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Expanding the PD-L1 Paradigm: A Comprehensive Systematic Review and Meta-Analysis of Scoring Systems and Additional Biomarkers Influencing Immune Checkpoint Inhibitor Outcomes in Breast Cancer

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Abstract

Objectives: The study aimed to conduct an in-depth analysis of the influence of PD-L1 status and expression levels and other variables on the effectiveness of immune checkpoint inhibitors (ICIs) in treating breast cancer.

Methods: A total of 19 articles, involving 16 trials and 7899 patients, were included in the analysis. The outcomes of interest were odds-ratio (OR) for pathological complete response (pCR) in early breast cancer, and hazard ratio (HR) for progression-free survival (PFS) and overall survival (OS) in advanced breast cancer.

Results: In early breast cancer, individuals with PD-L1-positive tumors were more likely to benefit from ICIs than those with PD-L1-negative tumors. Furthermore, patients with PD-L1 positivity in immune cells (IC) had superior outcomes compared to those scoring positively on combined positive score (CPS), with ORs for ICIs benefit being 2.28 for IC-positive patients vs 1.78 for CPS-positive patients. Regarding the impact of breast cancer subtypes on the efficacy of ICIs, our findings indicated that triple-negative breast cancer (TNBC) exhibits the greatest therapeutic response with OR of 1.93, followed by the hormone receptor-positive (HoR+) / human epidermal growth factor receptor 2-negative (HER2−), while the HER2+ was the worst. Additionally, age was identified as a key predictive factor in responding to ICIs. In advanced breast cancer, there was an upward trend in CPS values associated with enhanced ICIs responsiveness, with the predictive value increasing from 12% at a CPS threshold of 10 to 13.6% at 20.

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Conclusion: The study concluded that the PD-L1 expression scoring system effectively discriminates between patients with breast cancer in terms of the degree of benefit they may attain from ICIs. Patients with little or no PD-L1 expression experienced a diminished therapeutic benefit from ICIs.

Keywords
breast cancer, immune checkpoint inhibitor, PD-L1, meta-analysis, biomarkers

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Introduction

Immune checkpoints are essential for immune function, but tumors exploit them to avoid immune attacks.[1] Immune checkpoint inhibitors (ICIs) have shown promise in treating various cancers,[2] with US Food and Drug Administration (FDA) approval for drugs like ipilimumab, pembrolizumab, and others.[3] Despite breast cancer being a prevalent and often lethal malignancy, ICIs have historically encountered limited success, with the disease traditionally regarded as exhibiting a state of immunological quiescence.[4,5] However, recent studies indicated that ICIs can be effective in specific patient groups,[6-8] highlighting the need for biomarkers to identify those most likely to benefit.[9]

The role of PD-L1 expression in selecting ICIs treatment for breast cancer is contentious. Some trials, like KEYNOTE-012, found higher rate of response when pembrolizumab was used in the PD-L1-positive patients (positive was defined as PD-L1 expression in the stroma or in $\geq 1\%$ of tumor cells),[10] while others, such as IMpassion131,[11] did not. The KEYNOTE-355 trial found significant benefits in patients with a combined positive score (CPS) of 10 or more, but not at 20.[12] Trials like IMpassion031 and KEYNOTE-522 suggested that PD-L1 status may not be crucial for patients with early triple-negative breast cancer (eTNBC).[13,14] Finally, the significance of PD-L1 status in different subtypes of breast cancer was inconsistent. The predictive value of PD-L1 varies across breast cancer subtypes, with some studies showing no benefit from ICIs regardless of PD-L1 status.[15,16]

The predictive power of PD-L1 and the optimal CPS threshold for ICIs treatment in breast cancer remain uncertain. Therefore, we conducted a systematic review and meta-analysis of randomized clinical trials (RCTs) to assess the impact of ICIs on early (eBC) and advanced (aBC) breast cancer outcomes, including pathological complete response (pCR), event-free survival (EFS), overall survival (OS), and progression-free survival (PFS). We evaluated the predictive value of PD-L1 status and other variables to provide a broader reference for patient selection, avoiding the pitfalls of single-variable subgroup analyses. Our goal was to determine the predictive value of PD-L1 and other baseline variables in RCTs comparing ICIs with or without chemotherapy in patients with breast cancer.

Methods

This systematic review and meta-analysis aimed to determine the optimal PD-L1 scoring system and cut-point in patients with breast cancer treated with anti-PD-1/PD-L1 ICIs, with or without chemotherapy. The study was registered in the international prospective register of systematic reviews (CRD42024587738) and conducted following the PRISMA guidelines.[17]

Eligibility Criteria and Data Items

Eligible studies for this review included RCTs of ICIs with or without chemotherapy vs chemotherapy alone in eBC or aBC, with available data on pCR, EFS, PFS, OS and their respective statistics.

Exclusions applied to non-RCTs, studies lacking data, preclinical studies, case reports, reviews, articles before January 1, 2000 and non-English articles.

Data extracted was presented in a structured form (Supplemental Table S1, S2), including trial identifiers, author details, publication year, treatment regimens, tumor characteristics, PD-L1 assessment, endpoints and stratification factors. When possible, outcomes were pooled for subgroups defined by PD-L1 status, tumor subtype, size, Eastern Cooperative Oncology Group performance status (ECOG) performance status, lymph node status, metastatic sites, age, liver metastases, prior chemotherapy, and chemotherapy regimen.

Information Sources and Search Strategy

A thorough literature search was conducted across PubMed, Embase, Scopus, Web of Science and The Cochrane Library from January 1, 2000, to August 12, 2023, with search strategy details available in the supplement.

Selection Process

Two independent reviewers (SM and YW) carefully assessed all search results, with disagreements resolved by a third reviewer (WX). After applying the selection criteria, duplicate studies were removed using Endnote X9 software, either automatically or manually.

Study Risk of Bias Assessment and Reporting Bias Assessment

The selection bias, performance bias, detection bias, attrition bias, reporting bias were assessed by the Cochrane Collaboration' s tool.[18] Risk of bias was independently evaluated by two reviewers (SM and YW), with any disagreements resolved by a third (WX). The results were summarized visually, and funnel plots were utilized to assess publication bias.

