The expression of programmed cell death ligand 1 (PD-L1) involves in the clinicopathologic characteristics and prognostic implications of testicular germ cell tumor (TGCT): a systematic review and meta-analysis

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Background: Testicular germ cell tumor (TGCT) is a type of tumor with relatively lower incidence but being more prevalent in young men. The expression of programmed cell death ligand 1 (PD-L1) serves as a potential biomarker for predicting the survival outcomes of other tumors. Some studies discovered higher prevalence of PD-L1 in TGCT patients who achieved favorable treatment outcomes, while other studies showed lower or absent expression of PD-L1 in TGCT with the better prognosis as well. Therefore, in order to address this controversy and clarify the association between the expression of PD-L1 and pathological features and prognosis of TGCT, this meta-analysis was conducted.

Methods: A comprehensive literature search was performed using following search terms: "testis", "testicle", "testicular", "cancer", "carcinoma", "tumor", "neoplasm", "programmed cell death ligand 1", "programmed death ligand 1", "PD-L1", "PDL1", "B7 homolog 1", "B7-H1", "B7H1" and "CD274". Relevant studies were retrieved according to the inclusion criteria from reputable databases including PubMed, Embase, Web of Science, Cochrane Library and China National Knowledge Infrastructure (CNKI). These studies investigated the expression of PD-L1 in both tumor cells and tumor infiltrating immune cells (TIICs) in TGCT. The overall proportion of PD-L1 positivity was assessed using R programming. Pooled hazard ratio (HR) and odds ratio (OR) with corresponding 95% confidence interval (CI) were calculated using Revman software to evaluate the involvement of PD-L1 expression in TGCT. The Newcastle-Ottawa Scale (NOS) was used to evaluate the quality assessment of included studies. Sensitivity analysis and publication bias evaluation were subsequently performed.

Results: A total of eight eligible studies compromising 1,589 patients diagnosed with TGCT were finally included in this study. PD-L1 positivity was detected in 31% and 41% of TGCT patients' tumor cells and TIICs, respectively. The pooled data demonstrated a significant association between elevated PD-L1 expression levels in TIICs and a favorable prognosis characterized by the reduced disease progression and relapse events (HR =0.21, 95% CI: 0.13–0.33). Furthermore, PD-L1+ TIICs exhibited higher prevalence rates in seminoma (OR =2.11, 95% CI: 1.57–2.84) and embryonal carcinoma (OR =6.23, 95% CI: 2.42–16.02) patients. Notably, PD-L1 expression in TIICs displayed a tendency to increase in TGCT patients with lower stages or without lymph node metastasis.

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Conclusions: PD-L1 expression was observed in choriocarcinoma tumor cells, while yolk sac tumor and teratoma tumor cells exhibited lower or absent expression of PD-L1. Conversely, PD-L1 expression in TIICs was associated with seminoma and embryonal carcinoma, which was more commonly observed in TGCT patients with lower stages and better prognosis, thereby providing a theoretical foundation for the application of immunotherapy in relapsed/refractory TGCT patients.

Keywords: Programmed cell death ligand 1 (PD-L1); testicular germ cell tumor (TGCT); clinicopathology; prognosis; meta-analysis

Introduction

Testicular germ cell tumor (TGCT), although accounting for approximately 1% of new male tumors annually, is the most prevalent tumor type among young men aged 15 to 40 years (1,2). Recently, the incidence among patients in their 40s is increasing (3). TGCT is derived from germ cell neoplasia *in situ* generated by delayed gonocytes, and it is categorized into two histological subtypes: seminoma and nonseminomatous germ cell tumor (NSGCT), consisting of embryonal carcinoma, choriocarcinoma, yolk sac tumor and teratoma (4). These subtypes can appear alone or in combination as mixed germ cell tumor (GCT). Seminoma, generally the predominant tumor type, is characterized by the elevated levels of human chorionic gonadotropin (HCG) and tends to show fewer metastatic events and a more favorable prognosis compared to NSGCT, in which alpha fetoprotein (AFP) and HCG are produced (2,5). Besides AFP, HCG and lactate dehydrogenase (LDH), genetic variants or other molecules, such as claudin 6 or programmed cell death ligand 1 (PD-L1)/programmed cell death 1 (PD-1) signaling protein, act as serum tumor biomarkers, playing pivotal roles in the diagnosis, treatment, and prognosis of TGCT (6,7).

TGCT is sensitive to radiotherapy and chemotherapy. The implementation of platinum-based chemotherapy in TGCT results in lower mortality rates compared to other tumor types, with a 5-year overall survival (OS) rate of up to 95% (8). However, despite radical orchiectomy and/or the combined treatment with radiotherapy or chemotherapy, approximately 10–15% of TGCT patients experience relapse, and salvage treatment fails to provide optimal outcomes for 50% of these individuals (9,10). Considering both the limited efficacy and the short- or long-term side effects associated with second-line and salvage therapies, alternative novel and effective treatment strategies are investigated. Compared to normal testicular tissues with macrophages as the predominant immune cell population, testicular tissues are primarily infiltrated by T cells in TGCT, thereby making immunotherapy possible (11). Recently, immunotherapy targeting immune checkpoint inhibitors such as PD-L1 or PD-1 is explored in the treatments of TGCT.

PD-1 is primarily expressed in lymphocytes and interacts with its ligand PD-L1, which can be found in tumor or immune cells. This interaction leads to the inhibition of

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Highlight box

Key findings

• The elevated levels of programmed cell death ligand 1 (PD-L1) in tumor infiltrating immune cells (TIICs) were significantly associated with the favorable prognosis characterized by the reduced disease progression and relapse event.

• PD-L1⁺ TIICs exhibited higher prevalence rates in seminoma and embryonal carcinoma patients.

What is known and what is new?

• It is known that PD-L1 expression is prevalent in testicular germ cell tumor (TGCT) specimens.

• We found that PD-L1 expression in TIICs was more prevalent in seminoma and embryonal carcinoma, which indicated a better prognosis.

What is the implication, and what should change now?

• This meta-analysis represents the first comprehensive investigation of the associations between PD-L1 expression and clinicopathological characteristics as well as prognosis in patients with TGCT, which indicates the possibility of immunotherapy.

• Future research should focus on conducting large-scale studies in TGCT with comprehensive clinical and follow-up information.

T cell-mediated antitumor activity (12). Currently, the use of anti-PD-L1/PD-1, either alone or in combination with conventional radiotherapy or chemotherapy, demonstrates promising results in the treatments of various tumors, including lung cancer, kidney cancer, melanoma, hematologic tumors, as well as TGCT (7,13). Several case reports showed stable conditions or positive responses, such as decreased serum biomarkers and tumor volumes, in patients with relapsed/refractory TGCT following the administration of anti-PD-1 antibodies (14-16). Conversely, other evidence observed that the inhibition of PD-L1 failed to elicit antitumor effects in relapsed/refractory TGCT patients (17).

To a large extent, the effect of immunotherapy in TGCT depends on the expression of PD-L1 or PD-1. Some experimental studies revealed a higher prevalence of PD-L1 in TGCT specimens compared to normal testicular tissues, supporting the potential of immunotherapy targeting the PD-L1/PD-1 pathway in TGCT (18,19). While, other studies obtained the contradictory results showing the absent or lower expression of PD-L1 in TGCT patients (20-22). In addition, TGCT patients with higher expression of PD-L1 often achieved favorable treatment outcomes (18,19). However, other studies indicated that TGCT patients with lower PD-L1 expression had better prognosis (21). Therefore, in order to clarify the general expression of PD-L1 in TGCT, and its correlation with tumor pathology and prognosis, this meta-analysis was conducted. We present this article in accordance with the PRISMA reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2302/rc) (23).

