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Research paper

Discordance of immunotherapy response predictive biomarkers between primary lesions and paired metastases in tumours: A multidimensional analysis

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ABSTRACT

Background: Several biomarkers predict the efficacy of immunotherapy, which is essential for selecting patients who would potentially benefit. Discordant status of these biomarkers between primary tumours and paired metastases has been increasingly revealed. We aimed to comprehensively summarize the incidence of this phenomenon.

Methods: Databases were searched to identify studies reporting primary-to-metastatic conversion of biomarkers, including programmed death ligand-1 (PD-L1), programmed cell death protein-1 (PD-1), PD-L2, tumour-infiltrating lymphocyte (TIL), tumour mutational burden (TMB), and microsatellite instability (MSI).

Findings: 56 studies with 2739 patients were included. The pooled discordance rate of PD-L1 was 22%. The percentage of PD-L1 changed from positive to negative was 41%, whereas that from negative to positive was 16%. The discordance rate for PD-1 and PD-L2 was 26% and 22%, respectively. TIL level was found with a discordance rate of 39%, and changes from high to low (50%) occurred more than that from low to high (16%). No significant difference in TMB was observed between two sites in most studies. MSI status discordance was found in 6% patients, with a percentage of 9% from MSI-high to microsatellite instable (MSS) and 0% from MSS to MSI-high.

Interpretation: Our study demonstrates that PD-L1, PD-1, PD-L2, and TIL level had high frequency of discordance, while TMB and MSI status were less likely to change between primary tumours and paired metastases. Therefore, evaluating those frequently altered biomarkers of both primary and metastatic tumours is strongly recommended for precise clinical decision of immune checkpoint treatment.

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1. Introduction

Immune checkpoint therapy targeting programmed death ligand-1/2 (PD-L1/2) and programmed cell death protein-1 (PD-1) has emerged as an effective strategy for various cancers, yielding significant improvement in progression-free and overall survival of patients with metastatic cancer [1,2]. Following the great success of therapeutic antibody ipilimumab in advanced melanoma in 2010 [3], several novel monoclonal antibodies (pembrolizumab, nivolumab, atezolizumab, durvalumab, and avelumab) against these targets have been trialed and approved by the U. S. Food and Drug Administration (FDA) in multiple malignancies [4]. Even so, a series of challenges such as severe immune-related adverse events and finite clinical benefits limited to a specific proportion of patients requires careful consideration [5].

Using biomarkers for the prediction of immune checkpoint therapy efficacy, therefore, have been investigated in various tumours [6,7]. For instance, PD-L1 expression on tumour/immune cells was identified as an ideal biomarker to select potential benefited patients with advanced cancer in different randomized clinical trials. In KEYNOTE-024, patients who had previously untreated advanced non-small cell lung cancer with PD-L1 expression on at least 50% of tumour cells could gain benefit from pembrolizumab monotherapy compared to platinum-based chemotherapy [8]. Atezolizumab combined with nab-paclitaxel revealed an improved overall survival in

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Research in context

Evidence before this study

With the widespread use of several biomarkers in predicting the efficacy of immune checkpoint therapy in multiple advanced cancers (PD-L1 for metastatic non-small-cell lung cancer, microsatellite instability test for metastatic colorectal cancer, tumour mutational burden for metastatic solid tumour, etc.), there is an increasing interest on discordance status of these biomarkers among primary tumours and their metastases. However, controversial data have been reported. Therefore, we conducted a comprehensive literature search for articles evaluating the discordance rate of immunotherapy response biomarkers between primary tumours and paired metastases from databases PubMed, Embase, the Cochrane library, and Web of Science by May 16, 2020. Six widely-studied immunotherapy response biomarkers were analyzed, including programmed death ligand-1 (PD-L1), programmed cell death protein-1 (PD-1), PD-L2, tumour-infiltrating lymphocyte (TIL), tumour mutational burden (TMB), and microsatellite instability (MSI).

Added value of this study

This study provides a comprehensive review of the discordance rates of immunotherapy response biomarkers between primary tumours and paired metastases. Elucidating the predictive value of primary tumour in determining the biomarker status of the metastatic lesion has profound implications in precision immunotherapy.

Implications of all the available evidence

This study demonstrates that PD-L1, PD-1, PD-L2, and TIL level had a high frequency of conversion, while TMB and MSI status were less likely to change between primary tumours and paired metastases. Therefore, evaluating those frequently altered biomarkers of both primary and metastatic tumours is strongly recommended for precise clinical decision of immune checkpoint treatment.

patients with PD-L1 expression on at least 1% of tumour-infiltrating immune cells in advanced triple-negative breast cancer [9]. The combined positive score (CPS), defined as the ratio of PD-L1-positive cells (tumour cells, lymphocytes, and macrophages) to the total number of tumour cells × 100, was used as a method to select patients with advanced cervical cancer for pembrolizumab monotherapy [10]. Additionally, PD-1, PD-L2, tumour-infiltrating lymphocyte (TIL) level, tumour mutational burden (TMB), and microsatellite instability (MSI) status have also been identified as effective predictive biomarkers for checkpoint inhibitor-based immunotherapy in various cancers [11-15]. To confer precise therapies, some biomarkers are routinely recommended and assessed before using immune checkpoint inhibitors [16]. For instance, assay of PD-L1 expression prior to immunotherapy for non-small cell lung cancer is recommend by National Comprehensive Cancer Network guidelines [17]. Pembrolizumab has received accelerated FDA approval for adult and pediatric patients with advanced or metastatic solid tumours with biomarker selected for MSI-high or TMB-high (>10 mut/Mb) who have progressed after the first-line therapy, irrespective of the location of the primary tumour [18,19]. Recently, several lines of evidence have disclosed extensive discrepancies of these immune response biomarkers among primary tumours and their paired metastases [20-24]. PD-L1 conversion was observed in 5−64% patients among primary and metastasis pairs [25,26]. Large-scale differences in the immune microenvironment of primary and metastatic lesions were also highlighted for the expression of PD-1 and PD-L2, with rates ranging from 6% to 50% [27,28] and 17% to 27% [22,29], respectively. Inconsistent TIL counts were reported in breast and lung tumours compared with their metastases [20,30]. High concordance of MSI status is found in primary colorectal cancers and their matched liver, lung, and distant lymph node metastases with a total incidence of 2−16% [31,32]. Although discordant status of these biomarkers between primary and metastatic sites has been extensively reported, results from different studies are yet controversial.

In particular, the discordance between primary tumours and metastases from negative to positive and vice versa potentially affects the treatment strategy [17,33]. Nonetheless, metastatic materials are hard to obtain in some circumstances, due to their deep locations (brain, vertebra, etc.) or poor physical condition of patients with advanced cancer. In most cases, only the archived primary tissue is available. As such, unraveling the discordance rate of these biomarkers among primary and metastatic tumour sites would offer critical guidance to tailor immune checkpoint treatments. Nevertheless, a comprehensive summary and critical appraisal of quantitative evidence on this topic is still lacking.

Therefore, we performed a systematic review and meta-analysis to evaluate the conversion rates of six widely-studied immunotherapy response markers (PD-L1, PD-1, PD-L2, TIL, TMB, and MSI status) among primary tumours and paired metastases, paying special attention to the origin of primary tumours, sites of metastasis, timing of metastasis, methods, and positivity threshold for assessment.

2. Methods

2.1. Search strategy

This systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [34]. The review protocol is available on PROSPERO under registration number CRD42020180589 (www.crd.york.ac.uk/PROSPERO).

The PICOTS system was used to describe the key items for framing the objective and methodology of this review:

- Population—patients with synchronous/metachronous metastatic solid tumour.
- Index factors—Immunotherapy response markers (PD-L1, PD-1, PD-L2, TIL, TMB, and MSI status).
- Comparator factors—not applicable for this review.
- Outcomes—discordance rate between primary lesions and paired metastases.
- Timing—biomarker measurements were performed either before or after tumour metastasis.
- Setting—hospital/treatment center.

A comprehensive search of online databases, including PubMed, Embase, the Cochrane library, and Web of Science was performed on May 16, 2020. The literature search included the following terms (with MeSH terms, synonyms, and closely related words): “cancer” and “metastasis”, combined with “programmed death ligand 1,” “programmed death-1,” “programmed death ligand 2,” “tumour infiltrating lymphocyte,” “tumour mutational burden,” “microsatellite instability,” and “conversion/discordance.” The detailed search strategy is presented in ***Supplemental Methods***. Reference lists of retrieved articles were screened manually to ensure sensitivity of the search strategy and to identify additional relevant studies.

