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

Yutian Zou\textsuperscript{a,1}, Xiaoqian Hu\textsuperscript{b,1}, Shaoquan Zheng\textsuperscript{a,1}, Anli Yang\textsuperscript{a}, Xing Li\textsuperscript{a}, Hailin Tang\textsuperscript{a}, Yanan Kong\textsuperscript{a,*}, Xiaoming Xie\textsuperscript{a,*}

\textsuperscript{a}Department of Breast Oncology, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, 651 East Dongfeng Road, Guangzhou 510060, People's Republic of China

\textsuperscript{b}School of Biomedical Sciences, Faculty of Medicine, The University of Hong Kong, 21 Sassoon Road, Hong Kong, People's Republic of China

ARTICLE INFO

Article History:
Received 11 September 2020
Revised 20 October 2020
Accepted 6 November 2020
Available online xxx

Keywords:
Immune checkpoint therapy
PD-L1
PD-1
Tumour-infiltrating lymphocyte
Tumour mutational burden
Microsatellite instability

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.

Fund: The National Natural Science Foundation of China (81872152).

© 2020 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

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...
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 x 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 material 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 tumors 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,

![PRISMA flow diagram](image-url)

**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 scrutinised 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 χ² value and P value), which was manifested if P < 0.1 [36]. In addition, I² 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 (StatCorp, 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 [20-22,25-68],