Methods

This meta-analysis was registered on the International Platform of Registered Systematic Review and Meta-analysis Protocols (INPLASY, registration number: INPLASY2023120108). Ethical committee approval was not necessary since the analyzed data were derived from published articles. Any discrepancies between reviewers were resolved through discussion.

Search strategy

A comprehensive literature search was independently conducted by two reviewers (P.L. and W.P.) on the following databases: PubMed, Embase, Web of Science, Cochrane Library and China National Knowledge Infrastructure (CNKI), until May 6, 2024. The search terms utilized included "testis", "testicle", "testicular", "cancer", "carcinoma", "tumor", "neoplasm", "programmed cell death ligand 1", "programmed death ligand 1", "PD-L1", "PDL1", "B7 homolog 1", "B7-H1", "B7H1" and "CD274". For the studies included that required full-text retrieval, their references were scrutinized to identify additional relevant publications. An illustrative example of the search strategy performed in the PubMed database is shown in Appendix 1.

Eligibility criteria

Following a thorough evaluation of the title, abstract, and full-text of the retrieved literature by two reviewers (P.L. and W.P.), studies investigating the correlation between PD-L1 expression and TGCT were selected based on the Population, Intervention, Comparison, Outcomes and Study (PICOS) principal: (I) population: patients were diagnosed with TGCT; (II) intervention: PD-L1 expression in TGCT patients was assessed using immunohistochemistry; (III) comparison: the expression of PD-L1 was categorized into positive (high) and negative (low); (IV) outcomes: the study provided adequate information regarding the pathology or prognosis of TGCT patients; (V) study: cohort or case control studies.

Conversely, other factors were employed to exclude irrelevant studies, which encompassed: (I) failure to meet the inclusion criteria; (II) studies involving animal research, cell biology experiments, bioinformatic analyses, reviews, meta-analyses, comments, meeting abstracts, or case reports; (III) small sample sizes (n<15); (IV) duplicate studies or data; (V) unavailability of the full-text of the literature.

Data collection

The characteristics of the included studies were extracted independently by two reviewers (P.L. and Yuwei Zhong) and checked by another reviewer (M.Z.). Patients were divided into different subgroups depending on their specific situation. The useful data included (I) study information: authors' name, publication year and country; (II) patient information: age, tumor type and stage, metastasis situation, International Germ Cell Cancer Collaborative Group (IGCCCG) risk classification, follow-up time and outcomes; (III) experiment information: staining method, PD-L1 antibody and cut-off value for PD-L1 positive; (IV)

outcome information: total number of included patients and number of PD-L1 positive patients in each subpopulation. We collected and tabulated these data.

Quality assessment

The quality assessment of the included studies was carried out independently by two reviewers (Yuwei Zhong and Yonghong Zheng) using the Newcastle-Ottawa Scale (NOS), a tool containing eight items distributed across three domains: selection (0–4 scores), comparability (0–2 scores) and exposure or outcome (0–3 scores) (24). The NOS assigns a maximum score of 9, with studies scoring 6 or above considered to be of high quality.

Statistical analysis

The overall proportion of PD-L1 was calculated using the R package "meta" in R software version 4.3.0 for studies reporting a single proportion. To ensure appropriate data transformation, the Freeman-Tukey Double arcsine transformation method was applied (25). For statistical analysis, Review Manager 5.4 software (Revman, the Cochrane Collaboration, Oxford, UK) was utilized to compute heterogeneity, hazard ratio (HR), odds ratio (OR), and corresponding 95% confidence intervals (CIs), and generate forest plots. Heterogeneity among the studies was assessed using the Chi-squared test and I^2 statistic. If the P value of the Chi-squared test was less than 0.1 or I^2 exceeded 50%, significant heterogeneity was considered present, and a random-effects model was employed. Otherwise, a fixed-effects model was utilized (26). To explore the association between PD-L1 positivity and survival in TGCT patients, pooled HR with its 95% CI was determined. Additionally, the correlation between PD-L1 expression and clinicopathological characteristics in TGCT patients was analyzed using pooled OR and its 95% CI. Publication bias was assessed when the analysis included a minimum of five studies by utilizing Egger's test, and sensitivity analysis was performed using the one-by-one elimination method through R programming (26). P<0.05 was considered statistically significant.

Results

Search results

Following the search strategy outlined above, a total of 992 articles were obtained from PubMed, Embase, Web of Science, Cochrane Library and CNKI databases. After removing duplicate studies, a preliminary screening of titles and abstracts was conducted. Non-original articles (n=427) and those not relevant to the scope of this study (n=268) were excluded. Subsequently, the full texts of the remaining studies were retrieved. Further exclusions were made based on specific criteria: utilization of duplicate patient samples (n=2), absence of dichotomization of PD-L1 expression into positive/high and negative/low groups (n=4), unavailability of analyzable data (n=2), and small sample size (n=3). Ultimately, a total of eight studies were included in this meta-analysis. The flow diagram illustrating the process of screening eligible publications for this study is presented in *Figure 1*.

Study characteristics

All studies included in this meta-analysis were conducted from 2015 to 2024 in European and American countries except one study investigated Asian patients. With the exception of one study by Cierna *et al.*, which involved specimens from nine patients with extragonadal GCT (21), the remaining studies focused on TGCT patients. The age of the patients ranged from 16 to 90 years. A total of 1,589 specimens were analyzed across these eligible studies. It is worth noting that both Cierna *et al.* and Chovanec *et al.* publications originated from the same research group. Consequently, there were 136 patients from the study by Chovanec *et al.* that were also included in the study conducted by Cierna *et al.* (21,27). Cierna *et al.* and Fankhauser *et al.* analyzed the expression of PD-L1 in tumor cells, while Chovanec *et al.*, Sadigh *et al.* and Pęksa *et al.* examined its expression in tumor infiltrating immune cells (TIICs), such as lymphocytes and macrophages (18,21,27-29). Lobo *et al.*, Möller *et al.* and Farahani *et al.* investigated PD-L1 expression in both tumor cells and TIICs (19,20,22). Immunohistochemistry was utilized in all these studies to determine the positivity of PD-L1, although different antibodies and cut-off values were employed. These variations contributed to the wide range of PD-L1 positive expression observed across different studies. The proportion of PD-L1 positive staining in tumor cells ranged from 0.0% to 69.6%, while in TIICs it ranged from 3.0% to 83.5%. Follow-up data on patient outcomes were available in four studies, where the prognostic value of PD-L1 expression was assessed using OS, progression-free survival
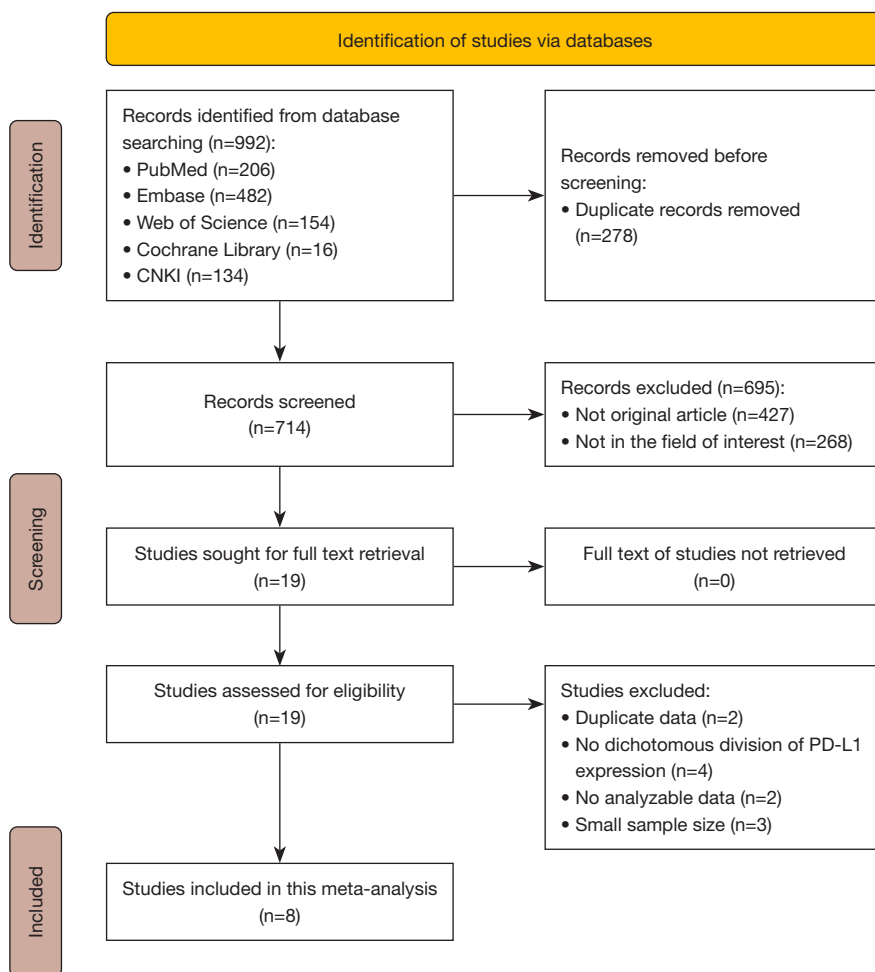
Figure 1 The flow diagram for selecting the eligible studies. CNKI, China National Knowledge Infrastructure; PD-L1, programmed cell death ligand 1.

