None	44	16.5	68/32
Balar 2017	Phase II	First/Cis Ineligible	Atezolizumab	1200 mg Q3 wks	None	119	11.7	81/19
Balar 2017	Phase II	First/Cis Ineligible	Pembrolizumab	200 mg Q3 wks	None	370	9.5	77/23
Bellmunt 2017	Phase III	Second	Pembrolizumab	200 mg Q3 wks	Docetaxel/Paclitaxel/vinflunine	542 (270 Pembrozilumab)	14.1	74/26
Massard 2016	Phase I/II	First/Second	Durvalumab	10 mg/kg Q2 wks	None	61	4.3	69/31
Plimack 2014	Phase Ib	Second	Pembrolizumab	10 mg/kg Q2 wks	None	33	13	70/30
Powles 2017	Phase III	Second	Atezolizumab	1200 mg Q3 wks	Docetaxel/Paclitaxel/vinflunine	931 (467 Atezolizumab)	17.3	76/24
Rosenberg 2016	Phase II	Second/Cis Ineligible	Atezolizumab	1200 mg Q3 wks	None	310	17.2	78/22
Sharma 2016	Phase I/II	Second	Nivolumab	3 mg/kg Q 2 wks	None	86	15.2	69/31
Sharma 2017	Phase II	Second	Nivolumab	3 mg/kg Q 2 wks	None	270	6	78/22

Overall and progression free survival

OS and PFS were reported in 5 studies. Weighted mean OS was 11.5 months (range 8.7–15.9 months), while weighted mean PFS was 2.4 months (range 2.0–2.8 months). Five studies provided 12 month survival data stratified based on PD-L1 status. There was no statistical difference in OS at 12 months based on PD-L1 status (OR = 0.81, 95%CI 0.47–1.40; p = 0.45); heterogeneity was non-significant (Fig. 3). Meta-regression showed that there was no difference in the prognostic effect for OS of PD-L1 status based on the cut-off used (Beta coefficient = −0.09; p = 0.53).

Discussion

For over two decades, cisplatin-based cytotoxic therapy has been the only first-line therapy proven to improve survival of patients with advanced urothelial carcinoma. Long term follow-up of the pivotal gemcitabine/cisplatin (CG) and methotrexate/vinblastine/doxorubicin/cisplatin (MVAC) clinical trial reported median OS of 14.0 months and 15.2 months, respectively, corresponding to a 5-year survival of 13% for CG and 15% for MVAC [1,2]. While response rates in the first-line setting are high (44–56%), duration of response is short and most patients will eventually progress [1]. Of patients who progress on first-line therapy, only a small fraction receive second-line therapy, usually because patients are not fit and because toxicity associated with second-line therapies preclude patients from further treatment. With the largest phase III trial, vinflunine is the best studied cytotoxic agent in the second-line setting documenting an ORR of 9% with a non-significant 2 month improvement in OS compared to best supportive care [3]. Docetaxel, paclitaxel, nab-paclitaxel and pemetrexed have documented ORR of 8–28% and OS of 7–9 months in the second-line setting [4,5,23,24].

The development of immune checkpoint inhibitors herald a new era in the treatment of urothelial cancers [25]. Five checkpoint inhibitors (Atezolizumab, Nivolumab, Durvalumab, Avelumab and Pembrolizumab) have been granted accelerated and conditional approval by the FDA based on phase II studies, regardless of PD-L1 expression. The recent publication of the phase III randomized study of the PD-1 inhibitor pembrolizumab versus standard chemotherapy (vinflunine/paclitaxel/docetaxel) has reported an improvement of overall survival closer to 3 months in favour of pembrolizumab (mOS 10.3 vs 7.4 months; HR = 0.73, 95% CI 0.59–0.91, p = 0.002) in the second-line setting for patients with metastatic urothelial carcinomas [16]; however, atezolizumab, a PD-L1 inhibitor, failed to report an advantage over chemotherapy for the primary endpoint of overall survival in patients with PD-L1 expression in ≥5% of tumour-infiltrating immune cells. mOS for atezolizumab was 11.1 months and 10.6 months for chemotherapy (HR = 0.87, 95%CI 0.63–1.21, p = 0.41) [21].