2.2. Inclusion and exclusion criteria

Inclusion and exclusion criteria were prespecified. Original full-text research articles reporting PD-L1, PD-1, PD-L2, TIL, TMB, or MSI status in both primary solid tumours and paired metastases were

included. Both prospective and retrospective studies were considered eligible. Articles published online "ahead of print" were included. Exclusion criteria were reporting receptors other than immunotherapy response markers, without paired lesions comparison, insufficient data, case reports, letters, commentaries, and reviews. When duplicate studies from the same cohort were identified, only the ones with the most complete and updated data were included. English was imposed as language limitation.

2.3. Study selection

All search results were independently inspected by two authors (Y.Z. and X.H.) and discrepancies were reevaluated by a third reviewer (S.Z.). Reviewers applied selection criteria after screening the potentially included studies. Duplicates were removed using Endnote X9 software or manually.

2.4. Data extraction

Baseline characteristics of each study (authors, year of publication, country of origin, study design, immune response biomarkers, cancer types, sample size, age, metastatic sites, timing of metastasis, scoring method, positivity threshold, specimen resource and number of observers) were recorded by two reviewers independently (Y.Z. and X.H.). The primary outcome was the total conversion rate. The secondary outcomes were the conversion rates of specific patterns: one is conversion rate from positive (primary site) to negative (metastatic site), another is conversion rate from negative (primary site) to positive (metastatic site). Data extracted from each study were presented as events and total number. Median mutation per mega base was extracted for studies that compare TMB between primary tumours and paired metastases.

2.5. Quality assessment of methodology

Quality assessment of each eligible study was conducted with the QUADAS-2 tool [35]. This tool consists of four key domains including patient selection, index test, reference standard, and flow of patients through the study (timing of the index test and reference standard). For each study, the first three items were assessed in terms of risk of bias and applicability, while risk of bias was considered for the flow of patients through the study. For patient selection, we evaluated the items including consecutive enrollment of patients, inappropriate inclusion/exclusion criteria, and prospective or retrospective design of the study. For the item of index test, we considered the clear description and standardization of the analysis (assessment method,

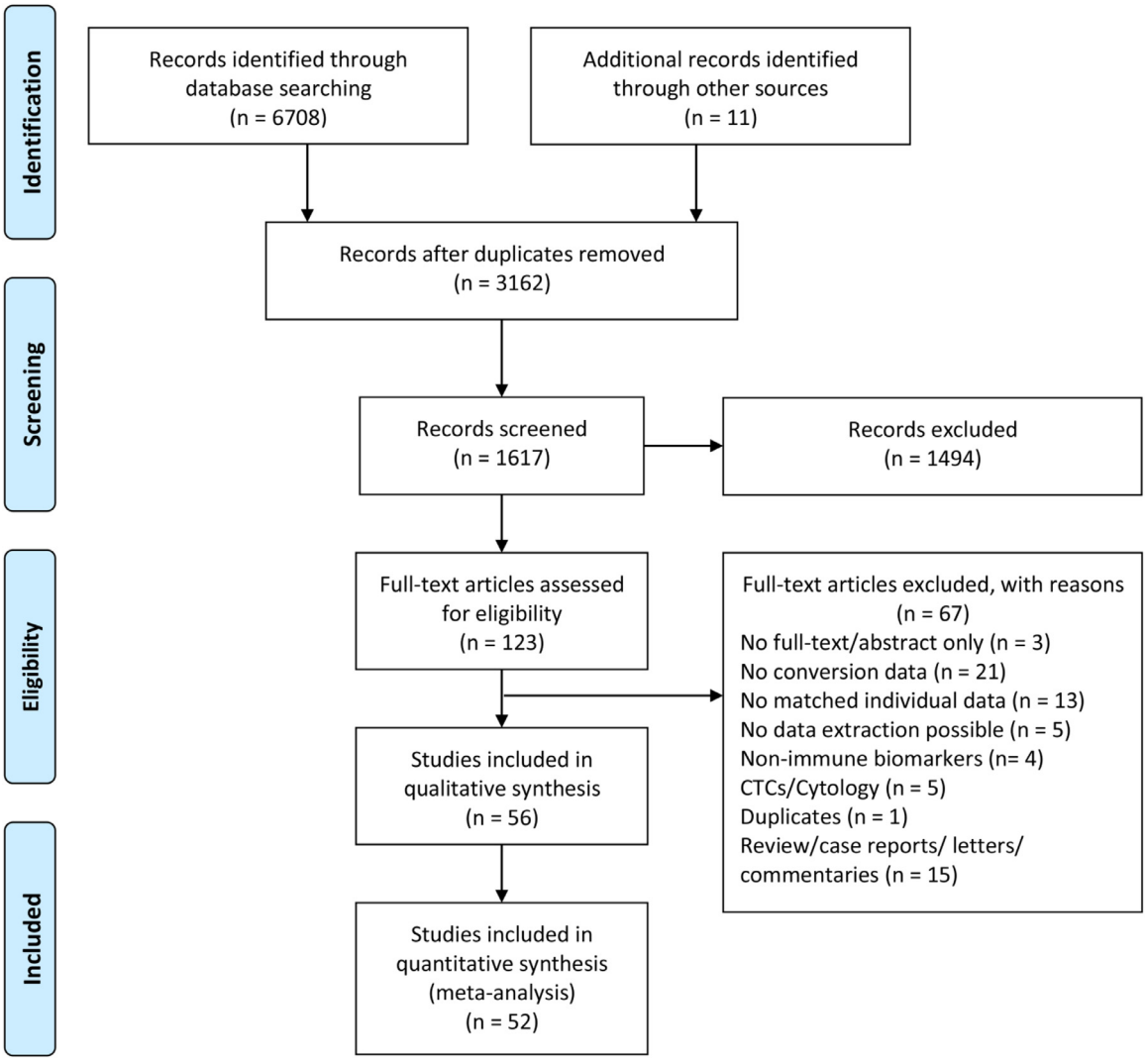


Fig. 1. PRISMA flow diagram of study selection and retrieval.
Abbreviations: CTC, circulating tumour cells.

scoring rule, threshold of positivity, blinding). Status of biomarkers in primary tumours was taken as the reference standard and scrutinized with the same criteria. Flow and timing were considered by the interval between index test and reference standard and the follow-up. Risk of bias and concern of applicability for each domain were rated as low, high, or unclear. In case of disagreement, the study was discussed until consensus was reached among the two investigators.

2.6. Data synthesis and analysis

Discordance rates and 95% confidence intervals (CI) of PD-L1, PD-1, PD-L2, TIL, and MSI status were extracted for each study. The random-effects model was applied to obtain pooled rates and the 'meta' package in R software (version 3.5.0) was used for data presentation. Heterogeneity of studies was estimated by Cochran's Q test (reported with a $\chi 2$ value and P value), which was manifested if $P < 0.1$ [36]. In addition, I^2 statistic with values over 50% or 75% is also used for indicating moderate or high heterogeneity respectively. Subgroup analysis was performed to evaluate the discordance rate in different subsets (positivity threshold, scoring method, metastatic site, etc.) and identify the possible sources of heterogeneity. Egger's test was performed with Stata software 15.1 (Stata Corp, College Station, Tex) to assess potential publication bias [37].

2.7. Role of funding source

The funding bodies had the role in interpretation and publication.

3. Results

3.1. Baseline characteristics of included studies

A total of 6708 potential articles were screened and 56 studies (2739 patients) were identified for systematic review, as details of our literature search are summarized in the PRISMA flow diagram (Fig. 1). Thirty-eight studies reported PD-L1 [20-22,25-28,38-68],

Table 1
Main characteristics of studies eligible for this systematic review and meta-analysis. Abbreviation: P, prospective study; R, retrospective study; PD-1, programmed death-1; PD-L1, programmed death ligand 1; PD-L2, programmed death ligand 2; TIL, tumor-infiltrating lymphocyte; TMB, tumor mutational burden; MSI, microsatellite instability; NA, not available.