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 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 2019   | USA     | P      | Colorectal cancer | 21          | Tumour | Synchronous | Liver, lymph node | MSI |
| Murata 2013    | Japan   | R      | Colorectal cancer | 26          | Lymph node | NA | MSI |
| Mateu 2014     | Korea   | R      | Melanoma | 54          | Lung, bone, liver, lymph node, skin | Metachronous | TIL |
| Baine 2015     | USA     | R      | Kidney clear cell carcinoma | 75          | Tumour | NA | TIL |
| Callea 2015    | USA     | R      | Kidney clear cell carcinoma | 53          | Tumour | NA | TIL |
| Kim 2015       | Korea   | R      | Non-small cell lung cancer | 74          | Tumour | NA | TIL |
| Cimino-Mathews 2016 | USA | R      | Breast cancer | 26          | Bone, liver, bone, visceral | NA | TIL |
| Heren 2016     | Netherlands | P | Cervical cancer | 39          | Blood | Metachronous | Liver, lymph node | MSI |
| Inoue 2016     | Japan   | R      | Non-small cell lung cancer | 132         | Tumour | NA | TIL |
| Mansfield 2016 | USA     | R      | Non-small cell lung cancer | 73          | Tumour | NA | TIL |
| Ogiya 2016     | Japan   | R      | Breast cancer | 25          | Bone, liver, bone, lung, skin | Metachronous | TIL |
| Pinedo 2016    | USA     | R      | Non-small cell lung cancer | 65          | Tumour | NA | TIL |
| Straub 2016    | Germany | R      | Head and neck squamous cell carcinoma | 28          | Tumour | NA | TIL |
| Urua 2016      | USA     | R      | Non-small cell lung cancer | 33          | Tumour | NA | TIL |
| Fujiwara 2017  | Japan   | R      | Colorectal cancer | 161         | Tumour | NA | TIL |
| Kim H 2017     | Korea   | R      | Non-small cell lung cancer | 37          | Tumour | NA | TIL |
| Kim S 2017     | Korea   | R      | Non-small cell lung cancer | 161         | Tumour | NA | TIL |
| Ogiya 2017     | Japan   | R      | Breast cancer | 46          | Tumour | NA | TIL |
| Pellicer 2017  | Austria  | R      | Bladder cancer | 17          | Tumour | NA | TIL |
| Roper 2017     | Australia | R      | Head and neck squamous cell carcinoma | 38          | Tumour | NA | TIL |
| Takamori 2017  | Japan   | R      | Non-small cell lung cancer | 21          | Tumour | NA | TIL |
| Eckstein 2018  | Germany | R      | Bladder cancer | 15          | Tumour | NA | TIL |
| Jong 2018      | Netherlands | R | Bladder cancer | 81          | Tumour | NA | TIL |
| Keller 2018    | Switzerland | R | Non-small cell lung cancer | 40          | Tumour | NA | TIL |
| Mansfield 2018 | USA     | R      | Non-small cell lung cancer | 33          | Tumour | NA | TIL |
| Miyamoto 2018  | Japan   | R      | Colorectal cancer | 50          | Tumour | NA | TIL |
| Ohada 2018     | Japan   | R      | Head and neck squamous cell carcinoma | 25          | Tumour | NA | TIL |
| Rousselle 2018 | France  | R      | Colorectal cancer | 32          | Tumour | NA | TIL |
| Schneider 2018 | Austria  | R      | Head and neck squamous cell carcinoma | 69          | Tumour | NA | TIL |
| Scognamiglio 2018 | USA | R | Head and neck squamous cell carcinoma | 34          | Tumour | NA | TIL |
| Shibutani 2018 | Japan   | R      | Colorectal cancer | 24          | Tumour | NA | TIL |
| Szekely 2018   | Italy   | R      | Breast cancer | 72          | Tumour | NA | TIL |
| Takamori 2018  | Japan   | R      | Colorectal cancer, bladder, breast cancer | 44          | Tumour | NA | TIL |
| Tawfik 2018    | USA     | R      | Breast cancer | 41          | Tumour | NA | TIL |
| Tretvaskova 2018 | USA | R      | Bladder cancer | 79          | Tumour | NA | TIL |
| Yang 2018      | China   | R      | Non-small cell lung cancer | 43          | Tumour | NA | TIL |
| Zhou 2018      | China   | R      | Non-small cell lung cancer | 25          | Tumour | NA | TIL |
| Alves 2019     | Portugal | R     | Breast cancer | 44          | Tumour | NA | TIL |
| Bao 2019       | USA     | R      | Kidney clear cell carcinoma | 49          | Tumour | NA | TIL |
| Baru 2019      | Turkey  | R      | Non-small cell lung cancer | 24          | Tumour | NA | TIL |
| Bao 2019       | Turkey  | R      | Breast cancer | 20          | Tumour | NA | TIL |
| He 2019        | China   | R      | Colorectal cancer | 55          | Tumour | NA | TIL |
| Kim 2019       | Korea   | R      | Non-small cell lung cancer | 13          | Tumour | NA | TIL |
| Mansco 2019    | Netherlands | R | Breast cancer | 49          | Tumour | NA | TIL |
| Pater 2019     | France  | R      | Breast cancer | 67          | Tumour | NA | TIL |
| Sun 2019       | China   | R      | Colorectal cancer | 33          | Tumour | NA | TIL |
| Tyran 2019     | France  | R      | Breast cancer | 14          | Tumour | NA | TIL |
| Yuan 2019      | China   | R      | Breast cancer | 47          | Tumour | NA | TIL |
| Zhang 2019     | China   | R      | Kidney clear cell carcinoma | 83          | Tumour | NA | TIL |
| Zhu 2019       | China   | R      | Breast cancer | 49          | Tumour | NA | TIL |
| Eckel-Passow 2020 | USA | R | Kidney clear cell carcinoma | 140         | Tumour | NA | TIL |
| Luo 2020       | China   | R      | Non-small cell lung cancer | 30          | Tumour | NA | TIL |
| He 2020        | China   | R      | Synovial sarcoma | 7           | Tumour | NA | TIL |
| Hutchinson 2020| USA     | R      | Breast cancer | 37          | Tumour | NA | TIL |
| Jiang 2020     | China   | R      | Lung cancer | 20          | Tumour | NA | TIL |
| Schlicker 2020 | Germany | R      | Colorectal cancer | 51          | Tumour | NA | TIL |
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 MSL status [24,31,32,76-78] 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 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 Supplementary 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 (Supplementary 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 Supplementary 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). Meta-chronous 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 Supplementary Table 2. The total conversion rates of PD-1 varied from studies between 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).