(PFS), relapse-free survival (RFS), or other indicators. The characteristics of the studies included in this meta-analysis are presented in *Table 1*.

Furthermore, all included studies were either cohort or case-control studies and obtained a NOS score between 6 and 7, indicating good methodological quality (*Tables 2,3*).

Overall proportion of positive PD-L1 expression in TGCT

PD-L1 expression has been observed in various types of cancer, including melanoma, lung cancer, gastric carcinoma, as well as TGCT (30). By pooling the data on patients with positive PD-L1 staining, we determined that 31% of TGCT patients exhibited high PD-L1 expression in tumor cells (random-effects, 95% CI: 0.06–0.65) (*Figure 2A*).

Additionally, PD-L1 was found to be overexpressed in TIICs in 41% of TGCT patients (random-effects, 95% CI: 0.16–0.69) (*Figure 2B*). All the studies included in this analysis showed significant heterogeneity.

Association of positive PD-L1 expression with prognostic implications in TGCT

Despite the inclusion of four studies that followed up with TGCT patients, two different studies examined the relationship between PD-L1 expression in tumor cells and the prognosis of TGCT. One study found that TGCT patients who exhibited a lack of PD-L1 expression or lower PD-L1 expression in the primary tumor had a better PFS compared to those with positive or higher PD-L1

Table 1 Characteristics of studies included in this meta-analysis

Study	Country	Tumor	Age, median [range], years	Number of patients	PD-L1 positive/ high, n (%)	Cut-off for positive	Measurement method	Antibody information Company	Clone	Outcome	Follow-up time, median [range]
Fankhauser 2015, (18)	Switzerland	Primary TGCT	33.5 [18–90]	329	229 (69.6)	>5%	IHC	Cell Signaling Technology	E1L3N	NA	NA
Cierna 2016, (21)	Slovak	Primary TGCT and extragonadal GCT	30 [17–67]	140	23 (16.4)	QS ≥10	IHC	Abcam	EPR1161(2)	PFS, OS	81.1 [0.3–235.8] months
Chovanec 2017, (27)	Slovak	Primary TGCT	30.6 [16–67]	240	61 (25.4)*	HS ≥160	IHC	Abcam	EPR1161(2)	PFS, OS	78.9 [0.4–293.7] months
Lobo 2019, (19)	Portugal	GCNIS-related TGCT	30 [25–36]	164	58 (35.4) or 137 (83.5)*	>0%	IHC	Dako	22C3	RFS	15 years
Sadigh 2020, (28)	USA	Primary TGCT	35 [18–69]	77	16 (20.8)*	NA	IHC	Cell Signaling Technology	E1J2J	NA	5.3 months
Pęksa 2021, (29)	Poland	Primary TGCT	32.9 [17–66]	180	89 (49.4)*	HS ≥41	IHC	Dako	22C3	EFS, CSS	31.4 months
Möller 2023, (20)	Germany	Primary TGCT	NA	562	0 (0.0) or 411 (73.1)*	>10%	IHC	MS Validated Antibodies	MSVA–711R	NA	NA
Farahani 2024, (22)	Iran	Seminoma	39.6	33	21 (63.6) or 1 (3.0)*	HS ≥15	IHC	Sinabiotech	NA	NA	NA

*, the positive staining of PD-L1 was detected in tumor infiltrating immune cells in this study. PD-L1, programmed cell death ligand 1; TGCT, testicular germ cell tumor; GCT, germ cell tumor; GCNIS, germ cell neoplasia in situ; IHC, immunohistochemistry; NA, not available; QS, quickscore; HS, histoscore; PFS, progression-free survival; OS, overall survival; RFS, relapse-free survival; EFS, event-free survival; CSS, cancer-specific survival.

Table 2 Quality assessment of included case control studies by NOS

Study	Selection Adequacy of case definition	Representativeness of the cases	Selection of controls	Definition of controls	Comparability Comparability of cases and controls on the basis of the design or analysis	Exposure Assessment of exposure	Same method of ascertainment for cases and controls	Non-response rate	Overall score
Fankhauser 2015, (18)	★	★			★★	★	★	★	7
Möller 2023, (20)	★	★			★★	★	★	★	7
Farahani 2024, (22)	★				★★	★	★	★	6

★, represents one score; ★★, represents two scores. NOS, Newcastle-Ottawa Scale.

expression (negative/lower verse positive/higher, HR =0.40, 95% CI: 0.16–1.01, P=0.0081) (21). While, the other study reported no significant difference of RFS among TGCT patients showing PD-L1 positive or negative tumor cells (19).

The majority of studies primarily focused on the correlation between PD-L1 expression in TIICs and the prognostic outcomes of TGCT patients, particularly in terms of disease progression or relapse events. Notably, Pęksa *et al.* analyzed this correlation in two specific subgroups, namely seminoma and NSGCT (29). Therefore, we segregated this study into two distinct sub-studies: (I) seminoma and (II) NSGCT. The results demonstrated that TGCT patients with PD-L1+ TIICs showed a more

Table 3 Quality assessment of included cohort studies by NOS

	Selection				Comparability	Outcome			
Study	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Outcome not present at start	Comparability of cohorts on the basis of the design or analysis	Assessment of outcome	Adequate follow-up period	Adequacy of follow-up	Overall score
Cierna 2016, (21)	★		★		★★	★	★	★	7
Chovanec 2017, (27)	★		★		★★	★	★	★	7
Lobo 2019, (19)	★		★		★★	★	★	★	7
Sadigh 2020, (28)	★		★		★★		★	★	6
Pęksa 2021, (29)	★		★		★★	★	★	★	7