Effect Measures and Synthesis Methods

Data and outcomes from each study were sourced from published articles and conference abstracts, comparing ICIs alone or with chemotherapy to placebo or chemotherapy alone. We assessed overall effects using odds ratios (OR) with 95% confidence intervals (CI) for pCR and hazard ratios (HR) with 95% CI EFS, PFS, OS. These were pooled in a meta-analysis with Review Manager 5.4.1, and forest plots were created for visual analysis. An OR > 1 or HR < 1 indicates better outcomes with ICIs. Predictive value was calculated as the difference in mean ORs or HRs, following a published formula (predictive value=$(HR \text{ or } OR)_A/(HR \text{ or } OR)_B$ - 1).[19] Heterogeneity was assessed by I^2 and statistical tests, with random-effects modeling for significant heterogeneity (Cochran Q $P < .10$ or $I^2 > 50\%$) and fixed-effect otherwise. Subgroup analyses also used random effects due to low statistical power. The significance level was set at $\alpha = 0.05$. Leave-one-out sensitivity analysis (excluding one study at a time) was conducted to access the robustness of the synthesized results (Fig. S5).

Result

Study Selection

The literature search identified 6468 records published between January 1, 2000 and August 12, 2023. Two additional articles were identified with NCT number of the specific trials through ClinicalTrials.gov. What's more, information of 2 studies was updated and 2 new studies were included which were presented on European Society for Medical Oncology (ESMO) 2023. After excluding 2025 duplicated articles, 4368 articles violating the eligible criteria, 47 trial registry records with unpublished outcomes, 34 full-text articles met the eligibility for assessment, of which 19 articles (16 trials) were included in the quantitative and qualitative synthesis.[6,7,11-16,20-30] Sixteen studies including 7899 patients with breast cancer totally were ultimately included in the analysis, with 9 studies evaluating the effect of ICIs in neoadjuvant therapy and the remaining 7 studies evaluating the impact of ICIs on survival in advanced breast cancer. And PRISMA flow diagram of study retrieval and selection was presented in Fig. S1.

Early Breast Cancer

There are 9 eligible trials (GeparNuevo, 2019; NCI-10013, 2022; I-SPY2, 2020; NeoTRIP, 2022, 2023; KEYNOTE-522, 2020, 2023; IMpassion031, 2020; IMpassion050, 2022; KEYNOTE-756, 2023; CheckMate 7FL, 2023), totally comprising 4520 patients with early breast cancer, of whom 2407 receiving ICIs in combination with chemotherapy and 2113 receiving chemotherapy alone in the analysis. One study included both patients with TNBC and HoR-positive/HER2-negative breast cancer, one study included HER2-positive breast cancer, two studies included TNBC and five studies only included TNBC. Four different types of ICIs treatments (atezolizumab, pembrolizumab, durvalumab, nivolumab) were used in the included studies. Eight studies utilized different assays to report the PD-L1 status. Among these studies, five employed the SP142 assay, which considered PD-L1-positive as 1% or more expression on immune cells (IC). Two studies utilized the 22C3 pharmDx assay, defining PD-L1-positive as 1 or more of a CPS. Lastly, one study used the SP263 antibody and defined PD-L1-positive as 1% or more expression in either the percentage of tumor cells with membranous staining or the percentage of tumor-infiltrating lymphocytes (TILs) with membranous or cytoplasmic staining. Pathological complete response (pCR) was extracted from all studies and the EFS was extracted only from 3 studies (NeoTRIP, KEYNOTE-522 and Impassion031). And the efficacy outcomes were reported overall and separately for the different subgroups about breast cancer with HER2-negative, including PD-L1 status, age, tumor size, ECGO PS, tumor subtype, dose-frequency chemotherapy regimen and nodal status. Table 1 provides the basic characteristics of the included studies and Table S1 in the Supplementary Data lists more detailed information.

Pathological complete response. The overall estimation (GeparNuevo, NCI-10013, I-SPY2, NeoTRIP, KEYNOTE-522, IMpassion031, IMpassion050, KEYNOTE-756, CheckMate 7FL) showed that a significant pCR benefit with a pooled OR of 1.78 (95% CI: 1.39 - 2.27, $P < .00001$, $I^2 = 58\%$), and TNBC patients had a significantly higher pCR rate than control patients (OR = 1.93, 95% CI: 1.34 - 2.79, $P = .0004$, $I^2 = 60\%$). However, compared with HoR-positive/HER2-negative, the predicted value of TNBC was 3.2% ([1.93 ÷ 1.87] - 1), indicating that the difference in benefits from immunotherapy between TNBC and HoR-positive/HER2-negative seemed to be minimal. (Fig. S6A) When analyzing the predictive value of PD-L1 status in different molecular subtypes, the presence of HER2-positive subtype was excluded, as only one trial provided data. Therefore, we conducted a subgroup analysis only for patients with HER2-negative status, as follows. Compared with PD-L1-negative subgroup (OR = 1.50, 95% CI: 1.14 - 1.98, $P = .004$, $I^2 = 0\%$), PD-L1-positive subgroup had an improved pCR with the addition of ICIs in patients with HER2-negative (OR = 1.91, 95% CI: 1.52 - 2.39, $P < .00001$, $I^2 = 11\%$) (Fig. S6B, C),

Table 1. Main Characteristics of the 16 Included Randomized Clinical Trials.

Trial	Tumor Subtype	Experimental Groups	Control Groups	Patients, Total	Phase
Early breast cancer					
GeparNuevo, 2019	TNBC	Anti-PD-L1 + chemotherapy	chemotherapy	174	phase II
NCI-10013, 2022	TNBC	Anti-PD-L1 + chemotherapy	chemotherapy	67	phase II
I-SPY2, 2020	HER2-	Anti-PD-1 + chemotherapy	chemotherapy	69	phase II
NeoTRIP, 2022/2023	TNBC	Anti-PD-L1 + chemotherapy	chemotherapy	280	phase II
KEYNOTE 522, 2020/2023	TNBC	Anti-PD-1 + chemotherapy	chemotherapy	1174	phase III
IMpassion031, 2020	TNBC	Anti-PD-L1 + chemotherapy	chemotherapy	333	phase III
IMpassion 050, 2022	HER2+	Anti-PD-L1 + chemotherapy	chemotherapy	454	phase III
KEYNOTE-756, 2023	HoR+/HER2−	Anti-PD-1 + chemotherapy	chemotherapy	1278	phase III
CheckMate 7FL, 2023	HoR+/HER2−	Anti-PD-1 + chemotherapy	chemotherapy	510	phase III
Advanced breast cancer					
SAFIR02-BREAST IMMUNO, 2021	HER2−	Anti-PD-L1	chemotherapy	199	phase II
NCT03051659, 2020	HoR+/HER2−	Anti-PD-1 + chemotherapy	chemotherapy	90	phase II
ALICE, 2022	TNBC	Anti-PD-L1 + chemotherapy	chemotherapy	68	phase II
IMpassion130, 2019/2021	TNBC	Anti-PD-L1 + chemotherapy	chemotherapy	902	phase III
KEYNOTE-355, 2022	TNBC	Anti-PD-1 + chemotherapy	chemotherapy	847	phase III
IMpassion131, 2021	TNBC	Anti-PD-L1 + chemotherapy	chemotherapy	651	phase III
KEYNOTE-119, 2021	TNBC	Anti-PD-1	chemotherapy	622	phase III