Study	Country	Design	Cancer types	Sample size	Metastatic sites	Timing of metastasis	Analyzed biomarkers
Messick 2010 [81]	USA	P	Colorectal cancer	21	Lymph node	Synchronous	MSI
Murata 2013 [80]	Japan	R	Colorectal cancer	26	Liver, lymph node	Synchronous/ Metachronous	MSI
Madore 2014 [68]	Australia	R	Melanoma	54	Brain, subcutaneous, bone, visceral	NA	PD-L1
Baine 2015 [75]	USA	R	Kidney renal clear cell carcinoma	20	Lung, bone, liver, lymph node, skin	Metachronous	TIL
Callea 2015 [21]	USA	R	Kidney renal clear cell carcinoma	53	Brain, lung, bone, soft tissues, lymph node	NA	PD-L1
Kim 2015 [67]	Korea	R	Non-small cell lung cancer	74	Lymph node	Synchronous	PD-L1
Cimino-Mathews 2016 [66]	USA	R	Breast cancer	26	Brain, lung, ovary, liver, peri-vertebral	NA	PD-L1
Heeren 2016 [65]	Netherlands	R	Cervical cancer	99	Lymph node	NA	PD-L1
Inoue 2016 [64]	Japan	R	Non-small cell lung cancer	132	Lymph node	Synchronous	PD-L1
Mansfield 2016 [63]	USA	R	Non-small cell lung cancer	73	Brain	Synchronous/ Metachronous	PD-L1, TIL
Ogiya 2016 [29]	Japan	R	Breast cancer	25	Skin, brain, liver, lung, bone	Metachronous	PD-L2, TIL
Pinato 2016 [62]	UK	R	Non-small cell lung cancer	65	Liver, adrenal, bone, brain	Synchronous	PD-L1, PD-L2
Straub 2016 [61]	Germany	R	Head and neck squamous cell carcinoma	28	Lymph node	Synchronous/ Metachronous	PD-L1
Uruga 2016 [60]	USA	R	Non-small cell lung cancer	33	Brain, liver, bone, lymph node	Synchronous	PD-L1
Fujiyoshi 2017 [79]	Japan	R	Colorectal cancer	161	Liver	Synchronous/ Metachronous	MSI
Kim H 2017 [59]	Korea	R	Non-small cell lung cancer	37	Brain, pleural	Metachronous	PD-L1
Kim S 2017 [58]	Korea	R	Non-small cell lung cancer	161	Brain, liver, lymph node, adrenal gland, bone, soft tissue	Synchronous/ Metachronous	PD-L1
Ogiya 2017 [74]	Japan	R	Breast cancer	46	Brain	Metachronous	TIL
Pichler 2017 [57]	Austria	P	Bladder cancer	27	Liver, lung, lymph node	Metachronous	PD-L1
Roper 2017 [56]	Australia	R	Head and neck squamous cell carcinoma	38	NA	NA	PD-L1
Takamori 2017 [55]	Japan	R	Non-small cell lung cancer	21	Brain, adrenal gland, spleen, jejunum	Synchronous/ Metachronous	PD-L1
Eckstein 2018 [73]	Germany	R	Bladder cancer	15	Liver	NA	TIL
Jong 2018 [54]	Netherlands	R	Bladder cancer	81	Lymph node	Synchronous/ Metachronous	PD-L1
Keller 2018 [26]	Switzerland	R	Non-small cell lung cancer	40	Lymph node	NA	PD-L1
Mansfield 2018 [23]	USA	R	Non-small cell lung cancer	13	Brain	Metachronous	TMB
Miyamoto 2018 [53]	Japan	R	Colorectal cancer	50	Lung	Synchronous/ Metachronous	PD-L1, PD-1
Okada 2018 [25]	Japan	R	Head and neck squamous cell carcinoma	25	Lung	Metachronous	PD-L1
Roussille 2018 [28]	France	R	Colorectal cancer	32	Brain	Synchronous/ Metachronous	PD-L1, PD-1
Schneider 2018 [52]	Austria	R	Head and neck squamous cell carcinoma	69	Lymph node	NA	PD-L1, PD-1
Scognamiglio 2018 [51]	USA	R	Head and neck squamous cell carcinoma	34	Lymph node	NA	PD-L1
Shibutani 2018 [72]	Japan	R	Colorectal cancer	24	Liver	Metachronous	TIL
Szekely 2018 [20]	Italy	R	Breast cancer	72	NA	Metachronous	PD-L1, TIL
Takamori 2018 [50]	Japan	R	Colorectal liver, bladder, breast cancer	44	Lung	Synchronous	PD-L1
Tawfik 2018 [49]	USA	R	Breast cancer	41	Lymph node	NA	PD-L1
Tretiakova 2018 [48]	USA	R	Bladder cancer	79	NA	NA	PD-L1
Yang 2018 [47]	USA	R	Melanoma	43	Skin	NA	PD-L1
Zhou 2018 [46]	China	R	Non-small cell lung cancer	25	Brain	Synchronous/ Metachronous	PD-L1, TIL
Alves 2019 [45]	Portugal	R	Breast cancer	44	Lymph node	NA	PD-L1
Basu 2019 [44]	USA	R	Kidney renal clear cell carcinoma	49	Lung, lymph node, adrenal glands, soft tissue	Synchronous/ Metachronous	PD-L1, PD-L2, PD-1
Batur 2019 [43]	Turkey	R	Non-small cell lung cancer	24	Brain	Metachronous	PD-L1, TIL
Erol 2019 [42]	Turkey	R	Breast cancer	20	Bone, lymph node, visceral organs	NA	PD-L1
He 2019 [32]	China	R	Colorectal cancer	55	Lung, liver, peritoneal	Metachronous	MSI
Kim 2019 [71]	Korea	R	Non-small cell lung cancer	13	Brain	Metachronous	TIL
Manson 2019 [27]	Netherlands	R	Breast cancer	49	Bone, brain, liver, lung/pleural, uterus	Metachronous	PD-L1, PD-1
Patel 2019 [41]	USA	R	Breast cancer	67	Lymph node	Synchronous	PD-L1
Sun 2019 [24]	China	R	Colorectal cancer	33	Brain, liver	Metachronous	MSI
Tyran 2019 [78]	France	R	Breast cancer	14	Brain	Metachronous	TMB
Yuan 2019 [40]	China	P	Breast cancer	47	Lymph node	NA	PD-L1, PD-1
Zhang 2019 [22]	China	R	Kidney renal clear cell carcinoma	83	Bone, lymph node, lung	NA	PD-L1, PD-L2, PD-1
Zhu 2019 [70]	USA	P	Breast cancer	49	Brain, bone, ovary, gastrointestinal tract	Metachronous	TIL
Eckel-Passow 2020 [39]	USA	R	Kidney renal clear cell carcinoma	140	Brain, bone, lymph node, adrenal, skin, lung, liver	Synchronous/ Metachronous	PD-L1, PD-1
Luo 2020 [38]	China	R	Non-small cell lung cancer	30	Brain, bone, liver, lymph node, pleura	NA	PD-L1
He 2020 [77]	USA	R	Synovial sarcoma	7	Lung, liver, pleura	Metachronous	TMB
Hutchinson 2020 [30]	USA	R	Breast cancer	37	Brain, bone, skin, lymph node	Metachronous	TIL, TMB
Jiang 2020 [76]	China	R	Lung cancer	20	Brain	Metachronous	TMB
Schlicker 2020 [31]	Germany	R	Colorectal cancer	51	Liver	Synchronous	MSI

eight studies reported PD-1 [22,27,28,39,40,44,52,53], four studies reported PD-L2 [22,29,44,62], twelve studies reported TIL [20,29,30,43,46,69-75], five studies reported TMB [23,30,76-78], and six studies reported MSI status [24,31,32,79-81] conversion between primary lesions and paired metastases. Nine different cancer types were considered among all eligible studies in this analysis, including fifteen for the non-small cell lung cancer, fourteen for breast cancer, nine for colorectal cancer, five for the kidney renal clear cell carcinoma, five for the head and neck squamous cell carcinoma, four for bladder cancer, two for melanoma, one for synovial sarcoma and one for cervical cancer. Studies comparing TMB between primary tumours and paired metastases were only summarized in systematic review, as no dichotomous variable data was available. Main characteristics of the included studies are presented in Table 1 and ***Supplemental Table 1-6***. Totally, 52 studies with 2685 patients were included for the final meta-analysis. The methodology quality of included studies was assessed by the QUADAS-2 tool (***Supplemental Table 7***).

