PD-L1 in gestational trophoblastic disease: an antibody evaluation

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Abstract

Introduction: Treatment with antibodies directed against programmed-cell death ligand 1 (PD-L1) is a novel therapy for patients with gestational trophoblastic disease. Assessment of PD-L1 expression in tumor tissue is commonly used to identify patients who might benefit from anti-PD-L1 treatment. Multiple antibodies are available to detect PD-L1-expressing cells, and percentages of PD-L1-expressing cells in samples of patients with gestational trophoblastic disease indicated by these antibodies differ substantially. This raises the question which PD-L1 antibody best reflects PD-L1 expression to select patients for treatment.

Material and methods: Seven commercially available antibodies for PD-L1 staining (E1L3N, 73–10, 22C3, CAL10, SP142, 28–8, SP263) were validated on Chinese hamster ovarian (CHO) cells transfected with PD-L1, PD-L2, wildtype CHO cells and tonsil tissue. Next, four complete hydatidiform moles and four choriocarcinomas were stained. Samples were independently assessed by two pathologists.

Results: All seven antibodies showed membranous staining in the PD-L1-transfected CHO cells. E1L3N and 22C3 scored the highest percentages of PD-L1-positive cells (70%–90% and 60%–70%, respectively). E1L3N stained the cytoplasm of non-transfected CHO cells and was excluded from analysis. The remaining six antibodies predominantly stained syncytiotrophoblast cells of both complete hydatidiform moles and choriocarcinomas. The percentage of PD-L1-stained trophoblast cells and staining intensity varied substantially per used PD-L1 antibody and between complete hydatidiform moles and choriocarcinomas. Agreement between pathologists was best with 22C3 (intraclass correlation coefficient 0.94–0.96).

Conclusions: Based on staining results of the CHO cells, gestational trophoblastic disease samples and intraclass correlation coefficient, 22C3 seems the most suitable for adequate detection of PD-L1-expressing trophoblast cells. All antibodies detected PD-L1-expressing cells in the gestational trophoblastic disease samples, though with...
Programed cell death ligand 1 (PD-L1) is an immune regulatory molecule which can act as a coregulatory signal through binding to the inhibitory programed death 1 (PD-1) receptor. Binding leads to inhibition of cytokine production and cytolytic activity of PD-1-expressing tumor infiltrating CD4+ and CD8+ T cells. During pregnancy, this mechanism has a crucial role in suppressing immune responses and allows development of the partly allogenic fetus and placenta.

Previous studies showed that PD-L1 is strongly expressed by trophoblast cells in different types of gestational trophoblastic disease (GTD), evading immune responses, and thereby allowing proliferation of these (pre-)malignant cells. GTD comprises a group of pregnancy-related disorders, originating from placental tissue. It potentially progresses into gestational trophoblastic neoplasia (GTN), a malignant form of GTD. Although GTN is highly curable with current chemotherapies, the mortality rate is still 5% due to chemotherapy resistance, necessitating novel treatment approaches.

Blockade of the PD-1/PD-L1 pathway with immune checkpoint inhibitors (ICI) has emerged as a novel therapy for various types of cancer. In cancer patients with, for example, head and neck malignancies or melanoma, durable responses after treatment with PD-1/PD-L1 ICIs of up to 2 years in 20%-40% of patients have been reported. In patients with unresectable chemotherapry-resistant GTN, pembrolizumab, an antibody against PD-1, can induce complete responses as described in several case reports. Selection of patients eligible for treatment with anti-PD-L1 ICIs, however, is challenging. Currently, there are multiple methods to assess expression of PD-L1 on tumor cells using different antibodies, platforms, scoring systems and cutoff values, often linked to a specific ICI. In most studies, patients eligible for anti-PD-L1 treatment are selected based on the immunohistochemical (IHC) expression of PD-L1 on tumor cells, inflammatory cells, or both. However, substantial response rates in patients with tumors lacking PD-L1, and minimal response rates in patients with tumors highly expressing PD-L1 have been reported. Studies analyzing PD-L1 expression in patients with GTD showed considerable variation in reported PD-L1 expression patterns. This variation may be explained not only by tumor heterogeneity but also by the use of different commercially available PD-L1 antibodies for IHC analysis. Therefore, it is currently insufficiently clear which PD-L1 antibody is most suitable for detection of PD-L1-expressing trophoblast cells, to assess whether PD-L1 would be a good marker for response to ICI in GTD patients.