★, represents one score; ★★, represents two scores. NOS, Newcastle-Ottawa Scale.
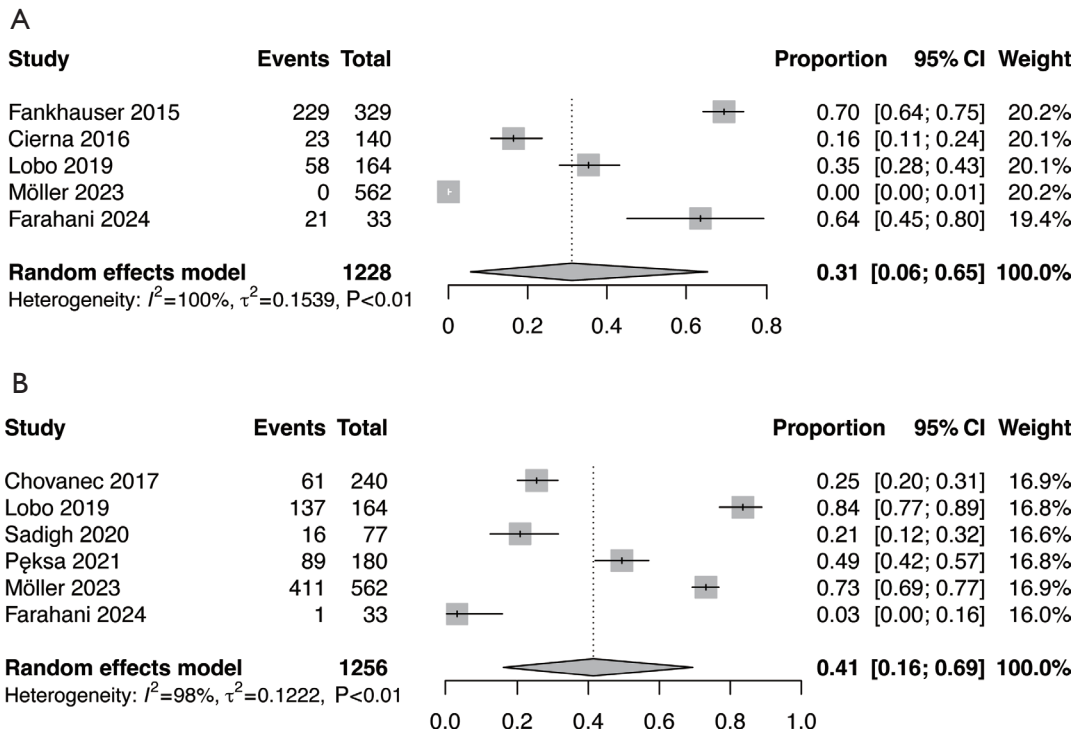

Figure 2 Forest plots of overall percentage of PD-L1 positive expression in tumor cells (A) and TIICs (B) in TGCT patients. Axis represents the proportion of PD-L1 positive expression in TGCT. CI, confidence interval; PD-L1, programmed cell death ligand 1; TIICs, tumor infiltrating immune cells; TGCT, testicular germ cell tumor.
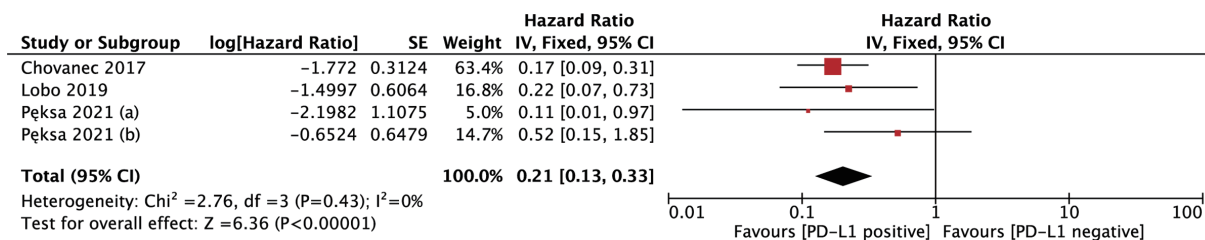
Figure 3 Forest plots of HR to evaluate the correlation between PD-L1 expression and event (progression or relapse) in TGCT. SE, standard error; IV, inverse variance; CI, confidence interval; HR, hazard ratio; PD-L1, programmed cell death ligand 1; TGCT, testicular germ cell tumor.

favorable prognosis and experienced fewer progression or relapse events compared to those with PD-L1-negative or lower TIIC expression (positive/higher verse negative/lower, HR =0.21, 95% CI: 0.13–0.33, P<0.00001) (*Figure 3*). No significant heterogeneity was observed. Thus, a fixed-effects model was utilized for this analysis.

Association of positive PD-L1 expression with clinicopathologic characteristics in TGCT

Histology

Seminoma is generally considered to be less aggressive in comparison to NSGCT (31). There was no significant difference found in the expression of PD-L1 in tumor cells of seminoma and NSGCT (*Figure 4A*). However, there was a significant correlation between positive PD-L1 staining in immune cells and seminoma. Among 757 seminoma patients and 466 NSGCT patients, 69% and 41% of them exhibited PD-L1 positive immune cell infiltration, respectively. The odds of PD-L1+ TIICs in seminoma were 111% higher than those in NSGCT (95% CI: 1.57–2.84) (*Figure 4B*). Afterwards, we analyzed the relationship between PD-L1 expression and individual subtypes of NSGCT. The pooled OR results revealed that PD-L1 overexpression in tumor cells was more prevalent in choriocarcinoma (OR =11.11, 95% CI: 4.82–25.60) (*Figure 4C*), while there was no advantage of PD-L1+ TIICs in choriocarcinoma (*Figure 4D*), as well as PD-L1 positive tumor cells in embryonal carcinoma (*Figure 4E*). The odds of PD-L1+ immune cell infiltration in embryonal carcinoma were significantly higher by a 5.23-fold change compared to other NSGCTs (OR =6.23, 95% CI: 2.42–16.02) (*Figure 4F*). In addition, lower expression of PD-L1 was commonly observed in yolk sac tumor cells (OR =0.54, 95% CI: 0.32–0.91), not in TIICs (*Figure 4G,4H*). Similarly, lower expression of PD-L1

was significantly associated with teratoma not only in tumor cells (OR =0.17, 95% CI: 0.05–0.54) but also in TIICs (OR =0.07, 95% CI: 0.01–0.44) (*Figure 4I,4J*).
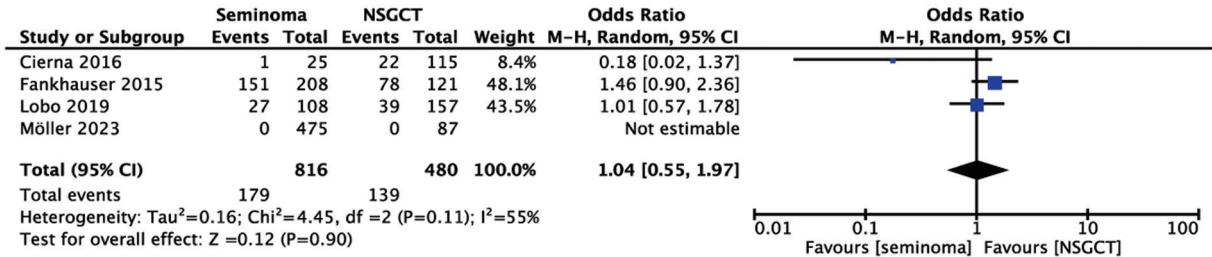
Stages