3.2. PD-L1 conversion rate between primary tumour and paired metastases

Conversion rates of PD-L1 were available in thirty-eight studies with a total of 2109 patients. Assessment details of these studies concerning PD-L1 conversion are shown in ***Supplemental Table 1***. The pooled total conversion rate of PD-L1 was 22% (95% CI = 18% to 26%) (Fig. 2a). The percentage of PD-L1 changed from positive to negative was 41% (95% CI = 33% to 49%), whereas from negative to positive was 16% (95% CI = 11% to 22%) (Fig. 2b-c). Subgroup analysis was performed and outcomes are shown in Fig. 3-4 and Supplemental Fig. S3-23. In subgroup analysis concerning different primary tumours, head and neck squamous cell carcinoma had the highest total conversion rate in paired metastases (35%, 95% CI = 21% to 48%) while bladder cancer had the lowest one (16%, 95% CI = 8% to 25%) among all cancers (Fig. 3). Analysis based on metastatic sites revealed that lung metastases (36%, 95% CI = 6% to 65%) showed a higher total PD-L1 conversion rate than brain metastases (15%, 95% CI = 9% to 20%). Additionally, heterogeneity decreased in varying degrees after dividing studies based on cut-off value and assessment method in IHC diagnosis, specimen source, number of observers and time of metastasis (Fig. 4). Metachronous metastasis and IHC assessment by multiple pathologists were related to a higher total PD-L1 conversion rate among primary tumour and paired metastases.

3.3. PD-1 conversion rate between primary tumour and paired metastases

Conversion rates of PD-1 were available in eight studies with a total of 562 patients. Assessment details of these studies concerning PD-1 conversion are shown in Supplemental Table 2. The total conversion rates of PD-1 varied between studies from 6% to 50%, with a pooled random effects percentage of 26% (95% CI = 15% to 36%) (Fig. 5a). The proportion of PD-1 converting from positive to negative was 38% (95% CI = 18% to 58%), and that from negative to positive was 23% (95% CI = 8% to 37%) (Fig. 5b-c). Subgroup analysis was performed and outcomes are shown in Supplemental Figure S1 and S24-26. In subgroup analysis based on different primary tumours, metastases of colorectal cancer ranked highest in the total conversion rate (64%, 95% CI = 30% to 100%) while that of head and neck squamous cell carcinoma showed the lowest rank (8%, 95% CI = 2% to 15%). Studies were further dichotomized into two groups by 1% or 5% positivity thresholds, showing a total pooled PD-1 conversion percentage of 43% (95% CI = 23% to 64%) and 8% (95% CI = 2% to 15%), respectively. Discordance of PD-1 was more common when samples came from tissue microarray (44%, 95% CI = 10% to 79%) than that from whole tissue (32%, 95% CI = 8% to 56%) (Supplemental Figure S1).

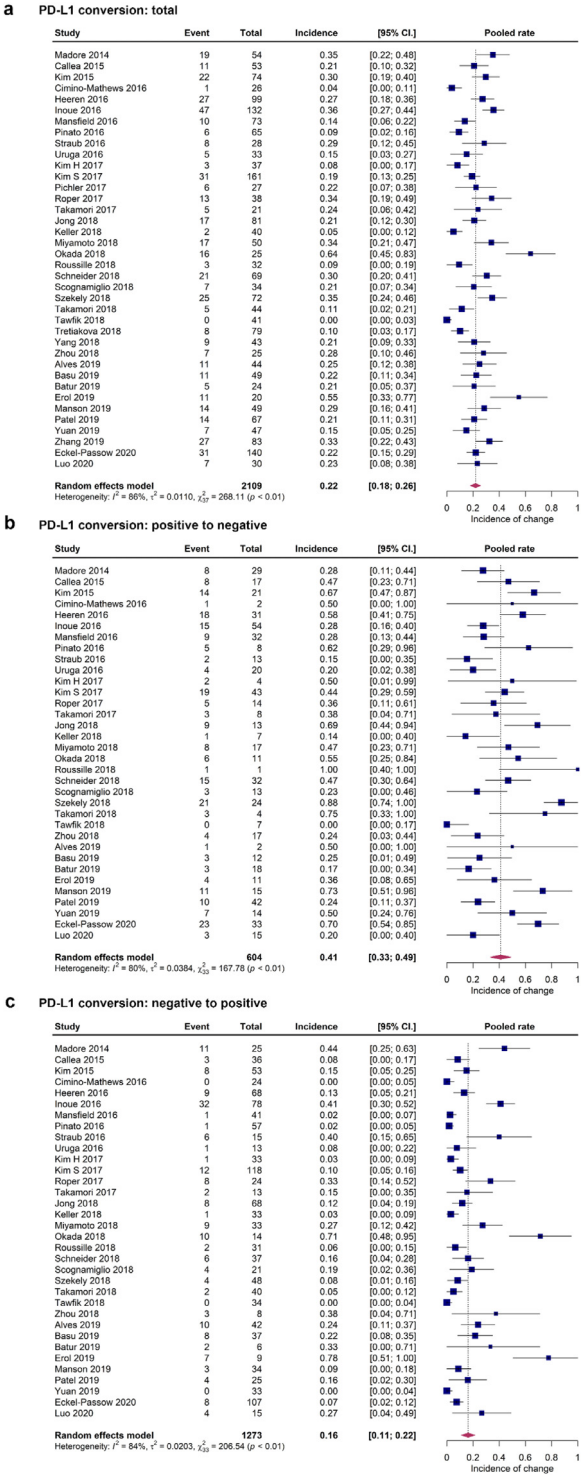


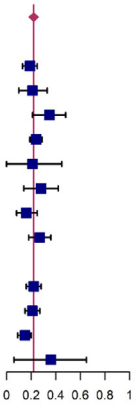
Fig. 2. Study-specific and pooled estimates for PD-L1 conversion rates among primary and metastatic sites. Discordance rates are shown for (a) total, (b) from positive to negative, and (c) from negative to positive conversion.

3.4. PD-L2 conversion rate between primary tumour and paired metastases

Conversion rates of PD-L2 were available in four studies with a total of 207 patients. The detailed assessment method of PD-L2 is

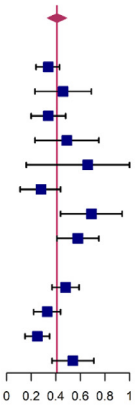
a PD-L1 conversion: total

Subgroup	No. of Study	Sample size	Incidence of change	I^2
All patients	**38**	**2109**	**0.22 (0.18-0.26)**	**86%**
Cancer type				
Non-small cell lung cancer	12	715	0.19 (0.13-0.25)	78%
Breast cancer	8	366	0.21 (0.10-0.33)	92%
Head and neck squamous cell carcinoma	5	194	0.35 (0.21-0.48)	72%
Kidney renal clear cell carcinoma	4	325	0.24 (0.19-0.29)	13%
Colorectal cancer	2	82	0.21 (0.00-0.45)	88%
Melanoma	2	97	0.28 (0.14-0.42)	60%
Bladder cancer	3	187	0.16 (0.08-0.25)	57%
Cervical cancer	1	99	0.27 (0.18-0.36)	/
Metastatic site				
Distant metastasis	18	978	0.22 (0.16-0.28)	79%
Locoregional lymph node metastasis	15	828	0.21 (0.15-0.27)	90%
Brain metastasis	4	154	0.15 (0.09-0.20)	22%
Lung metastasis	3	119	0.36 (0.06-0.65)	92%



b PD-L1 conversion: positive to negative

Subgroup	No. of Study	Sample size	Incidence of change	I^2
All patients	**34**	**604**	**0.41 (0.33-0.49)**	**80%**
Cancer type				
Non-small cell lung cancer	12	243	0.34 (0.24-0.43)	57%
Breast cancer	8	117	0.46 (0.23-0.69)	92%
Head and neck squamous cell carcinoma	5	83	0.34 (0.20-0.48)	52%
Kidney renal clear cell carcinoma	4	62	0.49 (0.23-0.75)	79%
Colorectal cancer	2	18	0.66 (0.16-1.00)	61%
Melanoma	1	29	0.28 (0.11-0.44)	/
Bladder cancer	1	13	0.69 (0.44-0.94)	/
Cervical cancer	1	31	0.58 (0.41-0.75)	/
Metastatic site				
Distant metastasis	15	230	0.48 (0.37-0.59)	67%
Locoregional lymph node metastasis	15	292	0.33 (0.22-0.44)	76%
Brain metastasis	5	68	0.25 (0.15-0.35)	58%
Lung metastasis	4	32	0.54 (0.37-0.71)	0%



c PD-L1 conversion: negative to positive

Subgroup	No. of Study	Sample size	Incidence of change	I^2
All patients	**34**	**1273**	**0.16 (0.11-0.22)**	**84%**
Cancer type				
Non-small cell lung cancer	12	472	0.12 (0.04-0.20)	84%
Breast cancer	8	249	0.14 (0.00-0.29)	86%
Head and neck squamous cell carcinoma	5	111	0.34 (0.16-0.53)	79%
Kidney renal clear cell carcinoma	3	180	0.10 (0.04-0.17)	48%
Colorectal cancer	2	64	0.16 (0.00-0.36)	82%
Melanoma	1	25	0.44 (0.25-0.63)	/
Bladder cancer	1	68	0.12 (0.04-0.19)	/
Cervical cancer	1	68	0.13 (0.05-0.21)	/
Metastatic site				
Distant metastasis	15	595	0.12 (0.05-0.18)	76%
Locoregional lymph node metastasis	15	536	0.16 (0.09-0.22)	85%
Brain metastasis	4	86	0.10 (0.00-0.22)	56%
Lung metastasis	3	87	0.33 (0.00-0.71)	94%