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
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...
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. 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.

| Subgroup                  | No. of Study Sample size | Incidence of change | %  |
|---------------------------|--------------------------|---------------------|----|
| All patients              | 38                       | 2109                | 0.22 (0.18-0.26) | 86% |
| Cut-off value             |                          |                     |    |
| 1%                        | 20                       | 1098                | 0.22 (0.16-0.27) | 88% |
| 5%                        | 21                       | 1170                | 0.24 (0.19-0.29) | 78% |
| 10%                       | 2                        | 123                 | 0.24 (0.13-0.35) | 54% |
| IHC assessment            |                          |                     |    |
| TPS                       | 27                       | 1300                | 0.21 (0.17-0.26) | 87% |
| CPS                       | 10                       | 728                 | 0.24 (0.16-0.31) | 82% |
| IPS                       | 9                        | 357                 | 0.29 (0.23-0.34) | 37% |
| Specimen Source           |                          |                     |    |
| Whole tissue              | 25                       | 1332                | 0.23 (0.16-0.27) | 87% |
| Tissue microarray         | 14                       | 772                 | 0.23 (0.15-0.30) | 87% |
| Observers                 |                          |                     |    |
| 1                         | 6                        | 407                 | 0.17 (0.10-0.23) | 69% |
| 2                         | 20                       | 1033                | 0.23 (0.17-0.28) | 76% |
| Time of metastasis        |                          |                     |    |
| Synchronous               | 6                        | 415                 | 0.21 (0.12-0.29) | 63% |
| Metachronous              | 6                        | 234                 | 0.29 (0.14-0.43) | 85% |

| Subgroup                  | No. of Study Sample size | Incidence of change | %  |
|---------------------------|--------------------------|---------------------|----|
| All patients              | 34                       | 604                 | 0.41 (0.33-0.49) | 80% |
| Cut-off value             |                          |                     |    |
| 1%                        | 17                       | 308                 | 0.43 (0.30-0.56) | 78% |
| 5%                        | 18                       | 331                 | 0.39 (0.31-0.46) | 41% |
| 10%                       | 3                        | 37                  | 0.43 (0.00-0.90) | 91% |
| IHC assessment            |                          |                     |    |
| TPS                       | 23                       | 354                 | 0.37 (0.26-0.45) | 68% |
| CPS                       | 16                       | 233                 | 0.49 (0.34-0.64) | 78% |
| IPS                       | 7                        | 155                 | 0.34 (0.14-0.53) | 90% |
| Specimen Source           |                          |                     |    |
| Whole tissue              | 23                       | 414                 | 0.40 (0.31-0.49) | 79% |
| Tissue microarray         | 13                       | 210                 | 0.55 (0.36-0.71) | 81% |
| Observers                 |                          |                     |    |
| 1                         | 6                        | 115                 | 0.37 (0.17-0.58) | 85% |
| 2                         | 17                       | 262                 | 0.42 (0.34-0.50) | 38% |
| Time of metastasis        |                          |                     |    |
| Synchronous               | 6                        | 145                 | 0.45 (0.27-0.62) | 75% |
| Metachronous              | 5                        | 72                  | 0.57 (0.30-0.84) | 91% |

| Subgroup                  | No. of Study Sample size | Incidence of change | %  |
|---------------------------|--------------------------|---------------------|----|
| All patients              | 34                       | 1273                | 0.16 (0.11-0.22) | 80% |
| Cut-off value             |                          |                     |    |
| 1%                        | 17                       | 657                 | 0.15 (0.08-0.22) | 82% |
| 5%                        | 18                       | 679                 | 0.19 (0.11-0.28) | 87% |
| 10%                       | 2                        | 86                  | 0.18 (0.08-0.24) | 0%  |
| IHC assessment            |                          |                     |    |
| TPS                       | 23                       | 714                 | 0.16 (0.10-0.23) | 81% |
| CPS                       | 16                       | 495                 | 0.17 (0.05-0.29) | 90% |
| IPS                       | 7                        | 181                 | 0.49 (0.20-0.78) | 98% |
| Specimen Source           |                          |                     |    |
| Whole tissue              | 23                       | 842                 | 0.15 (0.09-0.20) | 80% |
| Tissue microarray         | 13                       | 463                 | 0.17 (0.07-0.27) | 88% |
| Observers                 |                          |                     |    |
| 1                         | 6                        | 292                 | 0.08 (0.03-0.13) | 62% |
| 2                         | 17                       | 505                 | 0.18 (0.09-0.27) | 84% |
| Time of metastasis        |                          |                     |    |
| Synchronous               | 6                        | 270                 | 0.13 (0.01-0.26) | 91% |
| Metachronous              | 5                        | 135                 | 0.23 (0.00-0.47) | 88% |
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%), 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].
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.
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. 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.
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