There were two articles examining the correlation between PD-L1 expression in tumor cells and tumor-node-metastasis (TNM) or S stages in TGCT, revealing that higher stage TGCT patients had a higher rate of positive PD-L1 staining (18,21). However, upon extracting data from studies conducted by Lobo *et al.* and Pęksa *et al.* (19,29), we discovered that stage I tumors in TGCT patients showed a higher presence of PD-L1+ TIICs compared to stage II and III tumors (OR =0.70, 95% CI: 0.42–1.15) (*Figure 5A*). In the study by Chovanec *et al.* (27), they compared data from stage S0, S1 and S2 tumors with stage S3 tumors in TGCT, therefore it cannot be included in this meta-analysis. However, similar results were obtained, showing a significant association between PD-L1 positivity in TIICs and low-stage tumors but not high-stage tumors (P value =0.03) (27). Opposed to these results, the infiltration of PD-L1+ immune cells into TGCT with pT2–4 tumors demonstrated an advantage over tumors with pT1 stage, although no significant statistical differences were observed (*Figure 5B*). In addition, positive expression of PD-L1 in TIICs was more common in tumors that did not develop lymph node metastases or develop lymphovascular invasion (LVI), albeit without significant statistical difference (*Figure 5C,5D*).
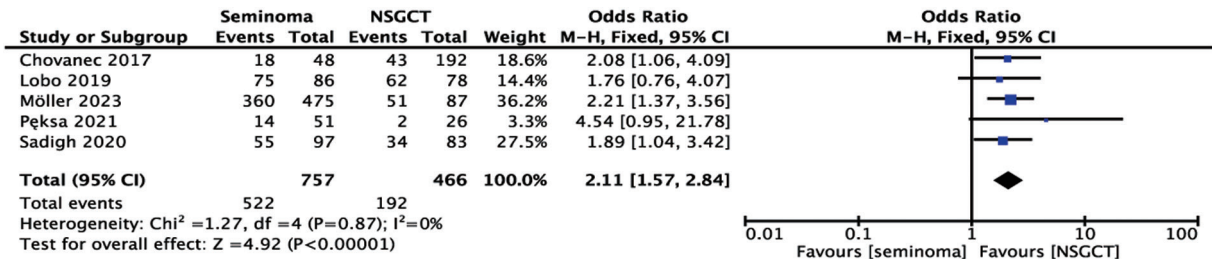
IGCCCG risk classification

Based on the histological subtype, metastasis, and levels of AFP, HCG and LDH, TGCT can be categorized into good, intermediate and poor outcome groups according to the IGCCCG classification. These groups correspond
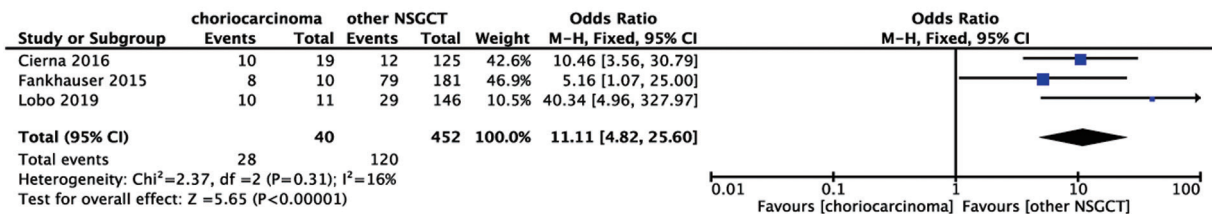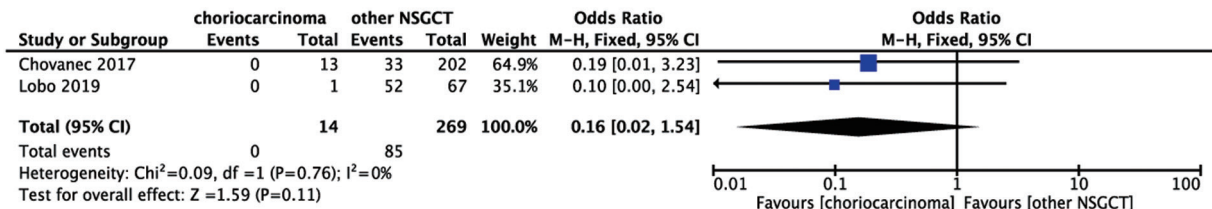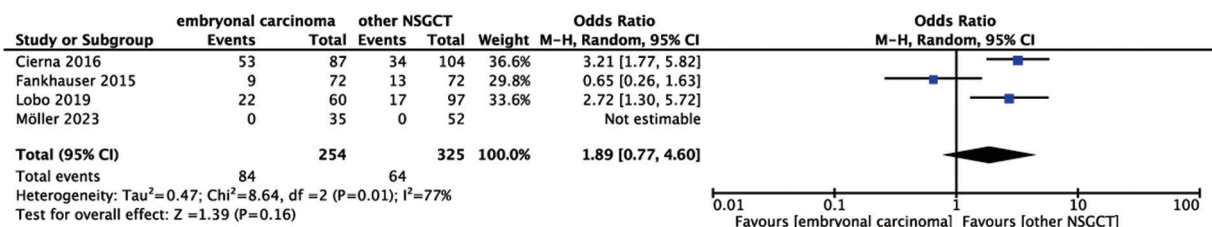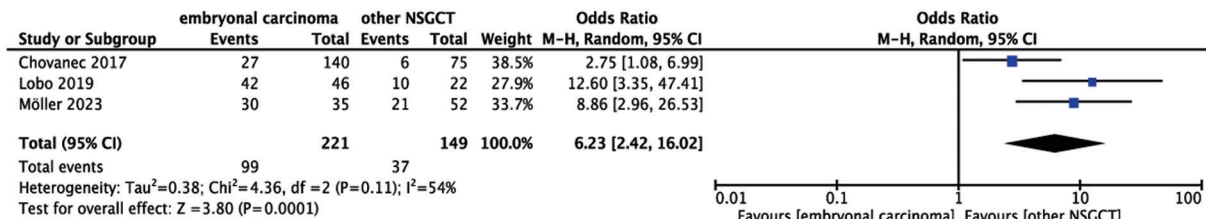
A

Study or Subgroup	Seminoma Events	Total	NSGCT Events	Total	Weight	Odds Ratio M–H, Random, 95% CI
Cierna 2016	1	25	22	115	8.4%	0.18 [0.02, 1.37]
Fankhauser 2015	151	208	78	121	48.1%	1.46 [0.90, 2.36]
Lobo 2019	27	108	39	157	43.5%	1.01 [0.57, 1.78]
Möller 2023	0	475	0	87		Not estimable
Total (95% CI)		**816**		**480**	**100.0%**	**1.04 [0.55, 1.97]**
Total events	179		139			

Heterogeneity: Tau²=0.16; Chi²=4.45, df =2 (P=0.11); I²=55%
Test for overall effect: Z =0.12 (P=0.90)

Odds Ratio M–H, Random, 95% CI
Favours [seminoma]   Favours [NSGCT]

B

Study or Subgroup	Seminoma Events	Total	NSGCT Events	Total	Weight	Odds Ratio M–H, Fixed, 95% CI
Chovanec 2017	18	48	43	192	18.6%	2.08 [1.06, 4.09]
Lobo 2019	75	86	62	78	14.4%	1.76 [0.76, 4.07]
Möller 2023	360	475	51	87	36.2%	2.21 [1.37, 3.56]
Pęksa 2021	14	51	2	26	3.3%	4.54 [0.95, 21.78]
Sadigh 2020	55	97	34	83	27.5%	1.89 [1.04, 3.42]
Total (95% CI)		**757**		**466**	**100.0%**	**2.11 [1.57, 2.84]**
Total events	522		192			

Heterogeneity: Chi² =1.27, df =4 (P=0.87); I²=0%
Test for overall effect: Z =4.92 (P<0.00001)

Odds Ratio M–H, Fixed, 95% CI
Favours [seminoma]   Favours [NSGCT]

C

Study or Subgroup	choriocarcinoma Events	Total	other NSGCT Events	Total	Weight	Odds Ratio M–H, Fixed, 95% CI
Cierna 2016	10	19	12	125	42.6%	10.46 [3.56, 30.79]
Fankhauser 2015	8	10	79	181	46.9%	5.16 [1.07, 25.00]
Lobo 2019	10	11	29	146	10.5%	40.34 [4.96, 327.97]
Total (95% CI)		**40**		**452**	**100.0%**	**11.11 [4.82, 25.60]**
Total events	28		120			

