

The Value of Programmed Death Ligand 1 Expression in Cancer Patients Treated with Neoadjuvant Chemotherapy

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أهمية ظهور ربيطة الموت المبرمج 1 في مرضى السرطان الذين تلقوا المعالجة الكيميائية المسبقة

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ABSTRACT: Programmed death ligand 1 (PD-L1) is an inhibitory molecule expressed by cancer cells to suppress T-cell activity and escape anti-tumour immunity. The role of PD-L1 in cancer has been studied extensively as it is considered an important immune checkpoint against immune over-activation through its interaction with Programmed death receptor 1 (PD-1) expressed on activated lymphocytes. PD-L1 expression was found to be enhanced by chemotherapy through different proliferation pathways. However, the predictive and prognostic value for PD-L1 expression in cancer patients treated with neoadjuvant chemotherapy (NAC) is not yet established. This review focused on the potential effects of chemotherapy on PD-L1 expression and the role of PD-L1 as a prognostic and predictive marker in NAC-treated cancer patients. In addition, the potential use of this marker in clinical practice is discussed.

Keywords: Programmed Cell Death 1 Ligand 1; Programmed Cell Death 1 Receptor; Neoadjuvant Therapy; Cancer.

المخلص: ربيطة الموت المبرمج-1 هو جزيء مثبط تعبر عنه الخلايا السرطانية لقمع نشاط الخلايا الليمفاوية من النوع (ت) للإفلات من المناعة التي تنشأ ضد الأورام السرطانية. تمت دراسة دور ربيطة الموت المبرمج-1 في السرطان على نطاق واسع لأنها تعتبر ضابطة مناعية هامة ضد التنشيط المفرط للجهاز المناعي من خلال تفاعلها مع مستقبل الموت المبرمج-1 التي تظهر على الخلايا الليمفاوية المنشطة. ووجد أن انتشار ربيطة الموت المبرمج-1 تتعزز بالمعالجة الكيميائية من خلال مسارات تكاثرية مختلفة. ومع ذلك، فإن القيمة التنبؤية الإنذارية لظهور ربيطة الموت المبرمج-1 عند مرضى السرطان الذين يتلقون معالجة كيميائية مسبقة لم تتحدد بعد. هذه المراجعة تناول تأثير المعالجة الكيميائية المحتملة على ظهور ربيطة الموت المبرمج-1 وأهمية انتشارها كعلامة تنبؤية إنذارية لدى مرضى السرطان الذين يتلقون المعالجة الكيميائية المسبقة. بالإضافة إلى ذلك، تمت مناقشة إمكانية استخدام هذا المؤشر في الممارسة السريرية.

الكلمات المفتاحية: لجين موت الخلية المبرمج-1؛ مستقبلات موت الخلية المبرمجة-1؛ العلاج المسبق؛ السرطان.

THE ROLE OF THE IMMUNE SYSTEM IN CANCER has been studied extensively; immunity in the cancer microenvironment has a role in different phases of cancer development.¹ Cancer cells usually express abnormal antigens which trigger the immune response to be activated against these cancerous cells.² Tumour-infiltrating lymphocytes (TIL), including cytotoxic T-cells, are usually involved in tumour antigen recognition and anti-tumour immunity to destroy cancer cells during immune-surveillance.^{1,3} Normally, the initiation of the immune response by T-cells is mediated through T-cell receptor (TCR) recognition of the antigen and regulated by inhibitory and stimulatory molecules expressed in these T-cells. The inhibitory molecules are considered as immune checkpoints for self-tolerance (i.e. protection from tissue damage during an immune response and prevention of developing

autoimmune diseases). However, the inhibitory effect mediated by these immune checkpoint molecules may also be used as a mechanism for tumour cells to escape the immune response.⁴ One of these immune checkpoints is the programmed death receptor 1 (PD-1)/programmed death ligand 1 (PD-L1) interaction [Figure 1].