Abbreviation: TNBC, triple-negative breast cancer; HER2: human epidermal growth factor receptor 2; HoR, hormone receptor; PD-L1, programmed death-ligand-1.

yielding a predictive value of 27.3% ([1.91 ÷ 1.50] - 1) favoring high PD-L1-positive patients. And then we made further analysis for different PD-L1 scoring systems to find an appropriate one to evaluate PD-L1 status to identify corresponding patients to receive ICIs. We discovered that patients with PD-L1-positive defined by CPS ≥ 1 had an OR of 1.78 (95% CI: 1.41 - 2.24, $P < .00001$, $I^2 = 0\%$), while there was a higher OR value in patients with PD-L1-positive defined by IC ≥ 1% (OR = 2.28, 95% CI: 1.30 - 4.00, $P = .004$, $I^2 = 46\%$) (Fig. S6B), yielding a predictive value of 28.1% ([2.28 ÷ 1.78] - 1) favoring IC ≥ 1% compared with CPS ≥ 1.

The efficacy of ICIs was then compared between younger and older patients. When the age of patients younger than 40, a pooled pCR OR of 2.72 (95% CI: 1.31 - 5.68, $P = .008$, $I^2 = 0\%$), more superior than patients with an age of 40 or older (OR = 1.51, 95% CI: 1.01 - 2.27, $P = .04$, $I^2 = 22\%$) (Fig. S6D, F), yielding a predictive value of 80.1% ([2.72 ÷ 1.51] - 1) favoring age younger than 40. Patients younger than 65 had a pooled pCR OR of 1.73 (95% CI: 1.37 – 2.18, $P < 0.00001$, $I^2 = 0\%$), while there was no benefit in the patients with an age of 65 or older (OR = 1.92; 95% CI: 1.01 – 3.62, $P = .05$, $I^2 = 0\%$) (Fig. S6G, H). Regarding lymph node status, The OR was also higher in metastatic lymph node positive patients than in metastatic lymph node negative patients, suggesting a slightly higher predictive value of metastatic lymph node status of 25.6% ([2.06 ÷ 1.64] - 1) in favor of metastatic lymph node positive patients (node-positive: OR = 2.06; 95% CI: 1.68 - 2.51, $P < .00001$, $I^2 = 46\%$; node-negative: OR = 1.64; 95% CI: 1.17 - 2.29, $P = .004$, $I^2 = 13\%$) (Fig. S6H, I). Moreover, more specific subgroup analyses for other variables were performed in the ITT population: (I) ECOG PS 0; (II) ECOG PS 1; (III) T1-T2 (T1, diameter: 1.0 cm to 2.0 cm; T2, diameter: 2.0 cm to 5.0 cm); (IV) T3-T4 (T3, diameter: >5.0 cm; T4, locally advanced disease); (V) anthracycline plus cyclophosphamide administered once every 2 weeks; (VI) anthracycline plus cyclophosphamide administered once every 3 weeks. (Fig. S6J, K, L, M, N, O) Besides, the pooled results on multiple subgroup analyses of pathological complete response (pCR) in the early breast cancer were presented in Figure 1.

Event-free survival. As for EFS (NeoTRIP, KEYNOTE-522 and Impassion031), the overall estimation showed that a marginal benefit with a pooled HR of 0.71 (95% CI: 0.58 - 0.88, $P = .002$, $I^2 = 48\%$) (Fig. S7).

Advanced Breast Cancer

Advanced breast cancer comprised inoperable locally advanced breast cancer and metastatic breast cancer. Overall, 7 studies (KEYNOTE-119, SAFIR02-BREAST IMMUNO, NCT03051659, ALICE, IMpassion130, KEYNOTE-355, IMpassion131) comprised 3379 patients, involving patients with HoR-positive/HER2-negative and TNBC in the present analysis. The experimental arm was ICIs in combination with chemotherapy in 5 studies (NCT03051659, ALICE, IMpassion130, KEYNOTE-355, IMpassion131) and ICIs alone in 2 studies (KEYNOTE-119 and SAFIR02-BREAST IMMUNO). In the KEYNOTE-119 and SAFIR02-BREAST IMMUNO, 443 patients received pembrolizumab or durvalumab while 378 patients received chemotherapy depending