Heterogeneity: Chi²=2.37, df =2 (P=0.31); I²=16%
Test for overall effect: Z =5.65 (P<0.00001)

Odds Ratio M–H, Fixed, 95% CI
Favours [choriocarcinoma]   Favours [other NSGCT]

D

Study or Subgroup	choriocarcinoma Events	Total	other NSGCT Events	Total	Weight	Odds Ratio M–H, Fixed, 95% CI
Chovanec 2017	0	13	33	202	64.9%	0.19 [0.01, 3.23]
Lobo 2019	0	1	52	67	35.1%	0.10 [0.00, 2.54]
Total (95% CI)		**14**		**269**	**100.0%**	**0.16 [0.02, 1.54]**
Total events	0		85			

Heterogeneity: Chi²=0.09, df =1 (P=0.76); I²=0%
Test for overall effect: Z =1.59 (P=0.11)

Odds Ratio M–H, Fixed, 95% CI
Favours [choriocarcinoma]   Favours [other NSGCT]

E

Study or Subgroup	embryonal carcinoma Events	Total	other NSGCT Events	Total	Weight	Odds Ratio M–H, Random, 95% CI
Cierna 2016	53	87	34	104	36.6%	3.21 [1.77, 5.82]
Fankhauser 2015	9	72	13	72	29.8%	0.65 [0.26, 1.63]
Lobo 2019	22	60	17	97	33.6%	2.72 [1.30, 5.72]
Möller 2023	0	35	0	52		Not estimable
Total (95% CI)		**254**		**325**	**100.0%**	**1.89 [0.77, 4.60]**
Total events	84		64			

Heterogeneity: Tau²=0.47; Chi²=8.64, df =2 (P=0.01); I²=77%
Test for overall effect: Z =1.39 (P=0.16)
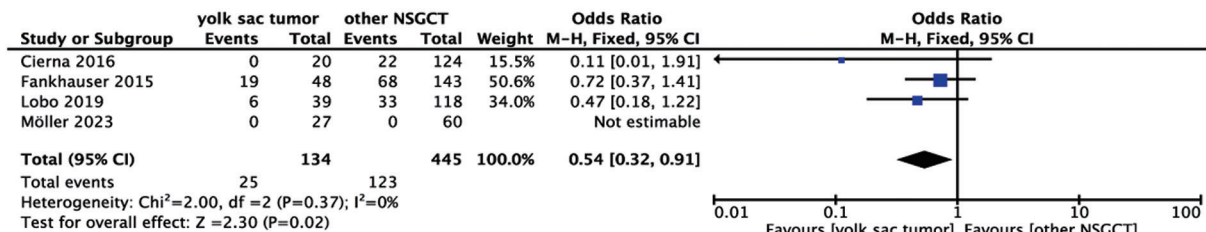
Odds Ratio M–H, Random, 95% CI
Favours [embryonal carcinoma]   Favours [other NSGCT]

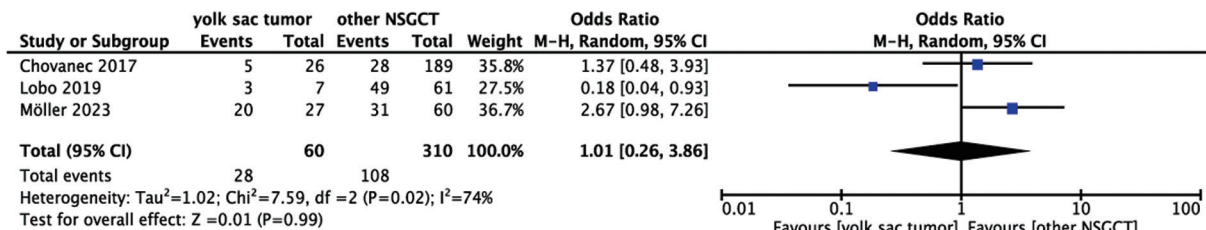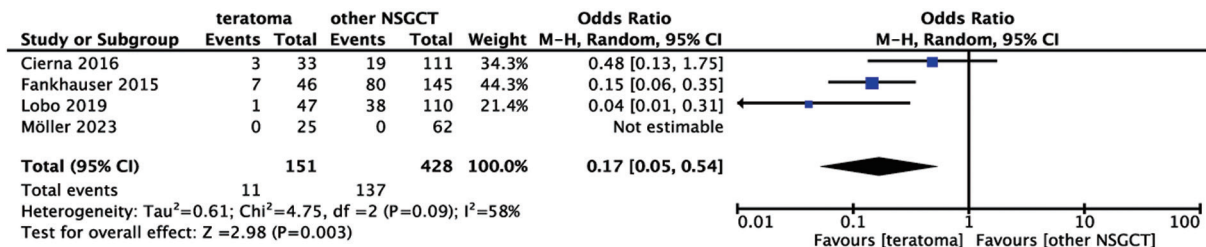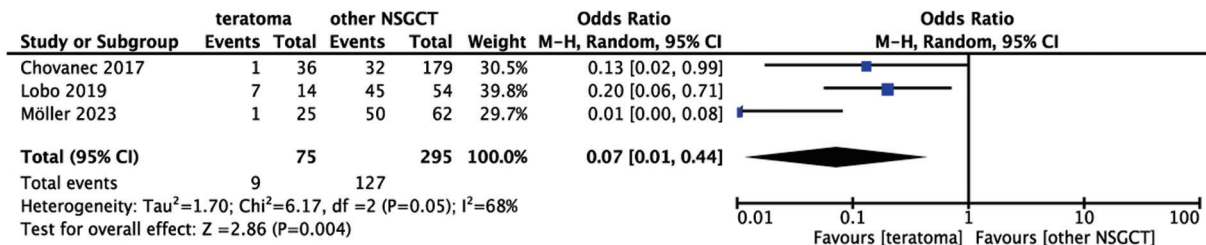**Figure 4** Forest plots of OR to evaluate the correlation between PD-L1 expression and histological characteristics in TGCT. Comparison of PD-L1 expression in tumor cells (A,C,E,G,I) or TIICs (B,D,F,H,J) between seminoma and NSGCT (A,B), choriocarcinoma and other types of NSGCT (C,D), embryonal carcinoma and other types of NSGCT (E,F), yolk sac tumor and other types of NSGCT (G,H), as well as teratoma and other types of NSGCT (I,J). NSGCT, nonseminomatous germ cell tumor; M-H, Mantel-Haenszel; CI, confidence interval; OR, odds ratio; PD-L1, programmed cell death ligand 1; TGCT, testicular germ cell tumor; TIICs, tumor infiltrating immune cells.