Given the multitude of studies that have already been reported, and dozens that are currently recruiting, there are several questions relevant to clinical practice. First, is there a preferred agent in the second-line setting? Secondly, can a biomarker (PD-L1 expression) inform the proper selection of patients who may have an increased chance of response? A recent analysis has reported outcomes associated with the use of chemotherapy, anti-VEGF inhibitors and immunotherapy demonstrating gains in OS with newer therapeutic approaches [26]. Since pembrolizumab is the only agent for which the promising benefit observed in phase II trials has been confirmed in a phase III trial, this agent should be the preferred agent to use in the clinic. Additionally, in the first-line setting, atezolizumab [15] and pembrolizumab [14] have been granted accelerated approval in first-line cisplatin-ineligible patients, pending results from phase III trials. Overall ORR for checkpoint inhibitors are similar and consistent with ORR reported in other indications (i.e. nivolumab in RCC, 25%, pembrozilumab in NSCLC, 18%, and nivolumab in head and neck malignancies, 13% [27–29]).

A critical unmet need is the development of predictive biomarkers,

Table 2
Study outcomes, PD-L1 assays and PD-L1 cut-off.

Study Name	PD-L1 Cut-off	PD-L1 Assay	Primary Outcome	ORR (%)	OS (months) (95% CI)	PFS (months) (95% CI)
Apolo 2016	> 1%	Dako 73-10	ORR	18.20	13.7 (8.5-NR)	2.6 (1.4–4.0)
Balar 2017	IC0 (< 1%); IC1 (1 < to < 5); IC2/3 (> 5%)	Ventana SP142	ORR	23.00	15.9 (10.4-NR)	2.7 (2.1–4.2)
Balar 2017 (Pembrolizumab)	CPS ≥10%	DAKO 22C3	ORR	29.00	NR	NR
Bellmunt 2017	CPS ≥10%	DAKO 22C3	OS and PFS	21.00	10.3 (8.0–11.8)	2.1 (2.0–2.2)
Massard 2016	> 25%	Ventana SP263	Safety	31.00	NR	NR
Plimack 2014	> 1%	DAKO K8012; DAKO 22C3	ORR	26.00	13.0 (5.0–20.0)	2.0 (2.0–4.0)
Powles 2017	IC0 (< 1%); IC1 (1 < to < 5); IC2/3 (> 5%)	Ventana SP142	OS	13.00	8.6 (7.8–9.6)	NR
Rosenberg 2016	IC0 (< 1%) IC1 (1 < to < 5); IC2/3 (> 5%)	Ventana SP142	ORR	15.00	11.4 (9.0-NR)	2.1 (2.1–2.1)
Sharma 2016	> 1%	DAKO 28-8 PharmaDx Kit	ORR	24.40	9.7 (7.3–16.2)	2.8 (1.5–5.9)
Sharma 2017	> 1%	DAKO 28-8 PharmaDx Kit	ORR	19.60	8.7 (6.05-NR)	2.0 (1.87–2.63)

ORR: objective response rate; NR: not reported; CI: confidence interval; IC: immune cell; OS: overall survival; PFS: progression-free survival.

which may allow for identification of patients that are likely to respond to these agents and therefore reduce exposure and risk of toxicities for those patients with little potential for benefit of response. Given the high-cost of these agents, in the era of value-based cancer care this becomes even more relevant. Based on our analysis, there does seem to be a statistically significant difference in the ORR between PD-L1 positive and PD-L1 negative patients. However, it should be noted that 17% of patients who were PD-L1 negative did see a response, and this should be considered when a treatment is chosen. Unfortunately, data for OS survival was limited and we were not able to confirm a correlation. Our results are further complicated by the fact that each agent is associated with a different PD-L1 companion assay with differing cut-offs for PD-L1 positivity. This lack of standardization for what constitutes positive or negative PD-L1 expression and the variability of expression amongst tumor samples, make analyses even more difficult. However, it should be noted that our meta-regression analyses did not find any difference in the prognostic effect of PD-L1 status for ORR or OS based on the cut-off that was used. A report evaluating 160 specimens from patients with urothelial carcinoma found that PD-L1 positivity (defined as PD-L1 > 5%) did not correlate with OS, instead PD-L1 staining in tumor infiltrating mononuclear cell was associated with longer survival [8]. These findings raise the question of whether PD-L1 testing should be conducted on both tumor cells and immune cells. Studies have as such, started incorporating composite scores of immune and tumor cell PD-L1 expression. Moreover, PD-L1 expression appears to be a continuum, which makes it difficult to define a cut-off for a positive or negative test result.