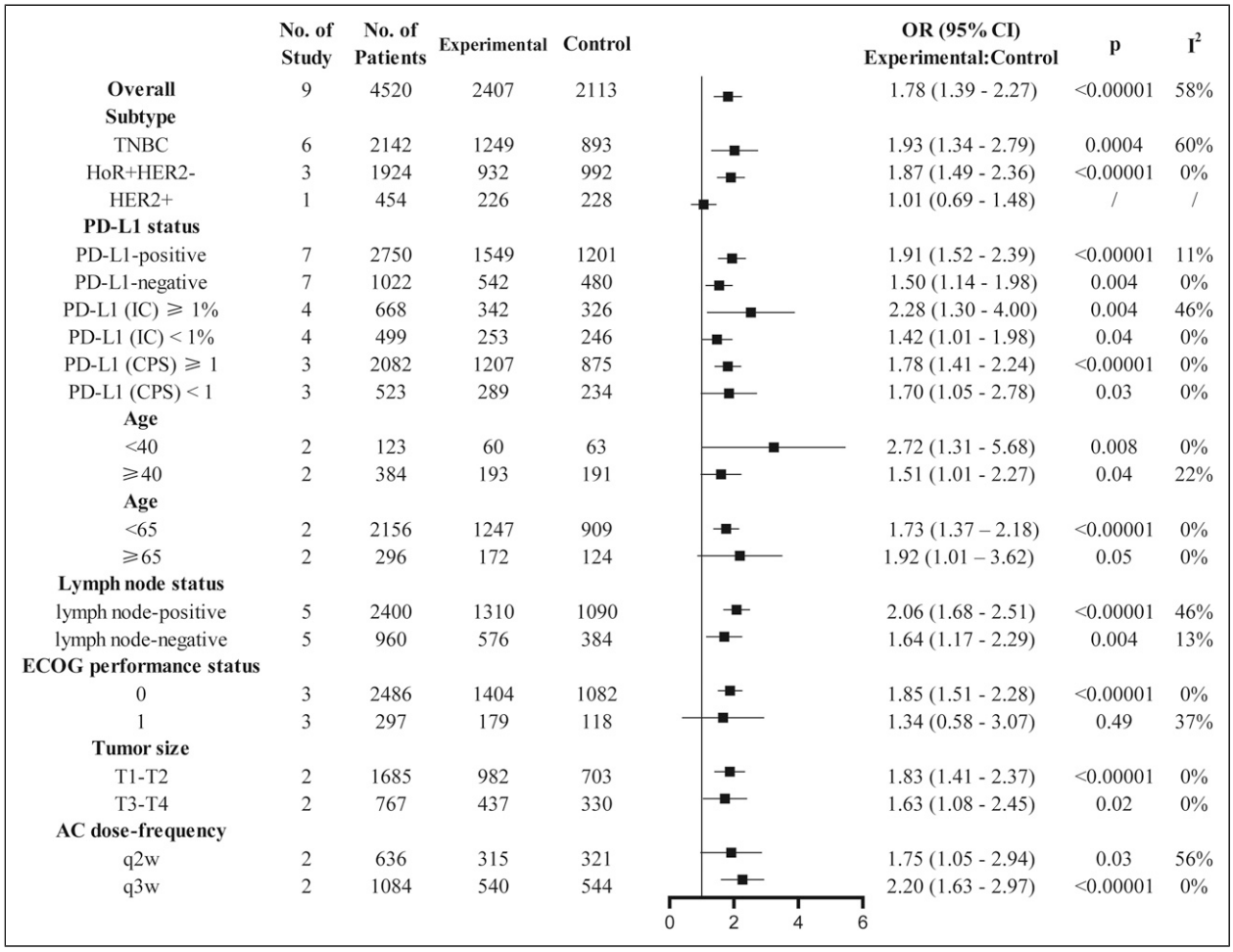

Figure 1. Multiple subgroup analyses of pathological complete response (pCR) in the early breast cancer. Forest plot of subgroup analysis of OR in different subtypes, PD-L1 status, ages, lymph node status, ECOG performance status, tumor size, dose-frequency AC. OR, odds-ratio; TNBC, triple-negative breast cancer; HoR, hormone receptor; HER2, human epidermal growth factor receptor 2; PD-L1, programmed death-ligand-1; IC, immune cells; CPS, combined positive score; ECOG performance status, Eastern Cooperative Oncology Group performance status; T1, diameter: 1.0 cm to 2.0 cm; T2, diameter: 2.0 cm to 5.0 cm; T3, diameter >5.0 cm; T4, locally advanced disease; AC, anthracycline plus cyclophosphamide; q2w, once every 2 weeks; q3w, once every 3 weeks.

on the investigators' choices. And 1532 patients received ICIs plus chemotherapy while 1026 received chemotherapy alone in the other 5 studies. There were 3 different ICIs treatments (pembrolizumab, durvalumab, atezolizumab) used in the included studies. All studies reported the PD-L1 status using various assays. In IMpassion130, 2 assays were used. Patients in 4 studies were tested using the SP142 assay, which defined PD-L1-positive status as 1% or more of PD-L1 expression on immune cells (IC). And patients in 4 other studies were tested using the 22C3 assay, which defined PD-L1-positive status as a combined positive score (CPS) of 1 or more. PFS and OS were the primary endpoints in the analysis. Efficacy outcomes were reported overall and separately for different subgroups, including PD-L1 status, age, number of metastatic sites, presence of liver metastases, previous receipt of neoadjuvant or adjuvant therapy, and ECOG PS. Table 1 provides the basic characteristics of the included studies and Table S2 in the Supplementary Data lists more detailed information.

Progression-free survival. For meta-analysis assessing PFS, the overall estimation (KEYNOTE-119, SAFIR02-BREAST IMMUNO, NCT03051659, ALICE, IMpassion130, KEYNOTE-355, IMpassion131) presented that no significant benefit with a pooled PFS HR of 0.96 (95% CI: 0.75 - 1.23, P = .74, I^2 = 87%) in the patients with HER2-negative (including HoR-positive/HER2-negative and triple-native) breast cancer. And a subgroup analysis presented no improvement in terms of PFS both in patients with HoR-positive/HER2-negative (HR = 1.29; 95% CI: 0.50 - 3.28, P = .60, I^2 = 87%) and with TNBC (HR = 0.89; 95% CI: 0.68 - 1.16, P = .40, I^2 = 89%) (Fig. S8A). To find out the original source of heterogeneity, we performed another subgroup analysis in patients, in conclusion that patients receiving ICIs plus