Therefore, the aim of this study was to identify the antibody most specific for detection of PD-L1-expressing cells, by evaluating the most frequently used commercially available PD-L1 antibodies for IHC.

In patients with unresectable chemotherapy-resistant GTN, pembrolizumab, an antibody against PD-1, can induce complete responses as described in several case reports. Selection of patients eligible for treatment with anti-PD-L1 ICIs, however, is challenging. Currently, there are multiple methods to assess expression of PD-L1 on tumor cells using different antibodies, platforms, scoring systems and cutoff values, often linked to a specific ICI. In most studies, patients eligible for anti-PD-L1 treatment are selected based on the immunohistochemical (IHC) expression of PD-L1 on tumor cells, inflammatory cells, or both. However, substantial response rates in patients with tumors lacking PD-L1, and minimal response rates in patients with tumors highly expressing PD-L1 have been reported. Studies analyzing PD-L1 expression in patients with GTD showed considerable variation in reported PD-L1 expression patterns. This variation may be explained not only by tumor heterogeneity but also by the use of different commercially available PD-L1 antibodies for IHC analysis. Therefore, it is currently insufficiently clear which PD-L1 antibody is most suitable for detection of PD-L1-expressing trophoblast cells, to assess whether PD-L1 would be a good marker for response to ICI in GTD patients.

**KEY MESSAGE**

Use of different antibodies to detect PD-L1-expressing cells in CHO, complete hydatidiform mole and choriocarcinoma samples leads to substantial variation in observed tumor cells expressing PD-L1. Uniformity in usage of PD-L1 antibodies is needed for comparison of studies analyzing PD-L1 expression in gestational trophoblastic neoplasia patients.

Therefore, the aim of this study was to identify the antibody most specific for detection of PD-L1-expressing cells, by evaluating the most frequently used commercially available PD-L1 antibodies for IHC.

**MATERIAL AND METHODS**

*Patient material*

Formalin-fixed paraffin embedded (FFPE) samples of four patients with a complete hydatidiform mole (CHM) which progressed to post-molar GTN after suction curettage, and four patients with a choriocarcinoma were selected from the Radboudumc pathology archives. Selection was based on the availability of FFPE specimens of the first uterine suction curettage at the Radboudumc, and concerned trophoblast tissue removed from patients before start of chemotherapy. Post-molar GTN was defined according to the FIGO 2000 guideline (ie mola hydatidosa with serum hCG plateauing for three consecutive weeks or rising over a period of two consecutive weeks).

*Flow cytometry*

Chinese hamster ovarian (CHO) cells (85050302, Sigma Aldrich) were transfected using Lipofectamine 3000 (L3000-015; Invitrogen) with the cDNA constructs cloned into the mammalian expression vector pcDNA3.1+/C-(K)-DYK encoding for PD-L1 and PD-L2 (OHu22144D and OHu04434D, respectively, both from Genscript) as described before. CHO cells were used since these cells have been the most commonly used mammalian host for (large)-scale commercial production of therapeutic proteins in the past.
Flow cytometry was performed on (transfected) CHO cells with conjugated antibodies suitable for flow cytometric assays: anti–PD-L1-BV421 (clone MIH1, 563738, BD Biosciences) and anti–PD-L2-PE (clone MIH18, 558066, BD Biosciences), with the FACS Verse (BD Biosciences). Flow cytometry data were analyzed using FlowJo software (v10; Tree Star). Transfection efficiency was ~80% for both PD-L1 and PD-L2 (Figure 1). The remainder of cells were embedded in paraffin with AgarCyto cell block preparation and used for staining.

2.3 | Immunohistochemistry

Sections of 4-μm thickness were cut and mounted on glass slides (900226, VWR). Seven commercially available PD-L1 antibodies were validated on wildtype Chinese ovarian hamster cells (CHO), and CHO cells transfected with PD-L1 and PD-L2. Tonsil tissue was used as a positive control, since PD-L1 and PD-L2 are expressed in lymphoid tissue.24 Primary antibodies included; anti-PD-L1 (clone E1L3N, 13684, Cell Signaling), PD-L1 Antibody Panel (clones 73–10, CAL10, SP142, 28–8, ab239749, Abcam), anti-PD-L1 (clone 22C3, M365329, DAKO) and anti-PD-L1 (clone SP263, Roche). IHC with anti-PD-L1 clone SP263 was performed on a VENTANA BenchMark ULTRA automated slide stainer as described by Huihkam et al.25 Other IHC protocols were carried out manually with antibody dilutions and epitope retrievals according to the manufacturers’ instructions (Appendix S1).