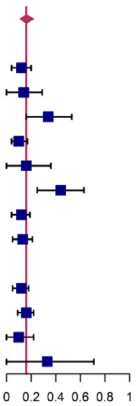


Fig. 3. Subgroup analysis of PD-L1 conversion rates based on cancer types and metastatic sites. Discordance rates are shown for (a) total, (b) from positive to negative, and (c) from negative to positive conversion.

showed in ***Supplemental Table 3***. The total discordance percentage for PD-L2 varied between studies from 17% to 27%, with a pooled random effects percentage of 22% (95% CI = 17% to 28%) (Fig. 6a). The percentage of PD-L2 changed from positive to negative was 41% (95% CI = 7% to 76%), and the percentage from negative to positive was 11% (95% CI = 5% to 18%) (Fig. 6b-c). Because few studies were identified and little heterogeneity was observed, subgroup analysis was not performed in PD-L2 conversion analysis.

3.5. TIL level conversion rate between primary tumour and paired metastases

Conversion rates of TIL level were available in twelve studies with a total of 333 patients. The detailed assessment method of TIL is shown in ***Supplemental Table 4***. Changes in TIL level between primary tumour and paired metastases were found with a pooled total discordance rate of 39% (95% CI = 29% to 49%) (Fig. 7a). The pooled

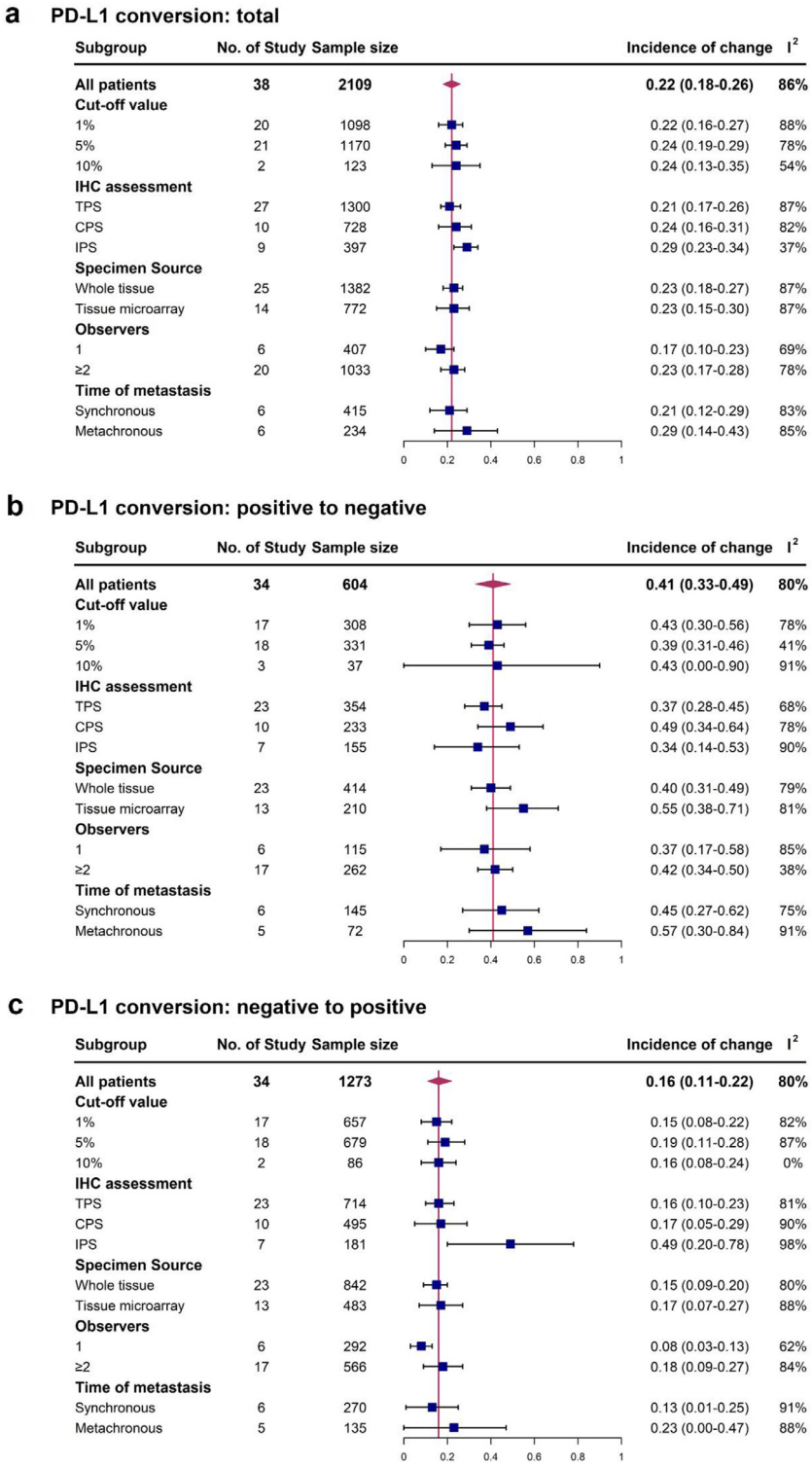


Fig. 4. Subgroup analysis of PD-L1 conversion rates based on IHC positivity threshold, assessment methods, specimen sources, the number of observers, and the time of metastasis. Discordance rates are shown for (a) total, (b) from positive to negative, and (c) from negative to positive conversion.

Abbreviations: CPS, combined positive score; TPS, tumour proportion score; IPS, immune cell proportion score.

proportion of positive to negative and negative to positive conversion was 50% (95% CI = 37% to 62%) and 16% (95% CI = 8% to 23%), respectively (Fig. 7b-c). Subgroup analysis was performed and outcomes are shown in ***Supplemental Figure S2 and S27-36***. Heterogeneity was considerably decreased when studies were divided into certain subgroups. Non-small cell lung cancer had the highest total conversion rate (70%, 95% CI = 46% to 94%) while breast cancer had the lowest total conversion rate (37%, 95% CI = 25% to 49%) among all cancers. Tumours with brain metastasis (66%, 95% CI = 48% to 84%) had higher total TIL level conversion rate than liver metastasis (50%, 95% CI = 29% to 70%). Compared with HE staining subgroup (55%), discordance was less frequently observed in bioinformatic method