chemotherapy had a better benefit than that only receiving chemotherapy (HR = 0.80; 95% CI: 0.73 - 0.88, $P < .00001$, $I^2 = 0\%$), while there was a pooled PFS HR of 1.55 (95% CI: 1.32 - 1.82, $P < .00001$, $I^2 = 0\%$) in the group where patients only received ICIs in the experiment arm (Fig. S8B). Therefore, it was believed that patients receiving ICIs alone may not benefit compared with chemotherapy alone. Hence, we only analyzed the PD-L1 status in association with patients receiving ICIs plus chemotherapy. Among patients who were PD-L1-negative, we did not find any significant PFS (Fig. S8D). However, for PD-L1-positive patients, the pooled PFS HR was 0.70 (95% CI: 0.63 - 0.78, $P < .00001$, $I^2 = 0\%$). Notably, patients with PD-L1-positive defined as 1% or more of PD-L1 expression on IC had a lower PFS HR of 0.67 (95% CI: 0.56 - 0.79, $P < .00001$, $I^2 = 0\%$) than who with PD-L1-positive defined as 1 or more of a CPS (HR = 0.72; 95% CI: 0.63 - 0.82, $P < .00001$, $I^2 = 0\%$) (Fig. S8C). This indicated a predictive value of 7.5% ([0.72 ÷ 0.67] - 1) favoring IC ≥ 1% compared with CPS ≥ 1. Further, there was an improvement of pooled PFS HR of 0.69 (95% CI: 0.57 - 0.82, $P < .0001$, $I^2 = 0\%$) in patients receiving ICIs in addition to chemotherapy with a CPS of 10 or more (Fig. S8E). This finding suggested a predictive value of 4.3% ([0.72 ÷ 0.69] - 1) favoring CPS ≥ 10 compared with CPS ≥ 1. In conclusion, patients used ICIs plus chemotherapy had a better benefit than ICIs alone and the predictive value of IC may higher than CPS. And more specific subgroup analyses were performed in the ITT population: (I) ECOG PS 0; (II) ECOG PS 1; (III) liver metastases; (IV) not liver metastases (Fig. S8F, G, H, I). Besides, the pooled results on multiple subgroup analyses of progression-free survival (PFS) in the advanced breast cancer were presented in Figure 2.

Overall Survival. As for OS, the overall estimation (KEYNOTE-119, SAFIR02-BREAST IMMUNO, NCT03051659, ALICE, IMpassion130, KEYNOTE-355, IMpassion131) showed that no significant benefit was observed in the patients with HER2−nagtive irrespective of PD-L1 status (including HoR-positive/HER2-negative and triple-negative) breast cancer (HR = 0.92, 95% CI: 0.83 - 1.02, $P = .10$, $I^2 = 15\%$). A subgroup analysis presented that there was no betterment in terms of OS not only in HoR-positive/HER2-negative group (HR = 1.02; 95% CI: 0.64 - 1.62, $P = .94$, $I^2 = 0\%$) but also in TNBC group (HR = 0.91; 95% CI: 0.81 - 1.02, $P = .12$, $I^2 = 32\%$) (Fig. S9A). Additionally, a subgroup analysis revealed that there was no significant difference between the two groups. Both ICIs, when used in combination with chemotherapy and as monotherapy, did not confer any benefit for patients compared to chemotherapy alone. (ICIs + chemotherapy vs chemotherapy: HR = 0.91, 95% CI: 0.82 - 1.01, $P =$

	No. of Study	No. of Patients	Experimental	Control	HR (95% CI) Experimental:Control	p	I^2
Overall	7	3379	1975	1404	0.96 (0.75, 1.23)	0.74	87%
Subtype							
TNBC	6	3172	1847	1325	0.89(0.68,1,16)	0.4	89%
HoR+HER2-	2	207	128	79	1.29(0.50,3.28)	0.6	87%
Experimental regimen							
ICIs+chemotherapy	5	2558	1532	1026	0.80 (0.73, 0.88)	<0.00001	0%
ICIs	2	821	443	378	1.55 (1.32, 1.82)	<0.00001	0%
PD-L1 status							
PD-L1-positive	5	1721	1020	701	0.70(0.63,0.78)	<0.00001	0%
PD-L1-negative	3	1143	572	571	0.93(0.79,1.09)	0.36	0%
PD-L1 (IC) ⩾1%	3	692	397	295	0.67(0.56,0.79)	<0.00001	0%
PD-L1 (IC) < 1%	2	569	285	284	0.80(0.51,1.27)	0.35	49%
PD-L1 (CPS) ⩾ 1	3	1029	623	406	0.72(0.63,0.82)	<0.00001	0%
PD-L1 (CPS) < 1	2	574	287	287	1.03(0.74,1.42)	0.87	0%
PD-L1 (CPS) ⩾ 10	2	648	/	/	0.69(0.57,0.82)	<0.00001	0%
ECOG performance status							
0	3	511	324	187	0.80(0.62,1.04)	0.1	0%
1	2	279	182	97	0.51(0.11,2.29)	0.38	80%
Liver metastases							
Yes	3	204	130	74	0.85(0.59,1.23)	0.38	0%
No	2	515	341	174	0.77(0.56,1.07)	0.12	0%

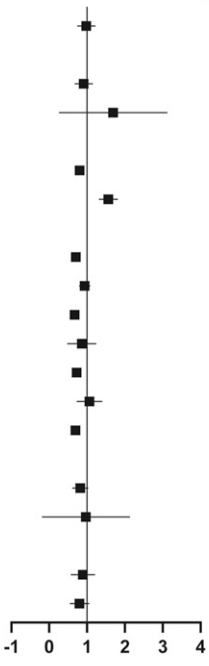

Figure 2. Multiple subgroup analyses of progression-free survival (PFS) in the advanced breast cancer. Forest plot of subgroup analysis of HR in different subtypes, experimental arms, PD-L1 status, ECOG performance status, whether liver metastases. HR, hazard ratio; TNBC, triple-negative breast cancer; HoR, hormone receptor; HER2, human epidermal growth factor receptor 2; ICIs, immune checkpoint inhibitors; PD-L1, programmed death-ligand-1; IC, immune cells; CPS, combined positive score; ECOG performance status: Eastern Cooperative Oncology Group performance status.

.07, I^2 = 0%; ICIs vs chemotherapy: HR = 0.95; 95% CI: 0.81 - 1.11, P = .54, I^2 = 0%; subgroup difference: I^2 = 0%) (Fig. S9B). Patients with PD-L1-positive status (defined as IC ≥ 1% or CPS ≥ 1) had an improvement with the addition of ICIs (HR = 0.84; 95% CI: 0.75 - 0.94, P = .002, I^2 = 12%). To find an appropriate scoring system and cut-point, we made a subgroup analysis for PD-L1-positive, patients with PD-L1-positive defined as 1 or more of a CPS presented an OS HR of 0.84 (95% CI: 0.75 - 0.94, P = .003, I^2 = 0%), but patients with PD-L1-positive defined as 1% or more of PD-L1 expression on IC had no improvement (HR = 0.83; 95% CI: 0.57 - 1.20, P = .31, I^2 = 52%) (Fig. S9C). Further, it decreased 25% risk of death in patients with a CPS of 10 or more (HR = 0.75; 95% CI: 0.63 - 0.89, P = .0009, I^2 =0%) (Fig. S9E), yielding a predictive value of 12% ([0.84 ÷ 0.75] - 1) favoring CPS ≥ 10 compared with CPS ≥ 1. And there was 34% reduction for death risk in patients with a CPS of 20 or more (HR = 0.66; 95% CI: 0.51 - 0.86, P = .002, I^2 =0%) (Fig. S9F), yielding a predictive value of 13.6% ([0.75 ÷ 0.66] - 1) favoring CPS ≥ 20 compared with CPS ≥ 10. There was a better improvement in terms of OS with a higher CPS seemingly. And more specific subgroup analyses were performed in the ITT population: (I) ECOG PS 0; (II) ECOG PS 1; (III) small number of metastatic sites (defined as 0-3 in IMpassion130 and IMpassion131 trials, 0-2 in KEYNOTE-355); (IV) large number of metastatic sites (defined as more than 3 in