![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.](image-url)

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

| Immune marker | No. of Study | Sample size | Incidence of change | I² |
|---------------|-------------|-------------|---------------------|----|
| 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.
**Declaration of Competing Interest**

The authors declare no potential conflicts of interest.

**Acknowledgements**

Not applicable.

**Funding Sources**

This study was funded by the National Natural Science Foundation of China (81872152, Xiaoming Xie).

**Supplementary materials**

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

**References**

[1] Sharma P, Allison JP. The future of immune checkpoint therapy. Science 2015;348(6230):56–61.

[2] Solinas C, Aiello M, Rozali E, Lambertini M, Willard-Gallo K, Migliori E. Programmed cell death-ligand 2: a neglected but important target in the immune response to cancer. Clin Oncol 2017;29(10):1008–11.

[3] Thommen DS, Koelzer VH, Herzig P, Roller A, Trefny M, Dimeloe S, et al. A transitory instability-high colorectal cancer (CheckMate 142): an open-label, phase 2 study. Nat Med 2016;17(12):e542–e51.

[4] Nishino M, Ramaiya NH, Hatabu H, Hodi FS. Monitoring immune-checkpoint blockade: response evaluation and biomarker development. Nat Rev Clin Oncol 2018;15(10):595–606.

[5] Wang DY, Salem JE, CohenJV, Chandra S, Menzer C, Ye F, et al. Fatal toxic effects associated with immune checkpoint inhibitors: a systematic review and meta-analysis. JAMA Oncol 2018;4(12):1721–8.

[6] Gibney GT, Weiner LM, Atkins MB. Prognostic biomarkers for checkpoint inhibitor–based immunotherapy. Lancet Oncol 2016;17(12):e542–e51.

[7] Subbiah V, Solit DB, Chan TA, Kurzrock R. The FDA approval of pembrolizumab for adult and pediatric patients with tumor mutational burden (TMB) >10: a decision centered on empowering patients and their physicians. Ann Oncol 2020;31(9):1115–8.

[8] Gubens MA, Davies M. NCCN guidelines updates: new immunotherapy strategies for improving outcomes in non-small-cell lung cancer. J Natl Compr Canc Netw 2017;15(5):574–8.

[9] Okada S, Itoh K, Ishihara S, Shimada J, Kato D, Tsumezuka H, et al. Significance of PD-L1 expression in pulmonary metastases from head and neck squamous cell carcinoma. Surg Oncol 2018;27(2):259–65.

[10] Dodge MD, Neppi CI, Irmak Y, Hall SR, Schmid RA, Langer P, et al. Adverse prognostic value of PD-L1 expression in primary resected squamous cell carcinomas and paired mediastinal lymph node metastases. Mod Pathol 2018;31(1):101–10.

[11] Mansor QF, Schrijver W, Ter Hoeve MD, Moelans CB, van Diest PJ. Frequent discordance in PD-L1 and PD-L1 expression between primary breast tumors and their matched distant metastases. Clin Exp Metastasis 2019;36(1):29–37.

[12] Ogiya R, Nikiura N, Kumatani B, Bianchini G, Kitano S, Iwamoto T, et al. Comparison of tumor-infiltrating lymphocytes between primary and metastatic breast tumors in patients with colorectal cancer. Sci Rep 2016;10(1):1730–5.

[13] Hutchinson KE, Yost SE, Chang CW, Johnson RM, Carr AR, McAdam PR, et al. Comprehensive profiling of poor-risk paired primary and recurrent negative breast cancers reveals immune phenotype shifts. Clin Cancer Res 2020;26(3):657–68.

