

### INCREASED PROGRAMMED CELL DEATH 1 LIGAND 2 (PD-L2) PROTEIN AND TRANSCRIPT LEVELS IN TRIPLE NEGATIVE BREAST CANCER PATIENTS IMPROVE PROGNOSTIC VALUE THROUGH IMMUNE PATHWAY ACTIVATION

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**Background** Recent advances in immunotherapy have shown promising results in triple-negative breast cancer (TNBC). Studies regarding PDL1/PD1 co-inhibitory pathway demonstrate that PDL1 positive subgroup of TNBCs had better prognosis and increased clinical benefit from immunotherapy.<sup>1</sup> Even then, other predictive biomarkers beyond PDL1 to better stratify immunotherapy responsive patients is necessary, where tumor infiltrating lymphocytes (TILs) may help predict prognosis when used in conjunction with PDL1 expression.<sup>2</sup> Thus, we explored the prognostic significance of another known ligand of PD1,<sup>3</sup> programmed cell death ligand 2 (PDL2), in TILs of TNBC patients.

**Methods** 389 TNBC cases diagnosed between 2003–2014 in Singapore General Hospital (SGH) were used. Tissue microarray blocks was stained with anti-PDL2 antibody (D7UHC). Immunostaining was scored based on TILs expression and positivity was defined as  $\geq 2$  TILs count. PDL2 mRNA (*PDCD1LG2*) and their association with survival outcomes were assessed in four cohorts (SGH/METABRIC/TCGA/FUSCC). Differentially expressed genes (DEGs) between PDL2 and *PDCD1LG2* sample groups were identified using Welch's t tests with multiple testing corrections. Protein-protein interaction (PPI) of identified DEGs was constructed to characterize hub-genes. Prognostic models were compared using ANOVA to evaluate the delta in log-likelihood of the models ( $\Delta LR_x^2$ ).

**Results** 22.9% (71/309) TNBCs expressed higher densities of PDL2 TILs, where PDL2+ patients demonstrated significantly better disease-free survival (DFS,  $P = 0.044$ ) and a trend towards improved overall survival (OS,  $P = 0.058$ ) (figure 1A,B). Similarly, higher *PDCD1LG2* transcript levels significantly improved survival rates in multiple cohorts (figure 1C,

D). Additionally, the inclusion of PDL2 immune infiltrates and *PDCD1LG2* expression, individually or combined, added significant prognostic value for DFS (PDL2:  $\Delta LR_x^2 = 0.71$ , *PDCD1LG2*:  $\Delta LR_x^2 = 3.3$ , PDL2 + *PDCD1LG2*:  $\Delta LR_x^2 = 2.36$ ) and OS (PDL2:  $\Delta LR_x^2 = 0.61$ , *PDCD1LG2*:  $\Delta LR_x^2 = 3.47$ , PDL2 + *PDCD1LG2*:  $\Delta LR_x^2 = 2.79$ ) beyond that provided by classical clinicopathological variables (table 1–2). Hub genes were identified between DEGs of PDL2 TILs (SGH: *PTPRC*, *CD4*, *CD8A*, *IL10RA*, *SELL*, *ITGB2*, *CTLA4*, *LCP2*, *IFNG*, *STAT1*) and *PDCD1LG2* (All cohorts: *PTPRC*, *CD4*, *CD86*, *IL10RA*, *IL1B*, *ITGB2*, *CD80*, *IL10*, *IFNG*, *CCL5*) (figure 2). All hub genes were remarkably downregulated in negative PDL2 TILs or low *PDCD1LG2* TNBCs with poorer survival rates (table 3).

**Conclusions** Overall, PDL2 expression in TILs could represent as an effective prognostic predictor through its involvement in antitumor immunity within the TNBC tumor microenvironment. These findings further corroborate PDL2 as a potential therapeutic target for TNBC, highlighting the importance of considering immune-related factors in TNBC prognosis and treatment.