Although data needs more maturity, and availability of this data should be made public for a more comprehensive analysis (atezolizumab RCT data not reported by PD-L1 expression), there are already strong signals that patients should be selected by PD-L1 expression before recommending immune checkpoint inhibitors. On June 20, 2018 the FDA issued an alert about the decreased survival of patients with low PD-L1 expression tumours seen in first-line randomized trials involving atezolizumab or pembrolizumab monotherapy arms. In our analysis, we report that PD-L1 was a prognostic factor independently of the line of treatment; reinforcing the relevance of this analysis for patient selection. On August 20, 2018, the FDA approved the companion diagnostic tests for atezolizumab and pembrolizumab to determine PD-L1 expression before these treatments can be offered to patients.

Currently, four PD-L1 assays are in use in clinical trials (Table 2). These have been developed in conjunction with different immune checkpoint inhibitors. Gaule and colleagues presented a quantitative comparison of the 4 assays. Although initial work had suggested heterogeneity between the assays especially amongst immune cell PD-L1 expression, Gaule and colleagues demonstrated high levels of concordance (R^2 = 0.76–0.99) when using isogenic cell lines [30,31]. They concluded that previous heterogeneity associated with the 4 assays is antibody independent and likely attributable to tumor heterogeneity. Finally there is evidence that PD-L1 expression is heterogeneous not only within tumors, but also between primary tumors and metastasis and evolves over time [32,33]. In the field of lung cancer, PD-L1 expression is used to select patients with higher chances of response. Solely based on this biomarker, pembrolizumab has been approved in

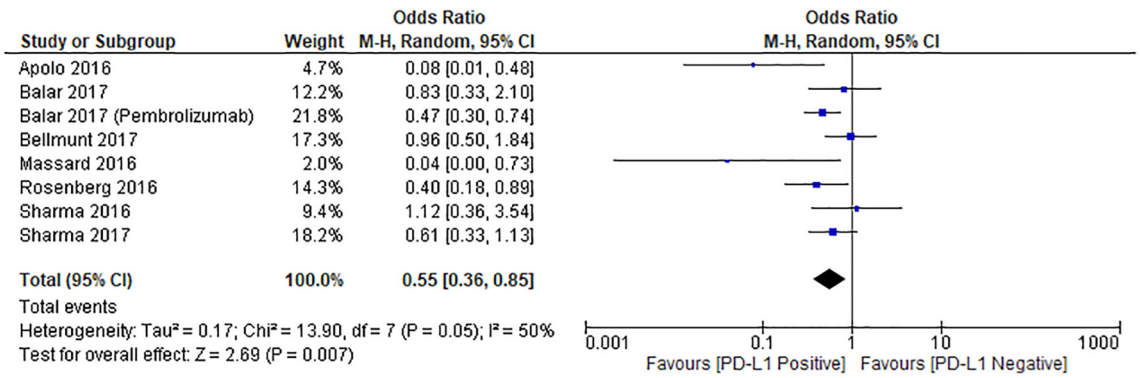

Fig. 1. Forest Plot of Comparison of ORR based on PD-L1 status. Odds ratio for each study is presented, and horizontal lines indicate the 95% Confidence Interval, CI.