	No. of Study	No. of Patients	Experimental	Control	HR (95% CI) Experimental:Control	p	I²
Overall	7	3379	1975	1404	0.92 (0.83, 1.02)	0.31	15%
Subtype							
TNBC	6	3172	1847	1325	0.91(0.81,1.02)	0.12	32%
HoR+HER2-	2	207	128	79	1.02(0.64,1.62)	0.94	0%
Experimental regimen							
ICIs+chemotherapy	5	2558	1532	1026	0.91 (0.82, 1.01)	0.07	0%
ICIs	2	821	443	378	0.95 (0.81, 1.11)	0.54	0%
PD-L1 status							
PD-L1-positive	7	2170	1251	919	0.84(0.75,0.94)	0.002	12%
PD-L1-negative	5	1449	739	710	1.03(0.90,1.18)	0.67	0%
PD-L1 (IC) ⩾1%	4	736	425	311	0.83(0.57,1.20)	0.31	52%
PD-L1 (IC) < 1%	3	658	343	315	0.95(0.77,1.15)	0.58	0%
PD-L1(CPS)⩾1	4	1434	826	608	0.84(0.75,0.94)	0.003	0%
PD-L1(CPS) < 1	3	791	396	395	1.11(0.92,1.35)	0.26	0%
PD-L1 (CPS) ⩾10	3	842	/	/	0.75(0.63,0.89)	0.0009	0%
PD-L1 (CPS) ⩾20	2	313	197	116	0.66(0.51,0.86)	0.002	0%
ECOG performance status							
0	3	833	/	/	0.87(0.75,1.01)	0.07	0%
1	3	567	/	/	0.83(0.71,0.99)	0.03	0%
Number of metastatic site							
Small	3	1266	721	545	0.86(0.74,0.99)	0.04	0%
Large	3	676	423	253	0.84(0.71,1.00)	0.05	0%
Age							
< 65	2	1350	790	560	0.90(0.79,1.02)	0.10	0%
⩾65	2	399	227	172	0.79(0.61,1.00)	0.05	0%
Liver metastasis							
Yes	2	339	187	152	0.80(0.61,1.03)	0.09	0%
No	2	762	395	367	0.87(0.72,1.05)	0.15	0%
Previous neoadjuvant or adjuvant chemotherapy							
Yes	2	1108	641	467	0.93(0.80,1.08)	0.34	0%
No	2	641	376	265	0.78(0.64,0.94)	0.01	0%

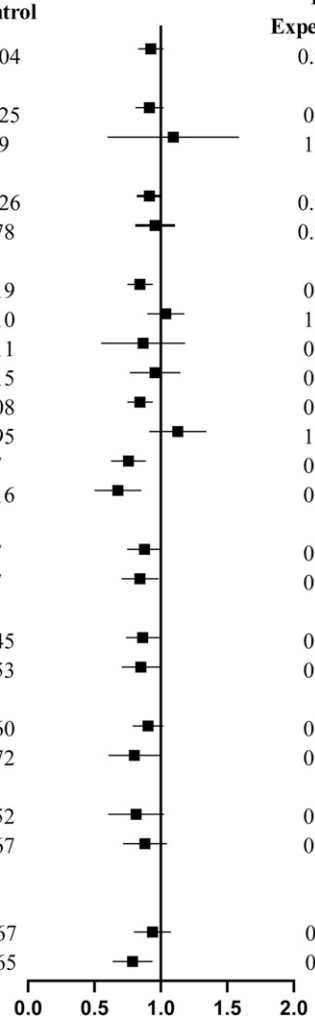

Figure 3. Multiple subgroup analyses of overall survival (OS) in the advanced breast cancer. Forest plot of subgroup analysis of HR in different subtypes, experimental arms, PD-L1 status, ECOG performance status, number of metastatic sites, ages whether liver metastases, previous neoadjuvant or adjuvant chemotherapy. TNBC, triple-negative breast cancer; HoR, hormone receptor; HER2, human epidermal growth factor receptor 2; ICIs, immune checkpoint inhibitors; PD-L1, programmed death-ligand-1; IC, immune cells; CPS, combined positive score; ECOG performance status, Eastern Cooperative Oncology Group performance status, small number of metastatic sites defined as 0-3 in IMpassion trials and 0-2 in KEYNOTE-355; large number of metastatic sites defined as more than 3 in IMpassion trials and more than 2 in KEYNOTE-355.