The IHC-stained CHO cell and tonsil slides were assessed individually by two pathologists specialized in gynecological oncology. Positivity of the PD-L1 protein was defined by membranous staining. The percentage of positive cells was determined. Additionally, cytoplasmatic staining and staining intensity were documented. We scored staining intensity as weak (+/-), mild (+), strong (+++) or very strong (+++). Scoring results of the slides were revealed after both pathologists scored all slides individually.

Antibodies considered specific for PD-L1 protein staining based on the results of the tonsil and transfected CHO samples, were used to stain four FFPE tumor tissue samples of patients with a CHM, and four FFPEs of patients with a choriocarcinoma. Samples were again analyzed by the two pathologists, and scoring results were revealed after they both scored all slides individually. To evaluate the agreement between the two pathologists in PD-L1-positive scored cells of the CHM and choriocarcinoma samples, we determined the intraclass correlation coefficient (ICC) for all tested antibodies individually.

2.4 | Statistical analyses

To calculate ICC, the two-way random-effects model with consistency was used.26 The ICCs were determined with both average and single measures. Average measures are used when measures of ≥2 raters are averaged to derive the result of a test. Single measures are used when a single rater performs the test, and is likely to be used in daily clinical practice. An ICC value of <0.50 is considered poor, between 0.50 and 0.75 moderate, between 0.75 and 0.90 good, and >0.90 red excellent.26 Analyses were performed with IBM SPSS statistics, version 25.

![Figure 1](image_url) Flow cytometry of (transfected) Chinese hamster ovarian (CHO) cells. PD-L1 and PD-L2 expression of wild type CHO cells (gray line), and transfected CHO cells (black line) measured by flow cytometry.
2.5 | Ethics statement

This study was approved by the local ethical committee of the Radboud University Medical Center (reference number 2018–4132) on June 7, 2018.

3 | RESULTS

3.1 | IHC on CHO and tonsil tissue

All antibodies showed positive membranous staining of the PD-L1-transfected CHO cells varying from 1% to 90%. The highest percentages of cells that scored positive for PD-L1 in the PD-L1-transfected CHO cells were observed for the E1L3N antibody (70%–90%) and 22C3 antibody (60%–70%), whereas the 73–10, CAL10, SP142, 28–8 and SP263 antibodies scored low percentages (Figure 2; Table 1). None of the PD-L1 antibodies showed positive membranous staining on the CHO cells transfected with PD-L2, or the wildtype CHO cells; however, the E1L3N antibody showed slight cytoplasmatic staining in the CHO PD-L2 cells and, according to one pathologist, also in the wildtype CHO cells. The E1L3N, 73–10, 22C3, SP142 and SP263 antibodies showed strong membranous staining of the tonsil tissue (Figure 2; Table 1). The CAL10 and 28–8 antibodies showed weak to mild membranous staining of the tonsil tissue (Figure 2; Table 1).

3.2 | PD-L1 staining of complete hydatidiform moles and choriocarcinomas

We observed qualitative differences in PD-L1 staining patterns between the antibodies in the same CHM samples (Figure 3; Table 2). Expression of PD-L1 was predominantly seen on the membrane of syncytiotrophoblast cells. Staining intensity was strongest with the SP263 antibody; however, besides membranous staining, strong cytoplasmatic staining was observed for all CHM samples with this antibody (Figure 3). The 73–10, 22C3 and SP142 antibodies showed mild staining intensity, and staining intensity was weakest with the CAL10 and 28–8 antibodies (Figure 3).

Comparable to the results in CHM tissue, expression of PD-L1 on choriocarcinoma tissue was predominantly seen on the membrane of syncytiotrophoblast cells. Percentages of tumor cells positive for PD-L1 appeared to be higher in the choriocarcinoma samples than the CHM samples (Table 2). Staining intensity was comparable to the results in the CHM samples: intensity was strongest with the SP263 antibody, mild with the 73–10, 22C3 and SP142 antibodies, and weakest with the CAL10 and 28–8 antibodies (Figure 4). Again, cytoplasmatic staining was observed for choriocarcinoma samples stained with the SP263 antibody.

3.3 | Pathologists’ agreement

The ICCs determined with average measures were excellent for the 73–10, 22C3, 28–8 and SP263 antibodies, and good for the CAL10 and SP142 antibodies (Table 2). ICCs determined with single measures were considered excellent for the 22C3 and SP263 antibodies, good for the 73–10 and 28–8 antibodies, and moderate for the CAL10 and SP142 antibodies (Table 2).