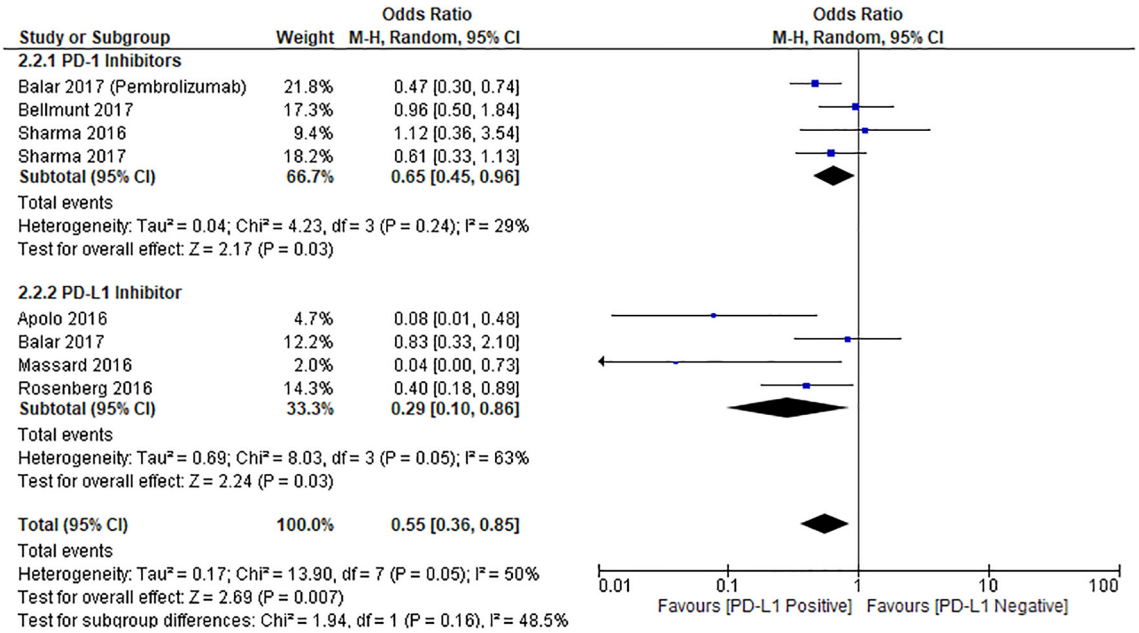

Fig. 2. Forest Plot of Comparison of ORR based on PD-L1 status in two subgroups (2.2.1 and 2.2.2) of PD-1 and PD-L1 Inhibitors. Odds ratio for each study is presented, and horizontal lines indicate the 95% Confidence interval, CI.

Table 3
Meta-regression and prognostic effect of ORR and OS by PD-L1 status and PD-L1 cut-off.

Outcome	Beta coefficient	P-Value
ORR	−0.036	0.93
OS	−0.09	0.53

ORR: objective response rate; OS: overall survival.

the first-line for patients with PD-L1 ≥50%, or PD-L1 ≥1% for second-line [7,34]. Therefore, in order to elucidate how different PD-L1 platforms perform, other studies have investigated their correlation. The first study of concordance was the Blueprint study where 39 cases were analyzed utilizing the 28-8 DAKO, 22c3 DAKO, SP142 Ventana and SPS253 Ventana platforms, followed by a similar study including 90 different cases utilizing the 28-8, 22c3, SP1442 abd tge EL2N Leica platforms [35,36]. Consistently the 28-8 and 22-3 platforms showed good correlation, while the SP142 platform was considered an outlier that detected significantly less PD-L1 expression in tumor cells and immune cells. These results support our approach to combine ORR results by PD-L1 expression in our analysis, and support the use of PD-L1 assessment for patient selection independently of the used platform. Additionally, when the studies involving the SP142 platform were removed from analysis, the results were consistent with what is being reported for all trials.

Our study has several limitations. Data was extracted retrospectively. Individual patient data was not available for analysis and the analysis was therefore conducted at the trial level as opposed to the patient level. Further, the majority of studies were phase I/II trials which add heterogeneity to our analysis. For the phase III trials currently reported, data was only available for the pembrolizumab study. Data presented in these trials are still immature with short median follow-up and additional follow-up is necessary. This is especially important for the analysis of OS, which was limited with data available only at 1 year. It is possible that with extended follow-up an effect of PD-L1 expression on OS would be observed.

Conclusion

PD-L1 expression is associated with improved response rate with anti-PD-1 and anti-PD-L1 checkpoint inhibitors. However, because of the limited available data on OS, the results of this meta-analysis do not show any association between PD-L1 expression and OS. Given that the activity of checkpoint inhibitors is limited to a specific population not yet identified, the development of biomarkers should become a priority in order to identify those patients with higher chances of response, while limiting exposure and potential immune-related toxicity for patients unlikely to respond, as well as to address the financial toxicity associated with this type of intervention.

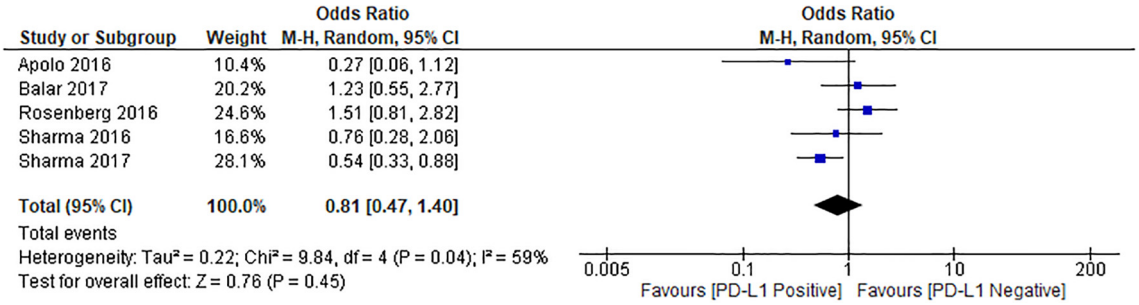

Fig. 3. Forest Plot of Comparison of OS based on PD-L1 status at 12 months. Odds ratio for each study is presented, and horizontal lines indicate the 95% Confidence Interval, CI.

Declaration of Competing Interest

None.

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