PD-1 is a member of cluster of differentiation (CD)28 family that is expressed in T-cells.⁵ It is also expressed in B-cells, professional antigen presenting cells and natural killer cells.⁶ The PD-L1 was firstly identified by Dong *et al.* in 1999 and showed that there was an increase in the T-cell proliferation and interleukin (IL)-10 secretion by T-cells stimulated with PD-L1/B7-H1 and low levels of anti-CD3.⁷ In 2000, Freeman *et al.* showed induction of PD-L1 expression by cytokines as interferon (IFN)- γ or other inflammatory stimuli could result in attenuation of

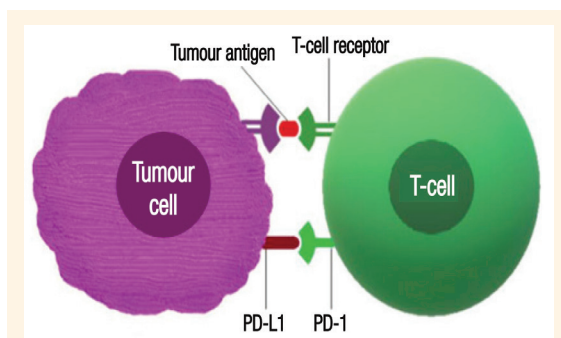


Figure 1: Depiction of the T-cell receptor recognition of the tumour antigen initiating the T-cell response which is regulated by inhibitory molecules expressed in T-cells such as programmed death receptor 1. However, tumour cells may use this inhibitory effect to escape the immune response.

PD-L1 = programmed death ligand 1; PD-1 = programmed death receptor 1.

TCR/CD28-mediated T-cell activation which may be significant in the regulation of inflammatory or auto-immune reactions.⁸ PD-L1, also called B7-H1, is a member of B7 molecules which are involved in the regulation of T-cell response either through stimulation or inhibition.⁹ PD-L1 is generally widely expressed in different tissues from different lineages including lymphoid (i.e. immune cells) or non-lymphoid (i.e. epithelial and endothelial) lineages and in cancer cells that originate from these lineages.^{8,10} PD-L1 was found to inhibit proliferation of T-cells and production of cytokines such as IFN- γ and IL-2, through interaction with PD-1 expressed on these cells.¹¹ In the cancer microenvironment, the interaction of PD-L1 which is expressed in cancer cells and PD-1 which is expressed in T-cells leads to the inhibition of cytotoxic T-cell activity, a decrease in cytokines production by T-cells and induction of T-regulatory cells. Therefore, this interaction protects tumour cells from destruction by cytotoxic T-cells and promotes tumour cell proliferation.^{12,12}

Many studies chose to investigate the role of PD-L1 expression in subjects not treated with neoadjuvant chemotherapy (NAC). For example, a study on triple negative breast cancer (TNBC) patients excluded patients treated with NAC.¹³ Similarly, several studies preferred to include patients who did not receive NAC.^{14–16} This exclusion was partly attributed to the possibility that chemotherapy may contribute to change the expression of specific markers; for example, the expression of progesterone receptor and cellular proliferation marker (Ki-67) in breast cancer was decreased after NAC.¹⁷

The effect of NAC on PD-L1 expression in cancer patients has not yet been established. Therefore, this review aimed to highlight the effect of chemotherapy on the expression of PD-L1, the potential use of PD-L1 expressed in pre-treated patients as a predictive marker

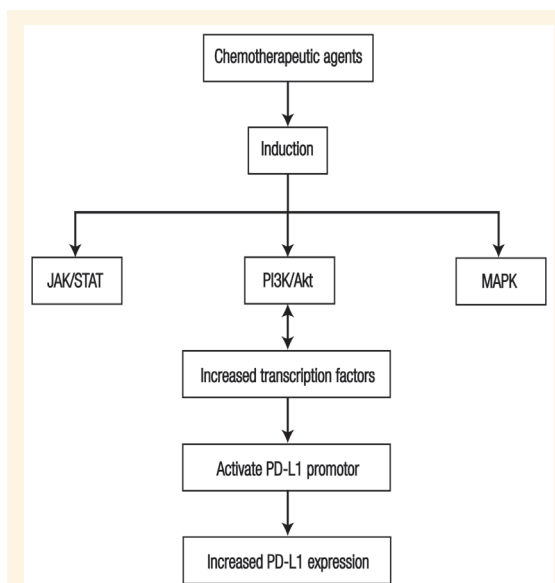


Figure 2: Flowchart showing the different pathways involved in programmed death ligand 1 upregulation induced by chemotherapy treatment.