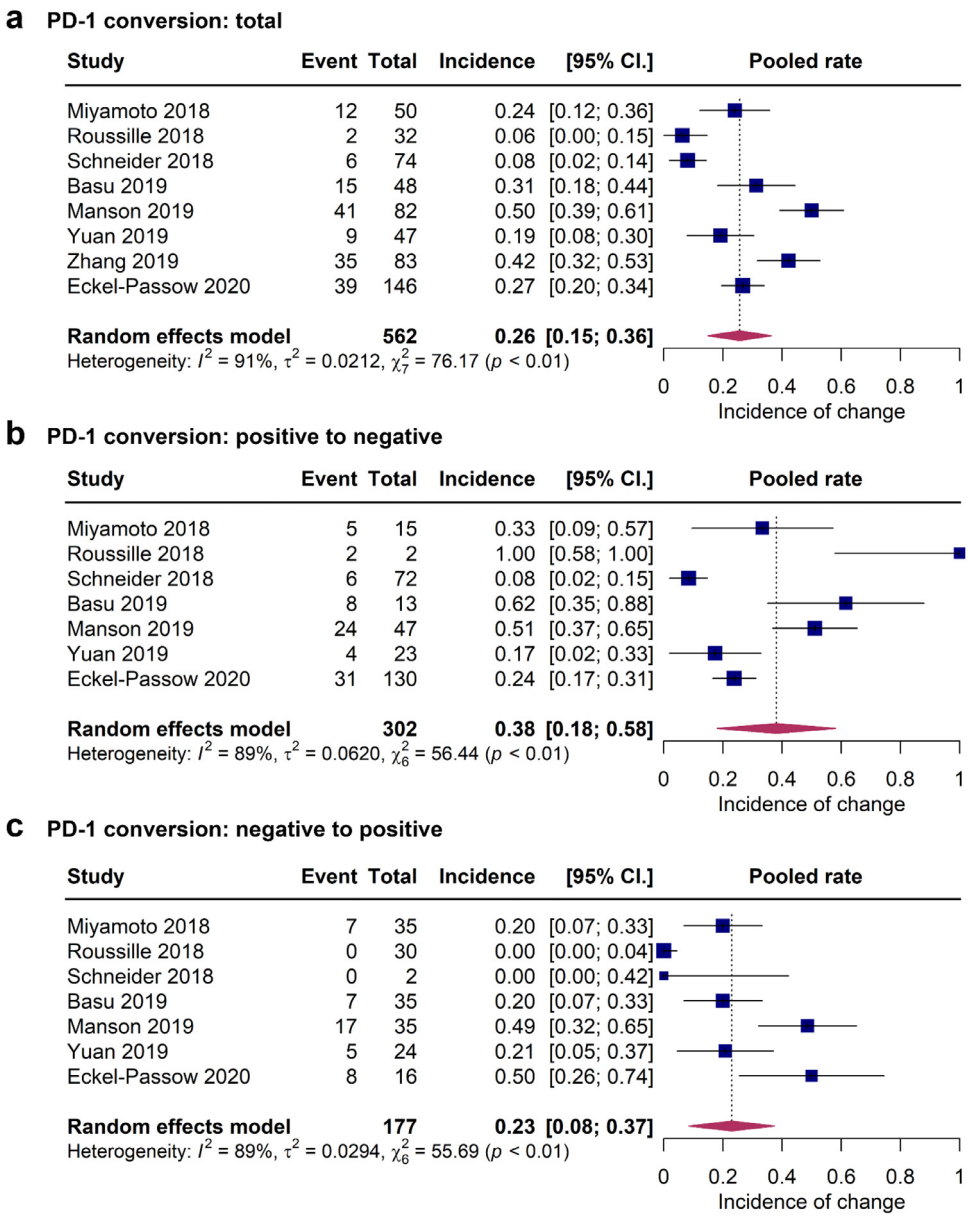


Fig. 5. Study-specific and pooled estimates for PD-1 conversion rates among primary and metastatic sites. Discordance rates are shown for (a) total, (b) from positive to negative, and (c) from negative to positive conversion.

subgroup (29%) between primary tumour and paired metastases. The frequency of total conversion was 56% (95% CI = 37% to 74%) for the 5% threshold and 38% (95% CI = 21% to 54%) for the 10% threshold (***Supplemental Figure S2***).

3.6. TMB status variation between primary tumour and paired metastases

The data for TMB status variation between primary tumour and paired metastases were available from five studies including 75 patients. The detailed outcomes of TMB status is shown in ***Supplemental Table 5***. In the study by Mansfield et al. there was a significantly higher TMB in brain metastases (median 24.9 / Megabase (Mb)) than in paired primary lung cancers (median 12.5 / Mb) [23]. However, another study with similar design reported a higher but nonsignificant TMB in brain metastases compared with primary lung cancers [76]. TMB was also higher in the brain metastases (median 10.2 / Mb) than in paired primary breast cancer (median 7.0 / Mb), but the difference was not significant either [78]. Additionally, no significant difference in TMB was observed between primary and metastatic pairs in triple-negative breast cancer [30]. In synovial sarcoma, median TMB was lower in matched metastatic lesions (median 3.2 / Mb) than in primary tumours (median 3.3 / Mb) [77].

3.7. MSI status conversion rate between primary tumour and paired metastases

Conversion rates of MSI status were available in six studies with a total of 347 patients. Assessment details of these studies concerning MSI status conversion are shown in ***Supplemental Table 6***. MSI status was classified into microsatellite instability-high (MSI-H) and microsatellite stable (MSS) in the included studies. MSI status was assessed by polymerase chain reaction in four studies and next-generation sequencing in two studies. The pooled total conversion rate of MSI status was 6% (95% CI = 1% to 11%) (Fig. 8a). The percentage of MSI status changed from MSI-H to MSS was 9% (95% CI = 0% to 17%),

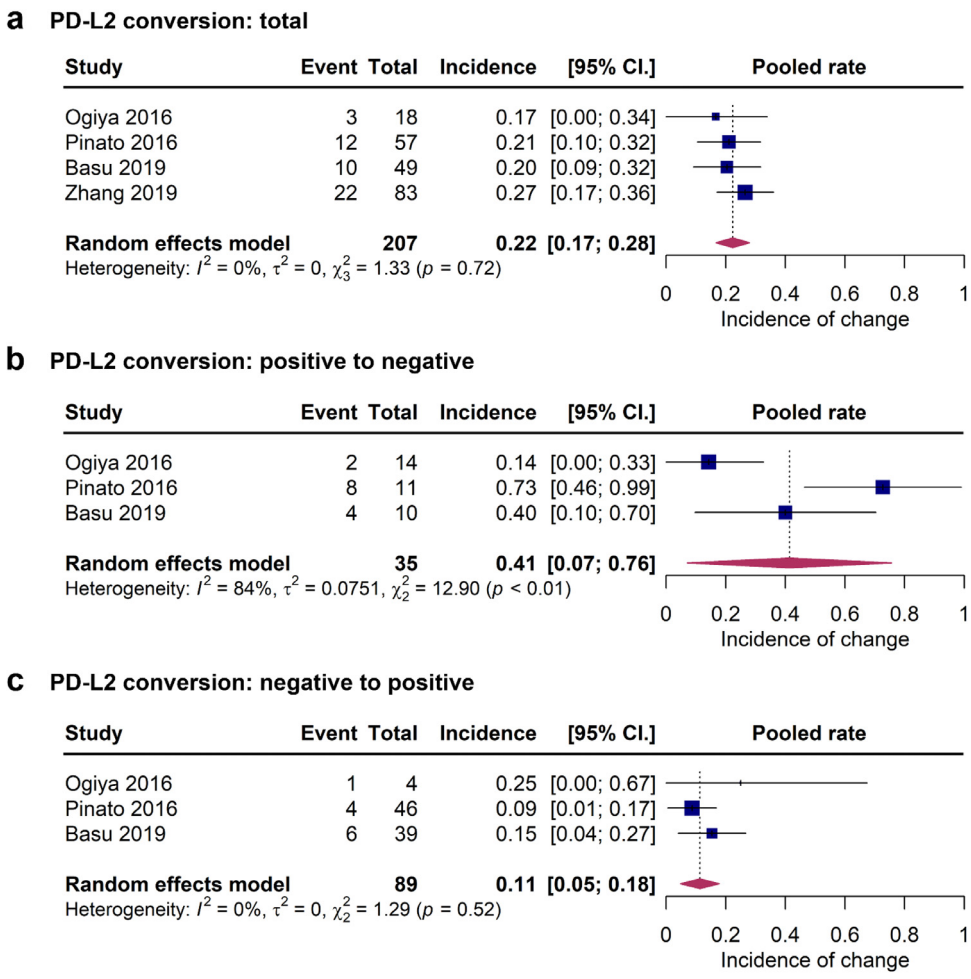


Fig. 6. Study-specific and pooled estimates for PD-L2 conversion rates among primary and metastatic sites. Discordance rates are shown for (a) total, (b) from positive to negative, and (c) from negative to positive conversion.

whereas that from MSS to MSI-H was 0% (95% CI = 0% to 1%) (Fig. 8b-c). Pooled statistics for the conversion rates of immunotherapy response markers among primary tumours and paired metastases in this study are summarized in Fig. 9.

4. Discussion

To date, this study is the first meta-analysis summarizing the conversion rates of immune checkpoint therapy response biomarkers between primary tumours and paired metastases. Six widely-studied biomarkers that are crucial for the efficacy of immune checkpoint therapy were included for analysis. Origin of primary tumours, sites of metastasis, timing of metastasis, as well as methods and positivity threshold for assessment were considered in subgroup analysis. Our results demonstrated that most of these biomarkers had varying degrees of discordance between primary tumours and metastases. Generally, PD-L1, PD-1, PD-L2, and TIL level had a high frequency of conversion, while TMB and MSI status were less likely to alter between primary tumours and paired metastases.