[14] Schlicker AL, Ellapayalam A, Reurre J, Snel MJH, Mittemperger L, Diodato R, et al. Investigating the concordance in molecular subtypes of primary colorectal tumors and their matched synchronous liver metastasis. Int J Cancer 2020;147(8):2303–15.

[15] Wang J, Gong Z, Jia Q, Wu Y, Yang ZZ, Zhu B. Programmed death ligand 1 expression and CD8(+) tumor-infiltrating lymphocyte density differences between paired primary and brain metastatic lesions in non-small cell lung cancer. Biochem Biophys Res Commun 2018;496(4):751–7.
Kim R, Keam B, Kim S, Kim M, Kim SH, Kim JW, et al. Differences in tumor microenvironment between primary lung tumors and metastases in lung cancer patients: therapeutic implications for immune checkpoint inhibitors. BMC Cancer 2019;19(1):19.

Shibutani M, Maeda K, Nagahara H, Fukukawa T, Matsuji S, Kashigawa S, et al. A comparison of the local immune status between the primary and metastatic tumor in colorectal cancer: a retrospective study. BMC Cancer 2018;18(1):371.

Eckstein M, Sikic D, Strissel PL, Erlemeier F. Evolution of PD-1 and PD-L1 gene and protein expression in primary tumors and corresponding liver metastases of metastatic breast cancer. Eur J Oncol 2018;7(4):527–9.

Ogiya R, Niikura N, Kumaki N, Yasojima H, Iwasa T, Kanbayashi C, et al. Comparison of immunohistochemical expression of PD-L1 in paired primary breast lesions and their corresponding liver metastases in patients with breast cancer. Oncotarget 2017;8(61):10361–71.

Baine MK, Turcu G, Zito CR, Adeniran AJ, Camp RL, Chen L, et al. Characterization of tumor infiltrating lymphocytes in paired primary and metastatic renal cell carcinomas specimens. Oncotarget 2017;8(50):24980–5002.

Jiang T, Cheng R, Pan Y, Zhang H, He Y, Su C, et al. Heterogeneity of neoantigen landscape between primary lesions and their matched metastases in lung cancer. Transl Lung Cancer Res 2020;9(2):246–56.

He Y, Zhang T, Baualh M, Tordan M, et al. Tumor mutation burden and checkpoint immunotherapy markers in primary and metastatic synovial sarcoma. Hum Pathol 2020;100:15–23.

Tyran M, Caruccia N, Garner S, Guille A, Adelajde J, Finetti P, et al. A comparison of DNA mutation and copy number profiles between primary breast cancers and paired brain metastases for identifying clinically relevant genetic alterations in brain metastases. Cancers (Basel) 2019;11(5).

Fukushima K, Yamamoto G, Takahashi A, Arii Y, Yamada M, Kukat M, et al. High concordance rate of KRAS/BRAF mutations and MSI-H between primary colorectal cancer and corresponding metastases. Oncol Rep 2017;37(2):785–92.

Murata A, Baba Y, Watanabe M, Shigaki H, Miyake K, Ishimoto T, et al. Methylation profiles of LINE-1 in primary and matched metastatic lesions of colorectal cancer. Br J Cancer 2013;109(2):408–15.

Messick CA, Church JM, Liu X, Ting AH, Kalady MF, Stage III colorectal cancer: molecular disparity between primary cancers and lymph node metastases. Ann Surg Oncol 2010;17(12):3299–305.

Pelekanov V, Barlow WE, Nahleh ZA, Wasserman L, Bo YC, von Wahle MK, et al. Tumor-infiltrating lymphocytes and PD-L1 expression in pre- and posttreatment breast cancers in the SWOG 50080 phase II neoadjuvant chemotherapy trial. Mol Cancer Ther 2013;12:3211–21.

Pelekanov V, Carvajal-Hausdorf DE, Altan M, Wasserman B, Carvajal-Hausdorf C, Wimberly H, et al. Effect of neoadjuvant chemotherapy on tumor-infiltrating lymphocytes and PD-L1 expression in breast cancer and its clinical significance. Breast Cancer Res Treat 2017;181(3):603–13.

Sunshine JC, Nguyen PL, Kaunitz CJ, Gottrell TR, Berry S, Esandrio J, et al. PD-L1 expression in melanoma: a quantitative immunohistochemical antibody comparison study. Clin Cancer Res 2017;23(15):3844–43.