JAK/STAT = janus-activated kinase/signal transducers and activators of transcription; PI3K/Akt = phosphoinositide-3 kinase/protein kinase B; MAPK = mitogen-activated protein kinase; PD-L1 = programmed death ligand 1.

for the response to chemotherapy and whether it could be used as a prognostic disease marker.

POST-NEOADJUVANT CHEMOTHERAPY CHANGES IN PROGRAMMED DEATH LIGAND 1 EXPRESSION IN DIFFERENT CANCERS

Immunohistochemical detection of PD-L1 expression can be either increased or decreased by NAC according to the tumour type. It is known that chemotherapy provokes an immune response but, once PD-L1 is over-expressed, this immune response might be diminished. Increased expression of PD-L1 after NAC suggests that the immune response is inhibited which may contribute to chemo-resistance and poor clinical outcome.¹⁸ Therefore, a combination of chemotherapy and immunotherapy was suggested to overcome this resistance. Conversely, decreased expression of PD-L1 may be associated with a better response to NAC treatment.

Cancers associated with increased Programmed Death Ligand 1 expression

Increased PD-L1 expression in association with NAC was demonstrated in post-treated specimens of ovarian, bladder and gastrointestinal cancers as well as head and neck squamous cell carcinoma (HNSCC).^{19–25} In epithelial ovarian cancer, 63% of PD-L1 negative tumours converted to positive PD-L1 tumours after NAC when patients received carboplatin and paclitaxel; the positivity rate in the PD-L1 positive samples increased from 30% in pre-treated specimens to 53% in post-treated

Table 1: Summary of publications showing the variation of programmed death ligand 1 expression in different cancer types before and after neoadjuvant chemotherapy^{19-23,25,26,28-32}

Author and year of publication	Type of cancer	Treatment regimen	Number of cases*	Cut-off point/ scoring system	Pre-treatment PD-L1	Post-treatment PD-L1	Effect of NAC in PD-L1 expression	Association with survival/prognosis	
								Pre-treatment	Post-treatment
Mesnage <i>et al.</i> ¹⁹ (2017)	Ovarian	Carboplatin and paclitaxel	51	≥5% is positive	IC = 30% TC = 0%	IC = 53% TC = 6%	Increased	PFS (P = 0.56)	PFS (P = 0.25)
Böhm <i>et al.</i> ²⁰ (2016)	Ovarian	Platinum-based NACT	26	IC: 0 if <1%, 1 if 1-5%, 2 if 5-10%, 3 if ≥10%	50% (cut-off point ≥5%)	73% (cut-off point ≥5%)	Increased	-	-
McDaniel <i>et al.</i> ²¹ (2016)	Bladder	Cisplatin and carboplatin	40	H-score	Mean score = 176.6	Mean score = 217.5	Increased	Not correlated with RFS	Not correlated with RFS
Van Der Kraak <i>et al.</i> ²² (2016)	Oesophageal	Cisplatin, 5-FU and radiation	10	TC ≥ 1% is positive	40%	0%	Decreased (cell line experiment showed increased PD-L1 expression, which was inconsistent with immunohistochemistry results)	-	-
Lim <i>et al.</i> ²³ (2017)	Rectal	Chemoradiotherapy	123	H-score (median value was used as cutoffs)	-	-	Increased	†	-
Oek <i>et al.</i> ²⁵ (2017)	HNSCC	Docetaxel, cisplatin or 5-FU; CCRT regimens	35	TC ≥ 5% is positive	63%	77%	Increased	-	-
Pelekanou <i>et al.</i> ²⁶ (2017)	Breast	Doxorubicin and cyclophosphamide followed by taxane	58	QIF score = 500 AU	51%	17%	Decreased	-	RFS (P = 0.14) OS (P = 0.64)
Sun <i>et al.</i> ²⁸ (2017)	Lung cancer	Platinum-based	26	≥5% is positive	65%	92%	Increased	-	-
Song <i>et al.</i> ²⁹ (2016)	Lung squamous cell carcinoma	Paclitaxel/platinum docetaxel/platinum	76	H-score (≥5 is positive)	53%	62%	Increased	Significant OS (P = 0.052) PD-L1 negative = 34.2 months; PD-L1 positive = 27.0 months	Significant OS (P = 0.003) PD-L1 negative = 36.5 months; PD-L1 positive = 27 months
Sheng <i>et al.</i> ³⁰ (2016)	NSCLC	Pemetrexed-based regimen and paclitaxel-based chemotherapy	32	H-score (≥ 5 is positive)	75%	37.5%	Decreased	-	-
Fujimoto <i>et al.</i> ³¹ (2017)	NSCLC	CCRT	35	≥1% is positive	63%	60%	Decreased	OS (P = 0.456) RFS (P = 0.939)	OS (0.746) [‡] RFS (0.784)
Remark <i>et al.</i> ³² (2016)	NSCLC	Platinum-based	21	Positivity assessed semi-quantitatively on tumour cells	Not mentioned	Not mentioned	Unchanged	-	-