4 | DISCUSSION

We observed substantial heterogeneity in the percentage of cells stained positive for PD-L1 within the same tonsil, CHO, CHM and choriocarcinoma samples between the different PD-L1 antibodies. Based on our results, the 22C3 antibody is the most suitable for adequate detection of PD-L1-positive cells in tissues of CHM, and in choriocarcinoma patients. With the use of the 22C3 antibody, we observed a high to very high membranous staining intensity positive for PD-L1 in 60%–70% within the, for 80% PD-L1-transfected CHO cells. No positive staining on the CHO cells transfected with PD-L2 or wildtype CHO cells was observed. The latter two observations underscore the specificity of the antibody. Additionally, with the 22C3 antibody, ICCs were excellent for the CHO, CHM and choriocarcinoma samples.

Based on the results with the transfected CHO cells, we excluded the E1L3N antibody, since unexpected positive cytoplasmatic staining was observed in the CHO cells transfected with PD-L2. Although according to the manufacturer cells with pure cytoplasmic immunoreaction (without membranous staining) should be ignored, we cannot fully exclude the possibility that E1L3N recognizes either a PD-L1 variant, or another structurally related protein like for instance PD-L2, and may therefore incorrectly indicate the presence of PD-L1-positive cells. Concerning this cytoplasmatic staining with the E1L3N antibody of the CHO cells that were transfected with PD-L2, the pathologists scored 30%–40% of CHO cells that were transfected with PD-L2 as falsely positive for PD-L1, underlining the risk of overestimation and incorrect observation of PD-L1-positive cells. Low percentages of PD-L1-positive cells with PD-L1-transfected CHO cells were observed upon staining with the antibodies 73–10, CAL10, SP142 and SP263. This suggests that these antibodies have a lower affinity for the PD-L1 protein than do 22C3 and 28–8 antibodies.

Many ongoing studies in patients with GTD, analyzing PD-L1 expression by trophoblast cells in order to predict clinical outcome or to select patients for treatment with PD-L1 ICIs, are based on percentage of PD-L1 expression in tumor samples of these patients, stained with, among others, the E1L3N, 28–8, SP263 and 22C3 PD-L1 antibodies. Since different antibodies are used to stain the same type of tumor samples, we compared the percentage of PD-L1-positive cells within the same CHM and choriocarcinoma samples using the 73–10, 22C3, CAL10, SP142, 28–8 and SP263 antibodies, to assess whether comparable results are obtained. As expected, PD-L1 expression was different in the CHM and choriocarcinoma samples. Fewer PD-L1-positive cells were present in CHM samples compared with the choriocarcinoma samples. Strikingly, we also observed a substantial variety in detection of PD-L1-expressing...
FIGURE 2  Immunohistochemical images of Chinese hamster ovarian (CHO) cells transfected with PD-L1, CHO cells transfected with PD-L2, wildtype (WT) CHO cells, and tonsil tissue, stained with the seven commercially available PD-L1 antibodies (EL13N, 73–10, 22C3, CAL10, SP142, 28–8 and SP263). Scale bars: 100μm.
cells within the same CHM and choriocarcinoma samples when we compared staining results with the different antibodies. These results suggest that the reported varieties in staining patterns and trophoblast subtypes expressing PD-L1 can be explained by the use of different PD-L1 antibodies.

As treatment eligibility of patients is frequently based on the percentage of PD-L1 expression within tumor samples, and since different PD-L1 antibodies showed various levels of PD-L1-expressing cells within the same samples, patient eligibility for treatment with anti-PD-L1 ICIs is actually determined by the choice of PD-L1 antibody, rather than by the actual expression percentages of PD-L1.

This is the first study to compare seven frequently used commercially available antibodies in order to select the antibody most suitable for detection of PD-L1-expressing cells in CHO cells and tissue of CHM and choriocarcinoma patients. To assess the specificity of the PD-L1 antibodies, we needed a "model" whose PD-L1 and PD-L2 expression percentages were known beforehand, in order afterwards to assess the percentage of