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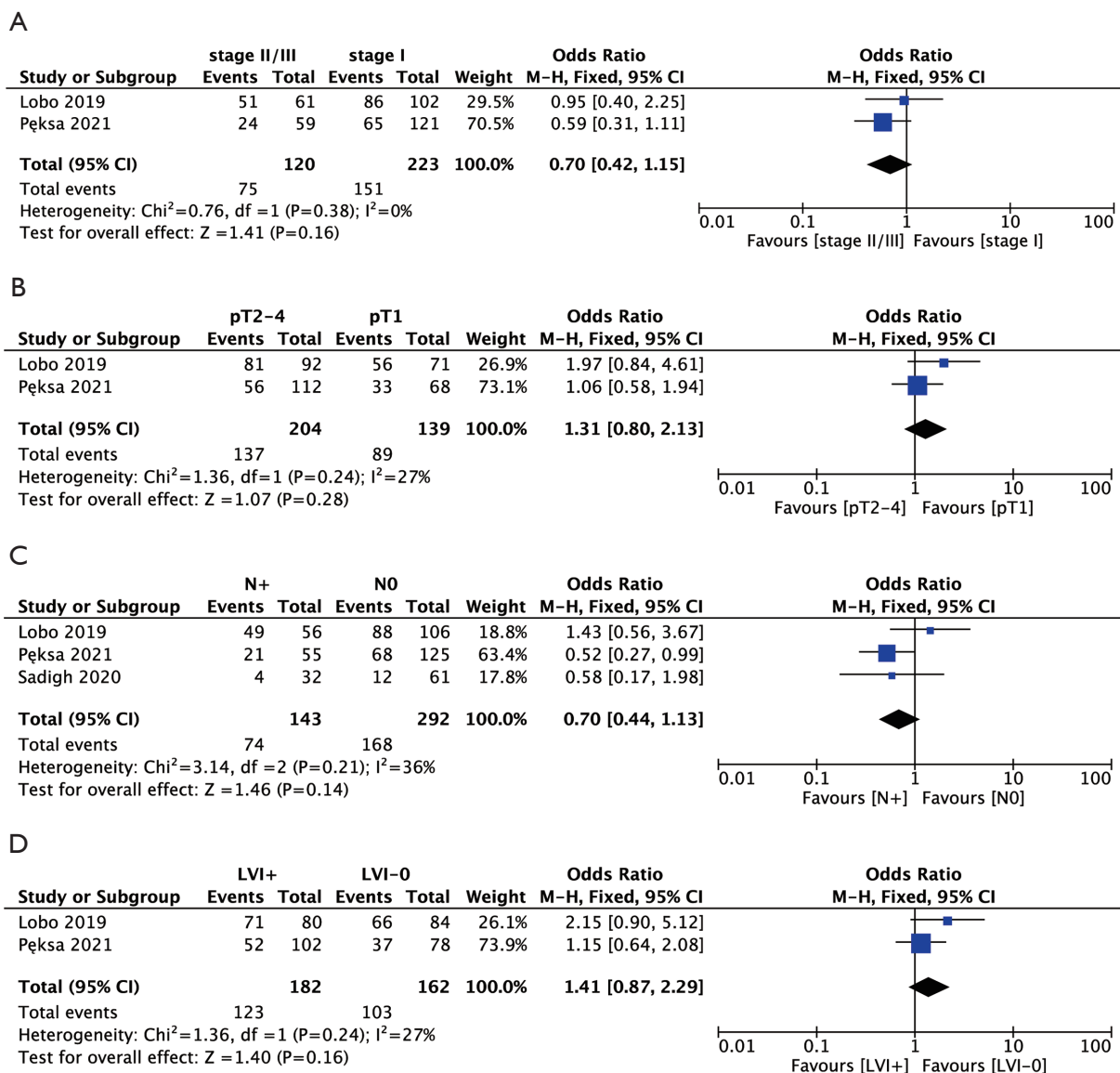
Figure 5 Forest plots of OR to evaluate the correlation between PD-L1 expression in TIICs and TNM stages (A), T stages (B), lymph node metastasis (C) and LVI (D) in TGCT. M-H, Mantel-Haenszel; CI, confidence interval; LVI, lymphovascular invasion; OR, odds ratio; PD-L1, programmed cell death ligand 1; TIICs, tumor infiltrating immune cells; TNM, tumor-node-metastasis; TGCT, testicular germ cell tumor.

to approximately 96%, 89%, and 67% of the 5-year OS rate (32,33). In this study, we conducted two comparisons due to the grouping differences between Chovanec *et al.* who combined the good and intermediate risk groups (27), and Lobo *et al.* who combined the poor and intermediate risk groups (19). Both comparisons revealed that positive expression of PD-L1 on TIICs was predictive of a favorable prognosis in TGCT (*Figure 6*). However, we did not analyze PD-L1 expression on tumor cells due to insufficient

data, as only one study addressed this aspect, which reported contrary results: higher expression of PD-L1 on tumor cells predicted a worse prognosis in TGCT patients (21).

Sensitivity analysis and publication bias

Sensitivity analysis was conducted to evaluate following comparisons: (I) the overall proportion of positive PD-L1 expression in TGCT as shown in *Figure 2*; and (II) the
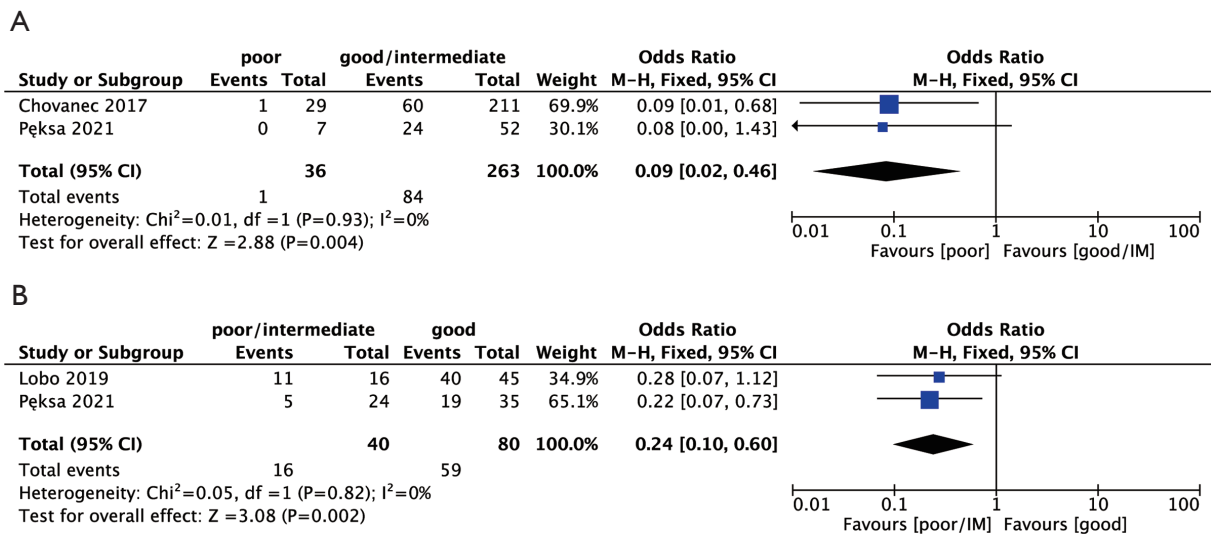
Figure 6 Forest plots of OR to evaluate the correlation between PD-L1 expression and IGCCCG risk classification in TGCT. Comparison of PD-L1 expression in TIICs between poor and good/IM group (A) as well as poor/IM and good group (B). M-H, Mantel-Haenszel; CI, confidence interval; OR, odds ratio; PD-L1, programmed cell death ligand 1; IGCCCG, International Germ Cell Cancer Collaborative Group; TGCT, testicular germ cell tumor; TIICs, tumor infiltrating immune cells; IM, intermediate.

comparison of positive PD-L1 expression in TIICs between seminoma and NSGCT as showed in *Figure 4B*. All comparisons included more than five studies. Upon excluding each individual study from the overall analysis, no significant differences were observed ([Figure S1](#)). Furthermore, publication bias was assessed using Egger's test for the aforementioned studies. Results indicated no evidence of publication bias in all of these studies, as P values were greater than 0.05 in all comparisons [P values of comparison (I) were 0.45 and 0.38, respectively; P value of comparison (II) was 0.37].