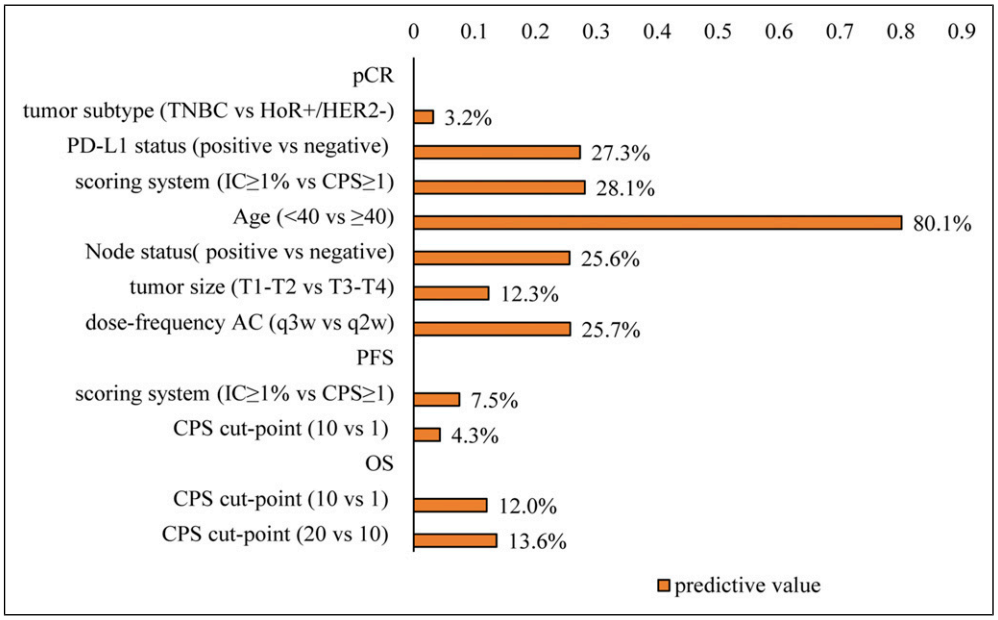

Figure 4. Predictive value of each variable with significant pooled result in early and advanced breast cancer. Predictive value, defined as the difference between the ratio of mean ORs or HRs and 1 across each level of that variable (predictive value = $(HR \text{ or } OR)_A/(HR \text{ or } OR)_B - 1$); pCR, pathological complete response; TNBC, triple-negative breast cancer; HoR, hormone receptor; IC, immune cells; CPS, combined positive score; T1, diameter: 1.0 cm to 2.0 cm; T2, diameter: 2.0 cm to 5.0 cm; T3, diameter >5.0 cm; T4, locally advanced disease; AC, anthracycline plus cyclophosphamide; q2w, once every 2 weeks; q3w, once every 3 weeks; PFS, progression-free survival; OS, overall survival.

IMpassion130 and IMpassion131 trials, more than 2 in KEYNOTE-355); (V) Age<65; (VI) Age ≥ 65; (VII) liver metastases; (VIII) not liver metastases; (IX) receiving previous neoadjuvant or adjuvant chemotherapy; (X) not receiving previous neoadjuvant or adjuvant chemotherapy (Fig. S9G, H, I, J, K, L, M, N, O, P). Besides, the pooled results on multiple subgroup analyses of overall survival (OS) in the advanced breast cancer were presented in Figure 3.

Predictive values of each variable associated with significance in the early and advanced breast cancer were presented in Figure 4.

Study Risk of Bias Assessment and Reporting Bias Assessment

The results of the bias analysis were visually represented in a risk of bias graph and summary figure, facilitating a transparent and systematic approach to evaluating the quality of the included studies in Fig. S2, S3. The eight studies were at low risk and only one study (GeparNuevo, 2019) was at unclear risk of the selection bias which did not mention the random sequence generation in the eBC team. Similarly, there was only one study (SatAFIR02-BREAST IMMUNO, 2021) at unclear risk of the selection bias in the aBC team. It is with regret that nearly half of the studies demonstrated suboptimal quality in the blinding of participants, personnel, and outcome assessment. The funnel plots showed publication bias in the Fig. S4. The symmetrical distribution of the data points along the vertical axis of the funnel plot indicated a low likelihood of publication bias, as it implied that both positive and negative findings have been published and included in the analysis.

Discussion

In our innovative approach to pinpoint patients poised to gain the most from ICIs, we introduce a cutting-edge meta-analysis of the most recent RCTs, delving into the predictive potency of various baseline variables. We've taken an unprecedented step by not only assessing these variables but also quantifying their predictive strength when our aggregated data reveals significance—a novel dimension added to the methodology detailed in the article's introduction. This pioneering move promises to enhance the precision of ICIs treatment strategies, offering a more nuanced understanding of patient response.

In eBC, our analysis revealed that both PD-L1-positive and -negative patients benefited from ICIs, with the PD-L1-positive group showing a higher overall predictive value of 27.1%. The odds ratios for PD-L1 IC and CPS were 2.28 and 1.78, respectively, indicating a greater benefit for PD-L1 IC-positive patients. A key finding was the substantial impact of age, with patients under 40 experiencing an 80.1% predictive value for improved outcomes with neoadjuvant ICIs, surpassing other variables and contrasting with a modest OS benefit difference between age groups in a previous study.[31] And in many published studies, that the older were less likely

to response to the ICIs was attributed to the immune dysfunctions.[32,33] It was found that various immune cells, such as $CD3^+CD45^+$ cells, $CD3^+CD4^+$ Th , $CD3^+CD8^+$ CTL and $CD19^+$ B cells, demonstrated a decreasing trend with age progression, which play an important role in immunotherapy.[34]

In advanced breast cancer, our findings indicated that ICIs alone provide no benefit, while their combination with chemotherapy offers a PFS advantage. The rationale for combining chemotherapy with ICIs is further supported by evidence that certain chemotherapeutic agents can induce immunogenic cell death, which is characterized by the release of damage-associated molecular patterns (DAMPs)[35] that can activate dendritic cells and stimulate an immune response. Additionally, chemotherapy may modulate the tumor microenvironment by reducing the number of immunosuppressive cells, such as regulatory T cells (Tregs),[36,37] thereby creating a more favorable environment for the action of ICIs. Unlike early-stage treatment, only PD-L1-positive patients see improvement in PFS and OS with the combination therapy. Our analysis of PD-L1 cut-points in aBC revealed varying data on OS, with 4 trials using a CPS of 1, three using a CPS of 10, and 2 using a CPS of 20. The predictive value of CPS increased from a cut-point of 1 to 10 by 12% and further to 20 by 13.6%, suggesting a correlation between higher CPS values and better ICI responses. The principal determinant contributing to this divergence is attributed to the distinct immunological microenvironment.