Hirsch FR, McElhinny A, Stanforth D, Ranger-Moore J, Janson M, Kulangara K, et al. PD-L1 immunohistochemistry assays for lung cancer: results from phase 1 of the blueprint of PD-L1 IFIC assay comparison project. J Thorac Oncol 2017;12(2):208–22.

Steeg PS. Targeting metastasis. Nat Rev Cancer 2016;16(4):201–18.

Puram SV, Tirosh I, Parikh AS, Patel AP, Yizhak K, Gillespie S, et al. Single-cell transcriptomic analysis of primary and metastatic tumor ecosystems in head and neck cancer. Cell 2017;171(1):1–16.

Varela JS, Driedger K, Fagan A, Purcell S, Cosgrove N, O’Halleran PJ, et al. Transcriptome characterization of matched primary breast and brain metastatic tumors to detect novel actionable targets. J Natl Cancer Inst 2019;111(4):388–401.

Sholl LM. Programmed death ligand 1 immunohistochemistry: can we agree on this? Histopathology 2020;76(2):189–90.

Giancotti FG, Dejana E. Pericyte and perivascular cell plasticity in non-small cell lung cancer tissues: a methodological study. Appl Immunohistochem Mol Morphol 2018;26(1):64–70.

Achrol AS, Bennett RC, Anders C, Soffietti R, Ahluwalia MS, Nayak L, et al. Brain metastases. Nat Rev Dis Primers 2019;5(1):1–15.

Gonzalez H, Hagerling C, Werb Z. Roles of the immune system in cancer: temporal and spatial discordance of programmed cell death-ligand 1 expression and the immune tumor microenvironment in primary and metastatic breast cancer. Oncoimmunology 2016;5(9):e1213934.

Pelekanou V, Carvajal-Hausdorf DE, Altan M, Wasserman B, Carvajal-Hausdorf C, Wimberly H, et al. Effect of neoadjuvant chemotherapy on tumor-infiltrating lymphocytes and PD-L1 expression in breast cancer and its clinical significance. Breast Cancer Res Treat 2017;181(3):603–13.

Sade-Feldman M, Yizhak K, Bjorgaard SL, Ray JP, de Boer CG, Jenkins RW, et al. Heterogeneity in the expression of programmed-death (PD) ligands in isogeneic tumour infiltrating T cells and the status of oncogenic drivers. Lung Cancer 2015;88(1):24–31.
Torshizi Esfahani A, Seyedna SY, Nazemalhosseini Mojarad E, Majd A, Asadzadeh Aghdaei H. MSI-L/EMAST is a predictive biomarker for metastasis in colorectal cancer patients. J Cell Physiol 2019;234(8):13128–36.

Schrijver W, Suijkerbuijk RPM, van Gils CH, van der Wall E, Moelans CB, van Diest PJ. Receptor conversion in distant breast cancer metastases: a systematic review and meta-analysis. J Natl Cancer Inst 2018;110(6):568–80.

Bhullar DS, Barriuso J, Mullamitha S, Saunders MP, O'Dwyer ST, Aziz O. Biomarker concordance between primary colorectal cancer and its metastases. EBioMedicine 2019;40:363–74.

Lindeman NI, Cagle PT, Aisner DL, Arcila ME, Beasley MB, Bernicker EH, et al. Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: guideline from the college of american pathologists, the international association for the study of lung cancer, and the association for molecular pathology. J Thorac Oncol 2018;13(3):323–58.

Krop I, Ismaila N, Andre F, Bast RC, Barlow W, Collyvar DE, et al. Use of biomarkers to guide decisions on adjuvant systemic therapy for women with early-stage invasive breast cancer: american society of clinical oncology clinical practice guideline focused update. J Clin Oncol 2017;35(24):2838–47.

Hong L, Negrao MV, Dibaj SS, Chen B, Reiben A, Bohac M, et al. Programmed death-ligand 1 heterogeneity and its impact on benefit from immune check-point inhibitors in NSCLC. J Thorac Oncol 2020;15(9):1449–59.