PD-L1 = programmed death ligand 1; NAC = neoadjuvant chemotherapy; IC = immune cell; TC = tumour cell; PFS = progression free survival; RFS = relapse free survival; FU = fluorouracil; HNSCC = head and neck squamous cell carcinoma; CCRT = concurrent chemoradiotherapy; QIF = Quantitative immunofluorescence; AU = arbitrary unit of fluorescence; OS = overall survival; NSCLC = non-small cell lung cancer.

*The cases involved in the analysis which had both pre- and post-treated biopsies and specimens. †Sustained high expression in pre- and post-treated specimens were associated with worse OS (P = 0.018) and RFS (P = 0.029). ‡Median OS of patients according to the change in PD-L1 expression: decreased = 85.1 months, unchanged = 92.8 months, increased = 14.6 months (P < 0.001). §Median disease-free survival of patients according to the change in PD-L1 expression: increased = 9.6 months, decreased = 25.9 months (P = 0.005).

specimens.¹⁹ This was further supported by findings showing that cisplatin treatment of ovarian cancer cell lines SKOV-3 and OVCAR-3 *in vitro* induced the expression of PD-L1.¹⁹ In addition, a high proportion of T-cells expressing PD-L1 were found in biopsies from ovarian high-grade serous carcinoma after NAC.²⁰ The expression of PD-L1 was increased in 76% of patients with urothelial carcinoma treated with cisplatin or carboplatin-based chemotherapy.²¹ Although the increased expression was not observed in post-treated oesophageal carcinoma specimens using immunohistochemistry, 5-fluorouracil induced surface expression of PD-L1 in oesophageal cancer (OE33) and colorectal cancer (HCT 116 p53^{+/+} and HCT 116 p53^{-/-}) cell lines.²² PD-L1 expression and TIL were increased after concurrent chemoradiation therapy (CCRT) in rectal carcinoma.²³ Notably, sustained high expression of PD-L1 in pre- and post-chemoradiotherapy specimens, associated with less increase in TIL, showed poorer overall survival (OS) and disease-free survival (DFS) compared to other patients.²³

Inhibition of PD-L1 may be beneficial only in some ovarian cancer patients. PD-L1 is expressed significantly more in high grade tumours than in low grade tumours; therefore, a subgroup of advanced disease ovarian cancer patients with high grade tumours, expressing PD-L1, may benefit the most from therapy targeting PD-L1.¹⁹

In HNSCC, immunohistochemical analysis of 13 negative cases of PD-L1 showed that nine cases (69.2%) showed increased PD-L1 expression after cisplatin treatment.²⁵ Furthermore, patients who received neoadjuvant cisplatin treatment showed more PD-L1 up-regulation compared to patients who did not receive cisplatin.²⁵ This was supported by findings showing increased PD-L1 expression in HNSCC cell lines (SNU-1066, SNU-1041, SNU-1076, Detroit-562, FaDu and PCI-13) treated with cisplatin alone.²⁵