PD-L1 is one of the most extensively studied predictive biomarkers for immune checkpoint therapy in clinical trials [16]. This study showed that changed expression of PD-L1 from primary to metastatic sites frequently occurred with a total pooled conversion rate of 22% in this study. Moreover, the pooled frequency of PD-L1 conversion from positive to negative (41%) was remarkably higher than that from negative to positive (16%). Similarly, about one quarter of primary-metastatic paired lesions had discordant expression of PD-1 or PD-L2, showing a more frequent change from positive to negative. Therefore, evaluating the biomarker status of both primary and metastatic tumours is of paramount importance for clinical decisions on systemic treatment strategy. Heterogeneity of studies on PD-L1, PD-1 and PD-L2, is mainly attributed to the discrepancy of IHC assessments. Considering assessing interval, metachronous metastases presented a higher PD-L1 total conversion rate when compared to synchronous metastases, which might result from selective pressure of systematic therapy. In line with our finding, extensively altered expression of PD-L1 after systematic treatment (e.g. chemotherapy) was previously reported [82,83]. Positivity threshold for biomarker assessment was found to be inconsistent in the different studies, which is also deemed as a source of heterogeneity in subgroup analysis. Heterogeneity could also be explained by IHC scoring methods, among which combined positive score (CPS), tumour proportion score (TPS), and immune cell proportion score (IPS) are commonly used for immunohistochemical assay of immune checkpoints [84,85]. Discordance was observed more frequently in studies using IPS, possibly due to the differences of microenvironmental immune infiltration between primary and metastatic tumours [86-88]. Up to now, standard IHC testing of these biomarkers (especially for PD-L1) are still lacking in terms of various antibodies, different scoring methods, and inconsistent positivity thresholds [89]. Besides, long-term (more than 1 year) storage of the samples resulted in the degradation of PD-L1 which can lead to a decrease in the quality of testing [90]. Such that, reaching to a consensus about details in biomarker assessment is also indispensable for the guidance of immunotherapy.

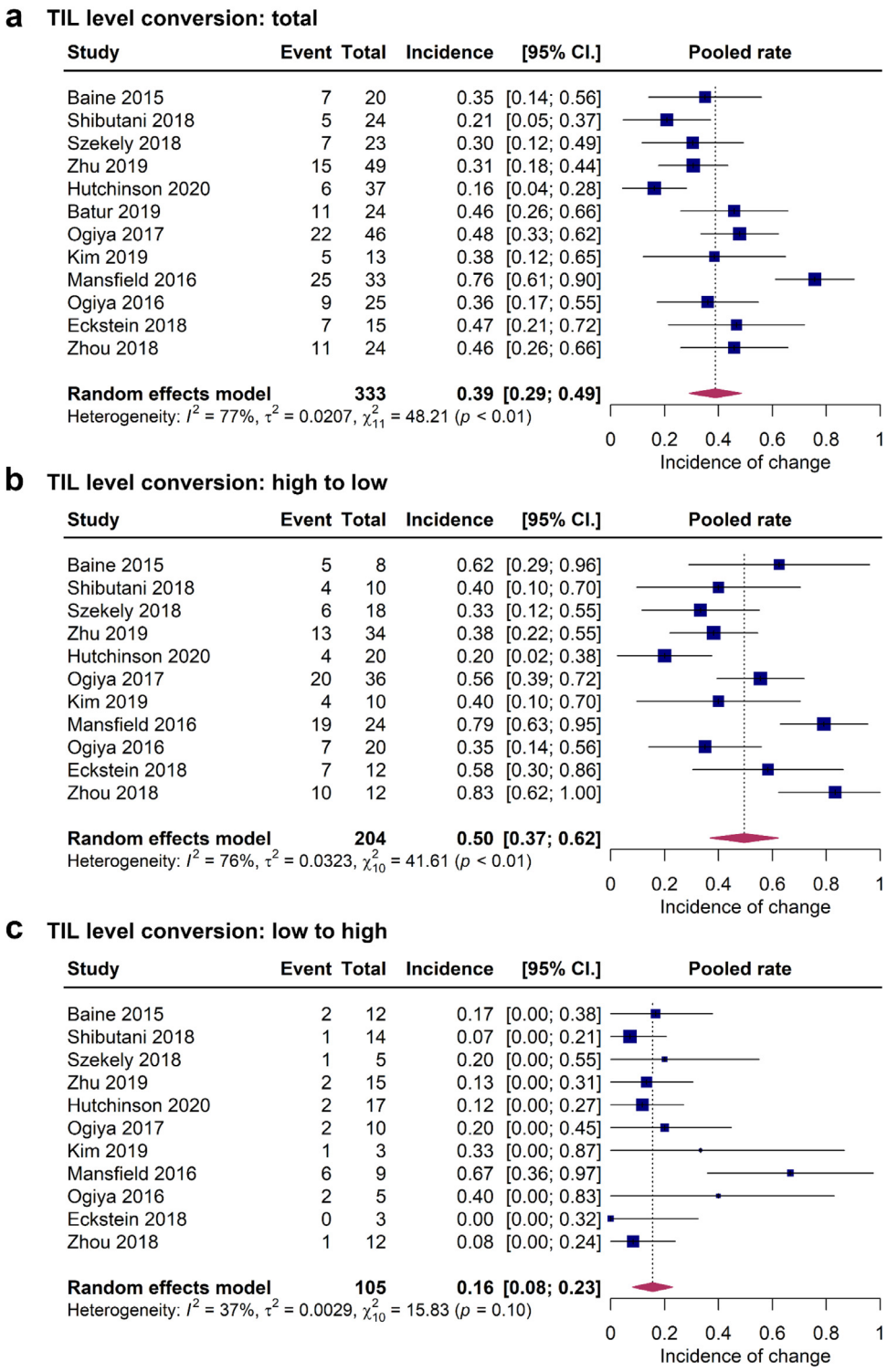


Fig. 7. Study-specific and pooled estimates for conversion rates of TIL level among primary tumours and paired metastases. Discordance rates are shown for (a) total, (b) from high to low, and (c) from low to high conversion.

Of note, TIL level was changed in more than one third of primary and metastasis pairs (39%), and the incidence was overwhelmingly higher in conversion from high to low level (50%) than from low to high level (16%) in the pooled analysis. Particularly, brain metastasis of tumours was more likely to occur high to low level swift of TIL (66%), which is concordant with the reduced T cell infiltration in immunosuppressive tumour microenvironment of brain metastases according to previous studies [91]. Immunological ignorance of metastatic tumours might derive from the lack of TILs or inactivation of CD8+ T cells [92]. Increasing evidence suggests that TIL density is strongly associated with therapeutic response to anti-immune check point treatment [93-95]; thus, a high incidence of TIL variation suggests that only using TIL profile of primary tumours for patient selection can be oversimplified. Getting a landscape of TIL in both primary and metastatic sites for clinical decision on immunotherapy is highly recommended.