| Antibody | Membranous staining | Cytoplasmatic staining | Staining intensity | % positive cells |
|----------|---------------------|------------------------|-------------------|-----------------|
| CHO PD-L1<sup>d</sup> | ++ | + | ++ | 70 90 |
| CHO PD-L2 | - | - | + | 40<sup>e</sup> |
| CHO WT | ++ | + | +++ | 50 60 |
| Tonsil | + | - | +++ | <5 1 |
| CHO PD-L1<sup>d</sup> | ++ | - | ++ | 60 70 |
| CHO PD-L2 | - | - | + | 1 <5 |
| CHO WT | - | - | + | |
| Tonsil | + | - | ++ | 10 30 |
| CHO PD-L1<sup>d</sup> | ++ | ++ | + | |
| CHO PD-L2 | - | - | + | 40 50 |
| CHO WT | - | - | + | |
| Tonsil | + | - | ++ | |
| CHO PD-L1<sup>d</sup> | ++ | ++ | + | |
| CHO PD-L2 | - | - | + | |
| CHO WT | - | - | + | |
| Tonsil | + | - | ++ | |

Abbreviations: CHO, Chinese hamster ovarian cells; WT, wild type; PD-L, programmed death ligand.

<sup>a</sup>Membranous staining scored as positive (+) or negative (−).
<sup>b</sup>Cytoplasmatic staining scored as positive (+) or negative (−).
<sup>c</sup>Membranous staining intensity scored as weak (+/−), mild (+), strong (++), or very strong (+++).
<sup>d</sup>80% of the CHO cells were transfected with PD-L1.
<sup>e</sup>Scoring percentages are based on cytoplasmatic staining within the CHO cells transfected with PD-L2.
PD-L1-expressing cells the antibody stained positive for expression of PD-L1. By transfecting CHO cells with PD-L1 and PD-L2, we were able to compare the IHC results with the flow cytometry results, here used as the gold standard. This gold standard is needed, since choriocarcinoma cell lines already express PD-L1 and PD-L2, and we cannot visualize the difference between cells transfected with PD-L1 and PD-L2, and the non-transfected (ie negative control) cells. Additionally, since the true percentage of PD-L1-expressing cells within the trophoblast tissues is unknown, it is not feasible to determine antibody specificity based on staining results in trophoblast samples.