Cancers associated with decreased Programmed Death Ligand 1 expression

Conversely, PD-L1 was downregulated in breast and squamous oral carcinoma, after treatment with NAC.^{26,27} In the analysis of 58 breast specimens (56% of patients treated with doxorubicin and cyclophosphamide followed by taxane and the remaining patients received other agents), the positivity rate of PD-L1 expression decreased from 51% to 17.2% and 17 cases converted from positive to negative status in spite of the increase of T-cells.²⁶ The comparison between NAC-treated and non-treated squamous oral carcinoma specimens showed that most of the treated specimens weakly expressed PD-L1, while non-treated specimens showed either strong or intermediate expression. Similarly, PD-1 expression was significantly less in NAC-treated specimens.²⁷

Programmed Death Ligand 1 expression in lung cancer

In lung cancer, the expression of PD-L1 showed variable results regarding PD-L1 expression after NAC treatment. Sun *et al.* showed that PD-L1 expression significantly increased in platinum-based-treated specimens.²⁸ PD-L1 expression also increased in nine patients and decreased in two patients with lung squamous cell carcinoma after NAC; the positivity rate changed from 52.6% to 61.8%.²⁹ Expectedly, expression of PD-L1 was associated with worse DFS compared to the decreased expression. However, PD-L1 expression was not an independent predictor of DFS as determined by multiple regression analysis.³⁰

Non-small cell lung cancer samples showed an overall decrease in PD-L1 expression after CCRT. The decreased PD-L1 expression was associated with better survival compared to samples with increased PD-L1 expression (85.1 months versus 14.6 months).³¹ These findings were comparable to another study involving patients with non-small lung carcinoma treated with a pemetrexed-based regimen and paclitaxel-based chemotherapy.³⁰ In this study, the positivity rate of PD-L1 changed from 75% to 37.5% with effect on immune cell infiltration; decreased PD-L1 expression in tumour cells was associated with a better response to NAC. Remark *et al.*'s study found that PD-L1 expression in lung cancer was not changed by cisplatin-based NAC treatment indicating that NAC did not contribute to the modification of PD-L1 expression.³² Various results from different studies suggest that PD-L1 expression, in response to NAC treatment, may be cancer specific and that different NAC modalities may trigger distinct changes in PD-L1 expression involving different mechanisms.

PROPOSED MECHANISMS OF CHEMOTHERAPY-MEDIATED-PROGRAMMED DEATH LIGAND 1 EXPRESSION CHANGES

Few studies have explored the mechanisms linked to PD-L1 expression changes associated with NAC. Chemotherapy may be involved in proliferative pathways that can lead to enhanced PD-L1 expression in cancer [Figure 2].³³

For instance, PD-L1 expression at the protein and mRNA levels was increased under the influence of 5-fluorouracil, gemcitabine and paclitaxel chemotherapy in pancreatic cancer cell lines (MIA PaCa-2, AsPC-1, Pan02).³⁴ These three anti-cancer agents increased PD-L1 through Janus-activated kinase/signal transducers and activators of transcription signalling pathways and through phosphorylation by phosphoinositide-3 kinase/protein kinase B (PI3K/Akt) and mitogen-activated protein kinase (MAPK) pathways.³⁴

In addition, HNSCC cancer cell lines (SNU-1066, SNU-1041, SNU-1076, Detroit-562, FaDu and PCI-13) treated with cisplatin showed an increase in PD-L1 expression along with up-regulation of extracellular signal-regulated kinase/MAPK. These pathways were found to contribute to chemoresistance. For example, activation of PI3K/Akt pathway was associated with chemotherapy resistance in urothelial carcinoma.³⁵ However, targeting the MAPK pathway enhanced the sensitivity of the tumour resistance to cisplatin and helped to overcome this resistance.³⁶

Inactivation of programmed cell death (i.e. *apoptosis*), is essential for cancer development. It was suggested that disabling apoptosis by anti-apoptotic factors may contribute to NAC resistance. For example, a gastric cancer cell line (SGC7901/5-FU) resistant to treatment with 5-fluorouracil was associated with overexpression of PD-L1 compared to a sensitive gastric cell line. A PD-L1 induced resistance was associated with up-regulation of anti-apoptotic proteins, including ATP binding cassette subfamily C member 1 and anti-apoptotic factor B-cell lymphoma 2, that may contribute to the resistance. The blockage of PD-L1 by siRNA was found to overcome this resistance and inhibit proliferation.³⁷