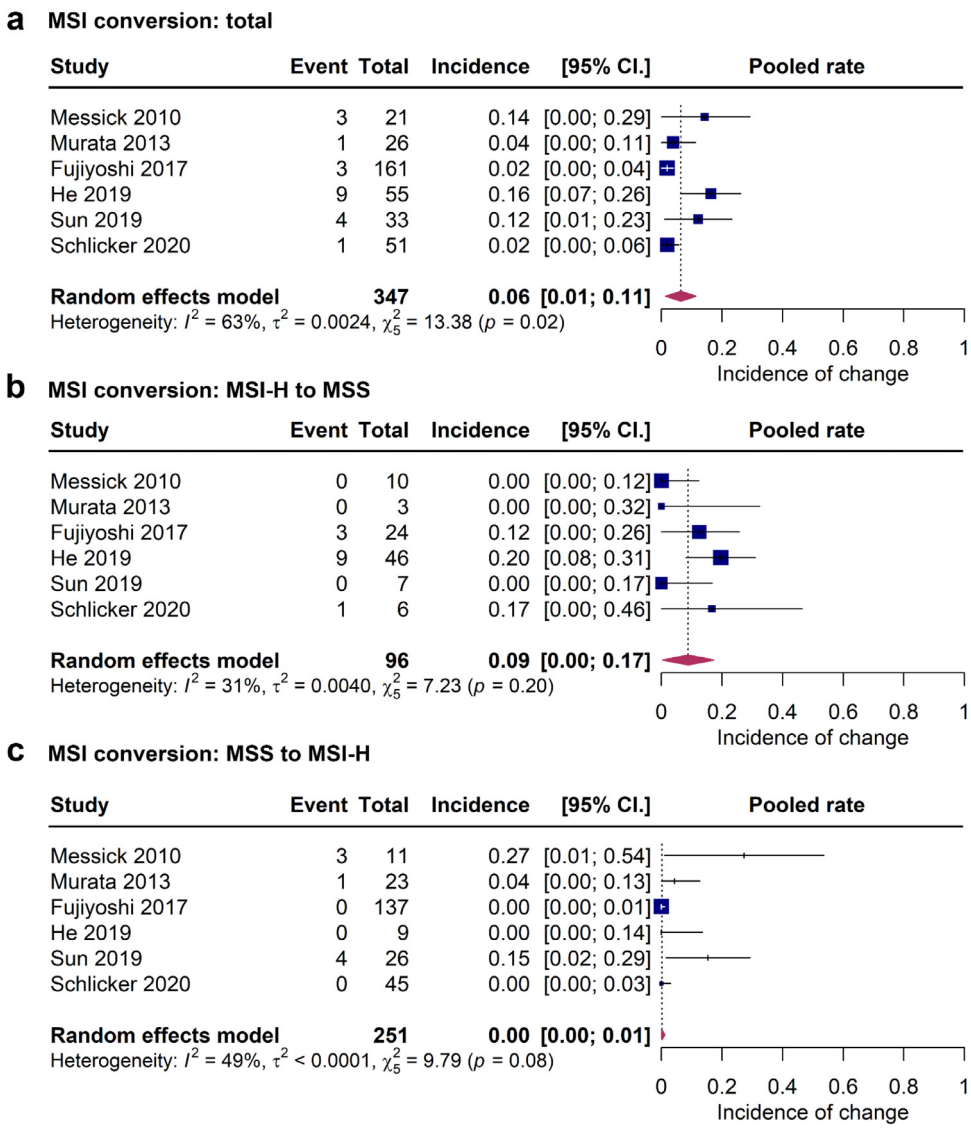


Fig. 8. Study-specific and pooled estimates for conversion rates of MSI status among primary tumours and paired metastases. Discordance rates are shown for (a) total, (b) from MSI-H to MSS, and (c) from MSS to MSI-H conversion.

Abbreviations: MSI, microsatellite instability; MSI-H, microsatellite instability-high; MSS, microsatellite stable.

Regarding genomic changes, TMB and MSI status are also considered as instructive features for anti-cancer immunotherapy [16]. Although higher non-synonymous TMB was observed in brain metastatic lesions than primary sites in 13 patients with lung cancer [23], no significant difference of TMB between primary and metastatic pairs was achieved in most studies. Careful interpretation is warranted, considering the small sample size and potential selection bias of studies included. MSI status conversion was found in a minority of patients (6%), with a percentage of 9% from MSI-H to MSS and 0% from MSS to MSI-H. Reasons for the discordance in MSI status between primary and metastatic lesions are still unclear [96]. Due to the intratumour heterogeneity of tumour tissue, metastasis might be a subclone derived from the primary tumour with a simplex genomic signature [97]. Therefore, MSI status of metastasis only represent a part of primary tumour, which can lead to false-positive or false-negative evaluations [98]. MSI-H tumour cells are less likely to develop metastasis, as a result of specific genetic and epigenetic changes [99,100]. It might be the reason for the rare incidence of conversion from MSS in primary tumours to MSI-H in metastatic tumours.

Discordance of biomarker status among primary and paired metastatic lesions is prevalent in multiple tumours, which challenges the treatment decision in clinical practice. In breast cancer, an alteration of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 status in distant metastases has frequently been reported [101]. Discrepancy of several biomarkers (KRAS, BRAF, PIK3CA, etc.) is rarely observed but still exist between primary colorectal cancer and its paired metastases [102]. Thus, reassessing metastatic tissue characteristics whenever possible is gradually recommended by several clinical guidelines in recent years [103,104]. However, solid clinical evidence or guidelines supporting the reassessment of immunotherapy response biomarkers in metastatic tumour is currently lacking. As is revealed in our study, reevaluation of immune checkpoint biomarkers is also strongly recommended, due to their high degree of inconsistency among primary and metastatic tumours. According to a recently published study, the PD-L1 status of metastatic specimens has better predictive value of immunotherapy response and survival, possibly due to the heterogeneity of cancer [105]. Thus, referring to the biomarker status of the metastatic site is recommended if controversial status is observed in two sites. However, one type of metastatic cancer should be noted, which is de novo metastatic cancer. Evaluation of both sites is recommended, and positivity determination only requires any of

Immune marker	No. of Study	Sample size	Incidence of change	I^2
PD-L1				
Conversion total	38	2109	0.22 (0.18-0.26)	86%
Positive to Negative	34	604	0.41 (0.33-0.49)	80%
Negative to Positive	34	1273	0.16 (0.11-0.22)	84%
PD-1				
Conversion total	8	562	0.26 (0.15-0.36)	91%
Positive to Negative	7	302	0.38 (0.18-0.58)	89%
Negative to Positive	7	177	0.23 (0.08-0.37)	89%
PD-L2				
Conversion total	4	207	0.22 (0.17-0.28)	0%
Positive to Negative	3	35	0.41 (0.07-0.76)	84%
Negative to Positive	3	98	0.11 (0.05-0.18)	0%
TIL level				
Conversion total	12	333	0.39 (0.29-0.49)	77%
High to Low	11	204	0.50 (0.37-0.62)	76%
Low to High	11	105	0.16 (0.08-0.23)	37%
MSI status				
Conversion total	6	347	0.06 (0.01-0.11)	63%
MSI-H to MSS	6	96	0.09 (0.00-0.17)	31%
MSS to MSI-H	6	251	0.00 (0.00-0.01)	49%

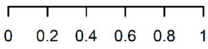


Fig. 9. Summary statistics for the conversion rates of immune checkpoint therapy response markers among primary tumours and paired metastases in pooled analysis.
Abbreviations: PD-L1, programmed death-ligand-1; PD-L2, programmed death-ligand-2; PD-1, programmed death-1; TIL, tumour-infiltrating lymphocyte; MSI, microsatellite instability; MSI-H, microsatellite instability-high; MSS, microsatellite stable.

positive site, nevertheless, it is lack of evidence. Therefore, future research should focus on the predictive value of biomarkers in each site, the consensual method for evaluation, and the consistent value of threshold.

This study had several limitations. First, given that most of the included studies were retrospective in design, bias was inevitable to some extent. Second, the small number of patients enrolled in some studies resulted in a high heterogeneity in the pooled analysis of conversion rates. Third, few studies reported the discordance rate among primary lesions and metastasis of specific sites, whereas understanding the possibility of conversion in specific metastatic sites is of great significance for clinical judgment. Fourth, certain systematic treatment (chemotherapy, radiation, endocrinotherapy, etc.) together with paired status of immune biomarkers in primary and metastatic lesions for each individual was not fully recorded in most studies, nor is it investigating the effect of these treatments on biomarker conversion available. Fifth, some other immunotherapy response biomarkers (e.g. mismatch repair status) were not able to analyze due to the lack of relevant research. Thus, more studies with high quality, perspective design, large sample size, detailed patient characteristics are warranted for further validation.

In conclusion, our study demonstrated that conversion of immunotherapy response biomarkers occurred frequently between primary lesions and their metastatic tumours, especially for PD-L1, PD-1, PD-L2, and TIL level. Therefore, evaluating the biomarkers of both primary and metastatic tumours is strongly recommended for precise clinical decision of immune checkpoint treatment. Further prospective studies are warranted to explore the mechanisms of this phenomenon and assess the clinical implications of biomarker conversion on immune checkpoint therapy.

Author Contributions

Conception and design, X.X. and Y.K.; Development of methodology, Y.Z., X.H. and H.T.; Acquisition of data, Y.Z., X.H., and S.Z.; Formal Analysis, S.Z., A.Y. and X.L.; Writing, Y.Z. and X.H.; Reviewing and Editing, X.X. and Y.K. All authors read and approved the final manuscript.

Abbreviations

FDA, Food and Drug Administration; MSI, microsatellite instability; ICI, Immune checkpoint inhibitors; PD-1, programmed cell death protein 1; PD-L1, programmed cell death-ligand-1; PD-L2, programmed cell death-ligand-2; TMB, tumour mutational burden; TIL, tumour-infiltrating lymphocyte

Declaration of Competing Interest

The authors declare no potential conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ebiom.2020.103099.

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