The microenvironment in eBC is often less immunosuppressive, allowing for the potential of ICIs to enhance the existing immune response, even in the absence of high PD-L1 expression. Additionally, the tumor burden is typically lower in early-stage disease, which may result in a more favorable context for ICIs therapy to exert its effects. Conversely, the landscape of the tumor microenvironment is significantly altered in aBC. The advanced disease is often associated with a more immunosuppressive milieu, characterized by increased levels of immunosuppressive cells such as Tregs and myeloid-derived suppressor cells (MDSCs),[38] which can dampen the effects of ICIs. In this context, the expression of PD-L1 becomes a critical biomarker, as it is more likely to be associated with tumors that have developed mechanisms to evade immune detection and destruction. Thus, ICIs are more effective in patients with PD-L1-positive tumors, where the interaction between PD-1 and PD-L1 can be effectively blocked, potentially unleashing a more potent antitumor immune response. Besides, PD-L1 positivity may indicate a higher presence of tumor-infiltrating lymphocytes (TILs), suggesting some level of immunogenicity. And higher tumor grades correlate with increased percentages of PD-1+ immune cells, implying that ICIs can enhance the immune response by activating these T cells.[34]

PD-L1 tumor proportion score (TPS) is a key predictor for ICIs in squamous cell carcinoma, while CPS is significant for adenocarcinoma in advanced gastroesophageal cancer.[19] However, the optimal PD-L1 cut-point for breast cancer remains undetermined. Prior meta-analyses have focused on the efficacy and toxicity of ICIs in breast cancer, particularly the impact of PD-L1 status, without delving into different scoring systems or cut-points. An earlier meta-analysis on eTNBC noted no significant difference between PD-L1-positive and -negative tumors,[39] without further exploration of PD-L1 cut-off values. It remains unclear if higher PD-L1 scores correlate with greater immunotherapy efficacy. Another meta-analysis for aTNBC with three RCTs showed PD-L1-positive patients (IC $\geq$ 1% or CPS $\geq$ 10) experienced significant PFS improvement with ICIs, with a trend towards better OS outcomes, while PD-L1-negative patients saw no benefits.[40] Both studies did not investigate various PD-L1 scoring systems or cut-points in depth.

Our study is the first to explore the relationship between PD-L1 expression and the benefits of ICIs in both eBC and aBC. We have taken into account various scoring systems and thresholds to assess this relationship. Additionally, we have examined other factors such as cancer subtype, age, lymph nodal status, and metastatic sites to determine their impact on the efficacy of ICIs. This meta-analysis was restricted to prospective, randomized, controlled trials, specifically phase

Table 2. Main Characteristics of the 6 Phase III Randomized Clinical Trials Ongoing.

Trial	Tumor Subtype	Experimental Groups	Control Groups	Patients, Total	Phase
Early breast cancer					
ASTEFANIA, 2021	HER2+	Anti-PD-L1 + trastuzumab	trastuzumab	1700	phase III
NCT03281954, 2017	TNBC	Anti-PD-L1 + chemotherapy	chemotherapy	1550	phase III
Advanced breast cancer					
IMpassion132, 2019	TNBC	Anti-PD-L1 + chemotherapy	chemotherapy	572	phase III
NCT04177108, 2019	TNBC	Anti-PD-L1 + Ipatasertib + chemotherapy	Ipatasertib + chemotherapy	1150	phase III
KEYNOTE-B49, 2021	HoR+/HER2-	Anti-PD-L1 + chemotherapy	chemotherapy	800	phase III
329TiP KATE3, 2021	HER2+	Anti-PD-L1 + trastuzumab	trastuzumab	350	phase III

Abbreviation: TNBC, triple-negative breast cancer; HER2: human epidermal growth factor receptor 2; HoR, hormone receptor; PD-L1, programmed death-ligand-1.

II and III studies, which included a total of 7899 patients with early and advanced breast cancer. The consistency observed in the pooled sensitivity analysis across different endpoints confirmed the reliability of the results. Our goal is to assist clinicians in identifying patients who are most likely to benefit from ICIs treatment. We have been closely monitoring ongoing clinical trials and anticipate further studies that will validate our findings, aiding in the more precise selection of breast cancer patients suitable for ICIs. Table 2 presents a compilation of the phase III RCTs currently investigating the efficacy of ICIs in the treatment of breast cancer.

Besides, there are several limitations and sources of heterogeneity in the RCTs evaluating ICIs for breast cancer. Not all trials assessed PD-L1 scores' impact, and pCR definitions varied.[6,7,14] The number of RCTs and sample sizes were often small, particularly for non-triple-negative subtypes, limiting the generalizability of findings. There is a need for more extensive RCTs to draw more reliable conclusions on the benefits of ICIs across breast cancer subtypes.

In Conclusion

The study underscored the significance of PD-L1 positivity, particularly in the context of immune cell expression, as a predictive biomarker for response to ICIs in eBC. The superior OR observed for IC-positive patients vs CPS-positive patients highlighted the importance of precise biomarker assessment in patient selection for ICIs therapy. The differential therapeutic response observed across breast cancer subtypes, with TNBC demonstrating the most favorable outcomes, suggested that tumor biology played a crucial role in the efficacy of ICIs. In aBC, the positive correlation between CPS values and ICIs responsiveness suggested that a higher PD-L1 expression may be associated with better outcome. However, the conclusion requires future studies to validate.

Appendix

List of abbreviations

ICIs	immune checkpoint inhibitors
FDA	US Food and Drug Administration
CPS	combined positive score
pCR	pathological complete response
eTNBC	early triple-negative breast cancer
HoR	hormone receptor
HER2	human epidermal growth factor receptor 2
ITT	intention-to-treat
RCTs	randomized clinical trials
aBC	advanced breast cancer
EFS	event-free survival
eBC	early breast cancer
OR	odds-ratio
CI	confidence intervals
HR	hazard ratio
PFS	progression-free survival
ECOG-PS	Eastern Cooperative Oncology Group performance status
ESMO	European Society for Medical Oncology
IC	immune cells

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Author Contributions

Shuangwei Mo: Conceptualization, Methodology, Software, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Visualization, Supervision. Yuxian Wang: Software, Visualization, Formal analysis, Investigation, Data Curation, Validation, Writing - Review & Editing, Supervision. Yaoling Wang: Writing - Review & Editing, Supervision. Xinhai Chen: Writing - Review & Editing, Supervision. Hongyi Zhu: Writing - Review & Editing, Supervision. Zhengrong Zou: Conceptualization, Validation, Writing - Review & Editing, Supervision, Resources, Project administration. Weikai Xiao: Conceptualization, Methodology, Validation, Resources, Data Curation, Writing - Original Draft, Project administration, Funding acquisition.

Declaration of Conflicting Interests

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Ethical Statement

Ethics Approval and Consent to Participate

This manuscript presents a systematic review and meta-analysis of randomized clinical trials. As this study involves the synthesis and analysis of existing data from published trials, no new data collection or patient involvement was conducted. Therefore, ethical approval was not required for this study.

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Supplemental Material

Supplemental material for this article is available online.

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