Selection of the 22C3 antibody was primarily based on the staining results of the CHO samples, and was underlined by the staining results of the CHM and choriocarcinoma samples. Although we used a low number of CHM and choriocarcinoma cases, we observed substantial variety in staining patterns using different PD-L1 antibodies within the same CHM and choriocarcinoma samples, emphasizing the need for uniformity in usage of a single PD-L1 antibody to detect PD-L1-expressing cells in patients with GTN. One could argue that the variety in staining patterns may partially be explained by PD-L1 heterogeneity within the tissues. This was also observed in other studies in which PD-L1 expression was determined on different tumor or trophoblast cells. We anticipated this possible inter-sample heterogeneity using subsequent samples of the same tissue-blocks for staining with the different antibodies. A possible limitation of our study is that except for SP263, samples were stained manually and therefore were more prone to staining inaccuracies. However, manufacturer protocols were strictly followed, and all samples were stained simultaneously. Secondly, in clinical settings, antibody staining protocols are frequently optimized to enhance staining intensity for easier detection of positively stained cells. For example, the CAL10 antibody hardly stained the tonsil or the PD-L1-transfected CHO cells and may have improved optimization. A limitation of “in-house” optimization of immunohistochemical staining methods is that these procedures should be described thoroughly to facilitate comparison between published studies, and to facilitate reproducibility in future studies. Therefore, we adhered to the manufacturer’s recommended protocols to determine the most optimal and reliable PD-L1 antibody, resulting in 22C3 being our recommended choice for future studies.
| Antibody  | Tissue type     | Pathologist 1% PD-L1+ | Pathologist 2% PD-L1+ | ICC (95% CI) Average measures | ICC (95% CI) Single measures |
|-----------|-----------------|-----------------------|-----------------------|-------------------------------|-----------------------------|
| 73-10     | CHO PD-L1       | 1                     | 20                    | 0.93 (0.74–0.98)              | 87 (0.58–0.96)              |
|           | CHO PD-L2       | 0                     | 0                     |                               |                             |
|           | CHO wildtype    | 0                     | 0                     |                               |                             |
|           | CHM 1           | 70                    | 60                    |                               |                             |
|           | CHM 2           | 10                    | 30                    |                               |                             |
|           | CHM 3           | 90                    | 60                    |                               |                             |
|           | CHM 4           | 50                    | 70                    |                               |                             |
|           | Choriocarcinoma 1 | 60                | 70                    |                               |                             |
|           | Choriocarcinoma 2 | 80                | 70                    |                               |                             |
|           | Choriocarcinoma 3 | 90                | 70                    |                               |                             |
|           | Choriocarcinoma 4 | 60                | 50                    |                               |                             |
| 22C3      | CHO PD-L1       | 60                    | 70                    | 0.98 (0.91–0.99)              | 0.96 (0.84–0.99)            |
|           | CHO PD-L2       | 0                     | 0                     |                               |                             |
|           | CHO wildtype    | 0                     | 0                     |                               |                             |
|           | CHM 1           | 0                     | 0                     |                               |                             |
|           | CHM 2           | 1                     | 10                    |                               |                             |
|           | CHM 3           | 10                    | 10                    |                               |                             |
|           | CHM 4           | 0                     | 0                     |                               |                             |
|           | Choriocarcinoma 1 | 50                | 60                    |                               |                             |
|           | Choriocarcinoma 2 | 30                | 60                    |                               |                             |
|           | Choriocarcinoma 3 | 80                | 80                    |                               |                             |
|           | Choriocarcinoma 4 | 10                | 20                    |                               |                             |
| CAL10     | CHO PD-L1       | 1                     | 0                     | 0.76 (0.10–0.93)              | 0.61 (0.05–0.88)            |
|           | CHO PD-L2       | 0                     | 0                     |                               |                             |
|           | CHO wildtype    | 0                     | 0                     |                               |                             |
|           | CHM 1           | 0                     | 0                     |                               |                             |
|           | CHM 2           | 1                     | 1                     |                               |                             |
|           | CHM 3           | 40                    | 20                    |                               |                             |
|           | CHM 4           | 0                     | 0                     |                               |                             |
|           | Choriocarcinoma 1 | 15                | 30                    |                               |                             |
|           | Choriocarcinoma 2 | 15                | 20                    |                               |                             |
|           | Choriocarcinoma 3 | 5                 | 20                    |                               |                             |
|           | Choriocarcinoma 4 | 1                 | 20                    |                               |                             |
| SP142     | CHO PD-L1       | 1                     | 5                     | 0.77 (0.15–0.94)              | 0.63 (0.08–0.88)            |
|           | CHO PD-L2       | 0                     | 0                     |                               |                             |
|           | CHO wildtype    | 0                     | 0                     |                               |                             |
|           | CHM 1           | 5                     | 5                     |                               |                             |
|           | CHM 2           | 1                     | 40                    |                               |                             |
|           | CHM 3           | 40                    | 30                    |                               |                             |
|           | CHM 4           | 1                     | 1                     |                               |                             |
|           | Choriocarcinoma 1 | 20                | 20                    |                               |                             |
|           | Choriocarcinoma 2 | 5                 | 30                    |                               |                             |
|           | Choriocarcinoma 3 | 50                | 40                    |                               |                             |
|           | Choriocarcinoma 4 | 20                | 30                    |                               |                             |
| 28-8      | CHO PD-L1       | 40                    | 50                    | 0.91 (0.66–0.98)              | 0.83 (0.49–0.95)            |
|           | CHO PD-L2       | 0                     | 0                     |                               |                             |
|           | CHO wildtype    | 0                     | 0                     |                               |                             |
|           | CHM 1           | 0                     | 0                     |                               |                             |
|           | CHM 2           | 0                     | 20                    |                               |                             |
|           | CHM 3           | 1                     | 1                     |                               |                             |
|           | CHM 4           | 0                     | 0                     |                               |                             |
|           | Choriocarcinoma 1 | 0                 | 0                     |                               |                             |
|           | Choriocarcinoma 2 | 0                 | 20                    |                               |                             |
|           | Choriocarcinoma 3 | 0                 | 1                     |                               |                             |
|           | Choriocarcinoma 4 | 0                 | 0                     |                               |                             |
Using different antibodies to detect PD-L1-expressing cells in CHM and choriocarcinoma samples leads to substantial variation in observed tumor cells expressing PD-L1. Based on our results, the 22C3 antibody seems most suitable for detecting PD-L1-positive cells in CHO cells transfected with PD-L1, and should therefore be used in future studies analyzing expression of PD-L1 in samples.
of patients with GTD. Clinical correlation with treatment response should be the next step.

AUTHOR CONTRIBUTIONS

All authors have been involved in the study design. YH collected the data. YH, MS, JB, MG, NO, JdeV and FS interpreted the data. YH wrote the manuscript and all authors were equally involved in reviewing and editing the manuscript.

CONFLICT OF INTEREST

None.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher’s website.

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