A novel mechanism of PD-L1 downregulation of expression by chemotherapy was discovered.³⁸ Anthracycline agents, including doxorubicin, were found to decrease the surface expression of PD-L1 in breast cancer cell lines (MDA-MB231 and SKBr3) in a dose and time-dependent manner. However, this effect was correlated with nuclear translocation of PD-L1 and increased nuclear phosphorylated Akt. PD-L1 was also found to have an anti-apoptotic function, in which its blockade by PD-L1 siRNA enhanced the effect of doxorubicin-induced apoptosis and which was PI3K/Akt pathway dependent.³⁸

PROGRAMMED DEATH LIGAND 1 AS A POTENTIAL PREDICTIVE MEASURE FOR THE RESPONSE TO NEOADJUVANT CHEMOTHERAPY

PD-L1 expressed in pre-treated specimen has been investigated to identify patients who would be most likely to respond to NAC. Several studies have found that PD-L1 expression is associated with a pathological complete response (pCR) after NAC in breast cancer.^{39–42} The analysis of 94 biopsies of breast cancer tissue revealed that pCR was associated with the epithelial and stromal expression of PD-L1. When patients were classified into groups, this association remained significant in hormone-positive and Her2-positive breast cancer.³⁹ Similarly, PD-L1 expression in tumour cells was associated with pCR in 54 biopsies of TNBC

which remained significant in multivariate analysis. All patients with more than 25% expression of PD-L1 and high TIL achieved pCR.⁴⁰ Expression of PD-L1 in tumour cells of Her2-positive breast cancer tumours showed a trend of association with pCR but did not reach significance.⁴¹ The evaluation of several predictive markers revealed that 20 out of 27 PD-L1-positive invasive breast cancer cases showed excellent response to NAC using the Residual Cancer Burden score.⁴¹ The mechanisms underlying the differential response amongst different molecular types of breast carcinoma may be due to the differing biology of the three types i.e. the tumours may or may not be immunogenic.

Only Baras *et al.*'s study of bladder cancer found that PD-L1 was not associated with pCR.⁴³ The expression of PD-L1 in invasive bladder carcinoma showed no significant difference between responders and resistant patients to cisplatin-based NAC.⁴³

PROGRAMMED DEATH LIGAND 1 AS A PROGNOSTIC MARKER IN PATIENTS TREATED WITH NEOADJUVANT CHEMOTHERAPY

The role of PD-L1 as a prognostic marker in patients treated with NAC remains largely unknown. The results of several studies identifying the expression of PD-L1 in residual tumours (i.e. post-treated specimens) have been concordant. It has been reported that high PD-L1 expression in residual tumours was associated with poor clinical outcome in locally-advanced breast cancer after NAC. According to molecular subtypes, the association was more significant in the TNBC subtype.⁴⁴ Patients with high PD-L1 expression and low CD8⁺ cells had a poor outcome compared to other groups.⁴⁴ However, some studies found that PD-L1 expression in residual breast tumours was not associated with the clinical outcome.^{26,41} In post-treated bladder cancer, disease-specific survival in PD-L1-positive patients was significantly lower than in PD-L1-negative patients.⁴⁵ In lung cancer, both pre- and post-treated expression of PD-L1 was associated with worse OS; however, the association was significant and independent in post-treated specimens indicating that PD-L1 status after NAC treatment is a better prognostic marker than PD-L1 status prior to NAC treatment.²⁹

Conclusion

Chemotherapy may cause an increase or decrease in the expression of PD-L1; this outcome seems to be cancer specific as shown by immunohistochemistry

analysis of pre- and post-treated specimens and cell line experiments. It is possible that PD-L1 is involved in chemoresistance through inhibiting the induction of T-cell activity pathways. Therefore, it may be important to consider combining immunotherapy and chemotherapy to improve the response to the treatment. Current evidence showed that increased PD-L1 expression in post-treated specimens is a poor prognostic marker; although, PD-L1 was shown to predict the response to NAC in breast cancer. Further studies are needed to validate this predictive role of PD-L1 in response to NAC in other cancers except breast cancer. Also, further broader investigations are required to explore the prognostic role of PD-L1.

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