Discussion

PD-L1, also known as PD-L1, is a protein that can be expressed constitutively or induced in various cells within different tissues, including the testis (34). In the physiological state, PD-L1 released by Sertoli cells, myoid peritubular cells, and germ cells directly or indirectly suppresses T cell activation and proliferation, thereby contributing to the establishment of immune privilege in the testes (35). Several studies reported that PD-L1 expression is generally lower in normal testis (18,21,36). Upregulation of PD-L1 expression is frequently observed in specific types of testicular tumors, such as GCTs and testicular lymphoma (37-39). In this meta-analysis, we

included a total of eight eligible studies based on our search strategy. Although additional studies also confirmed the significant upregulation of PD-L1 in TGCT, particularly in seminoma (40-43), we were unable to incorporate them into the final meta-analysis due to the lack of dichotomous expression data of PD-L1.

Immune cell infiltration is a crucial characteristic observed during the progression of TGCT (11). Among the infiltrating cells in TGCT, macrophages, CD8^{+} and CD45R0^{+} T cells, as well as dendritic cells, constituted the major population (44,45). High levels of TIICs were associated with a worse prognosis in TGCT, indicating an increased risk of tumor relapse (46). In addition to tumor cells, PD-L1 expression was also detected in TIICs in TGCT, such as lymphocytes and macrophages (27,28). The pooled results indicated that compared to the average of 31% PD-L1 positivity on tumor cells, PD-L1 was more frequently expressed on TIICs in TGCT, with an overall overexpression rate of 41%. Notably, Möller *et al.* reported the absence of PD-L1 positive staining on tumor cells, while up to 73% of TIICs in TGCT can express PD-L1 (20). However, Farahani *et al.* showed the contradictory results, in which only 3% of TIICs in TGCT patients presented PD-L1 (22).

Moreover, different conclusions were reported regarding the association of PD-L1 expression with patient outcomes

in TGCT. One study suggested that higher expression of PD-L1 in tumor cells correlated with PFS and OS rates, while another study found no significant difference in RFS rates between TGCT patients with PD-L1$^+$ and PD-L1$^-$ tumor cells (19,21). In contrast, our comprehensive meta-analysis demonstrated that the presence of PD-L1$^+$ TIICs was a significant predictor of favorable risk classification and prognosis, associated with reduced progression or relapse events. These findings suggest a contrasting role played by PD-L1 in tumor cells versus TIICs during the development of TGCT. One possible explanation for this discrepancy is that TGCT patients with PD-L1$^+$ TIICs exhibit heightened sensitivity to various therapeutic interventions, resulting in more effective responses and consequently improving prognosis. Additionally, activated T cells can trigger negative feedback loops, leading to the development of adaptive immune resistance. Some TGCT patients may display limited or even absent immune cell infiltration, resulting in lower PD-L1 positivity rates in TIICs. However, these individuals may exhibit overexpression of PD-L1 in tumor cells, contributing to the divergent findings regarding PD-L1 expression in TIICs versus tumor cells. Similarly, elevated levels of PD-L1 expression in conjunction with TIICs are also associated with favorable prognostic stages in other types of tumors, including breast cancer, lung cancer or colorectal cancer (47).

The distribution of PD-L1 positive tumor cells and immune cells varies across different pathological subtypes of TGCT. Choriocarcinoma exhibited a higher prevalence of PD-L1$^+$ staining in tumor cells, while seminoma and embryonal carcinoma displayed a higher frequency of PD-L1$^+$ staining in TIICs. Remarkably, the levels of PD-L1$^+$ tumor-associated macrophages were found to gradually decrease during the reprogramming process from pure seminoma to the seminoma component in mixed GCT, and subsequently to other NSGCT (43). Furthermore, irrespective of the PD-L1 expression pattern, its expression was significantly diminished in teratomas. These findings indicate that the PD-1/PD-L1 signaling pathway plays distinct roles among various subtypes of TGCT. Notably, PD-L1$^+$ TIICs not only predicted the improved prognosis but also correlated with lower pathological grades.

The aforementioned observations strongly suggest that PD-L1 is prominently expressed in TGCT, particularly in TIICs, thereby providing a theoretical foundation and target support for the immunotherapy of TGCT. Simultaneously, the considerable variability in PD-L1 expression among patients highlights the necessity for personalized treatment approaches tailored to different individuals with TGCT. To date, immunotherapy outcomes for platinum-refractory TGCT are unsatisfactory. In a study involving fourteen TGCT patients experiencing relapse or lacking alternative options, no partial or complete responses were observed following PD-1 antibody treatment, despite only two of them were PD-L1 positive (48). Similarly, eight enrolled patients with relapsed or refractory TGCT received PD-L1 antibody immunotherapy but exhibited neither objective responses nor disease stabilization (17). However, certain case reports have described stable disease or partial response in PD-L1 positive TGCT patients following PD-1 antibody treatment (14,15). The existing clinical trial results do not definitively establish a correlation between PD-L1 expression and the efficacy of anti-PD-1/PD-L1 immunotherapy, warranting further validation through additional experiments.

In addition, the expression of PD-L1 in TIICs does not serve as a reliable individual predictor for tumor pathology and prognosis in TGCT. A study demonstrated that there was no significant difference in the 5-year event-free survival (EFS) rate between TGCT patients with PD-L1$^+$ and PD-L1$^-$ TIICs. However, when the data of PD-L1 and V-domain Ig suppressor of T cell activation (VISTA) were combined, lower expression of PD-L1 and VISTA on TIICs significantly correlated with a worse EFS rate in TGCT (29). Furthermore, the systemic immune-inflammation index (SII) is identified as a useful tool for predicting survival outcomes in TGCT patients. Combining SII with the expression of PD-L1 in TIICs provided a more accurate prediction of TGCT patient prognosis (49,50).

However, there were some limitations in this meta-analysis. Firstly, the included studies were of insufficient quality and quantity to generate robust and accurate data. It is better to focus on conducting large-scale studies in TGCT with comprehensive clinical and follow-up information in the future research. Secondly, in terms of population ethnicity, only one study included Asian population, and other studies included patients from Europe and America. Therefore, it is essential to investigate TGCT populations from other regions such as Asia and Africa. Thirdly, there was heterogeneity in the meta-analysis due to the use of different clones of PD-L1 antibodies from various companies. The variation in antibody affinity may lead to different staining results in immunohistochemical experiments. Additionally, discrepancies in the criteria used to determine PD-L1 positivity among different studies can also impact the results. For example, Pęksa *et al.* considered

histoscores of 41 or higher as indicative of PD-L1 positivity, whereas the research group led by Chovanec *et al.* required a histoscore greater than 160 (27,29).

In summary, it is important to recognize the limitations of this meta-analysis, including the inadequate quality and quantity of studies, potential bias in population ethnicity, and heterogeneity in antibody selection and PD-L1 positivity criteria. Future research should address these limitations to enhance the reliability and generalizability of findings in TGCT. Despite the aforementioned limitations, this meta-analysis represents the first comprehensive investigation of the associations between PD-L1 expression and clinicopathological characteristics as well as prognosis in patients with TGCT.

Conclusions

Our findings revealed that PD-L1 was expressed in both tumor cells and TIICs in TGCT. Specifically, PD-L1 expression was observed in choriocarcinoma tumor cells in NSGCT, while yolk sac tumor and teratoma tumor cells exhibited lower or absent expression of PD-L1. Conversely, PD-L1 expression in TIICs was associated with seminoma and embryonal carcinoma, and was more commonly observed in TGCT patients with lower stages or without lymph node metastasis. Furthermore, positive PD-L1 expression in TIICs was found to be a favorable prognostic indicator in TGCT patients, as it correlated with a reduced likelihood of disease progression or relapse.

Acknowledgments

Footnote

Reporting Checklist: The authors have completed the PRISMA reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2302/rc

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2302/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2302/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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