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#### REFERENCES

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**Ethics Approval** Ethics approval was obtained from The Centralized Institutional Review Board of Singhealth, Singapore.

**Consent** Consent was waived for this study from The Centralized Institutional Review Board of Singhealth, Singapore.

Abstract 167 Table 1

**Table 1. Log-likelihood change of models with added individual prognostic terms**

Variables	Disease-free survival (SGH)				Overall survival (SGH)			
	$\Delta LR_x^2$	AIC	P value	Log rank test	$\Delta LR_x^2$	AIC	P value	Log rank test
CP	Reference	769.064716	1.27e-06	32.8641354	Reference	536.602275	1.15e-09	47.5918377
CP + PDL2 TILs total count	0.71051951	684.830772	4.28e-06	32.721082	0.60980708	469.923657	1.96e-08	44.3618034
CP + <i>PDCD1LG2</i>	3.30041424	715.397555	3.38e-07	38.2401752	3.47462996	513.558903	8.49e-11	55.9065847

CP, Clinicopathological parameters (patient age at diagnosis, tumor grade, tumor size >20mm, lymph node status); LR, Likelihood ratio; AIC, Akaike information criterion

Abstract 167 Table 2

**Table 2.** Log-likelihood change in the PDL2 model with added gene prognostic terms

Variables	Disease-free survival (SGH)				Overall survival (SGH)			
	$\Delta LR_x^2$	AIC	P value	Log rank test	$\Delta LR_x^2$	AIC	P value	Log rank test
CP <sup>PDL2</sup>	Reference	684.830772	4.28e-06	32.721082	Reference	469.923657	1.96e-08	44.3618034
CP <sup>PDL2</sup> + PDCD1LG2	2.3618892	633.933587	1.29e-06	37.6989277	2.78747079	449.107537	2.02e-09	51.8280136

CP<sup>PDL2</sup>, Clinicopathological parameters (patient age at diagnosis, tumor grade, tumor size >20mm, lymph node status, PDL2 TILs total count); LR, Likelihood ratio; AIC, Akaike information criterion

Abstract 167 Table 3

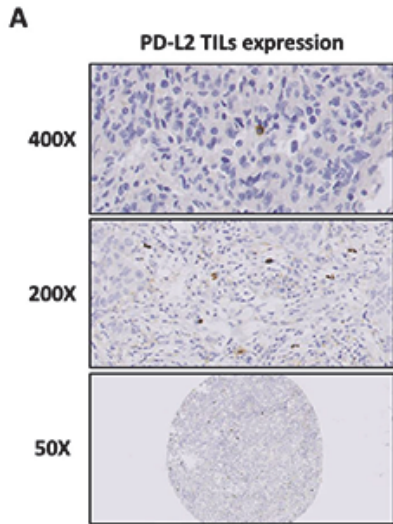
**Table 3A.** Summary of comparative survival analysis of hub genes in PDL2 TILs

PD-L2 TILs hub genes		SGH	
		Overall survival	Disease-free survival
PTPRC	P value	1.536e-03*	1.428e-03*
	Poorer prognosis expression	Low	Low
CD4	P value	1.056e-03*	7.553e-05*
	Poorer prognosis expression	Low	Low
CD8A	P value	1.767e-04*	3.069e-03*
	Poorer prognosis expression	Low	Low
IL10RA	P value	2.374e-07*	1.820e-06*
	Poorer prognosis expression	Low	Low
SELL	P value	2.028e-03*	1.065e-02*
	Poorer prognosis expression	Low	Low
ITGB2	P value	2.065e-03*	5.518e-05*
	Poorer prognosis expression	Low	Low
CTLA4	P value	1.764e-11*	8.605e-10*
	Poorer prognosis expression	Low	Low
LCP2	P value	3.519e-04*	8.284e-05*
	Poorer prognosis expression	Low	Low
IFNG	P value	7.153e-04*	1.029e-02*
	Poorer prognosis expression	Low	Low
STAT1	P value	1.347e-03*	2.762e-03*
	Poorer prognosis expression	Low	Low

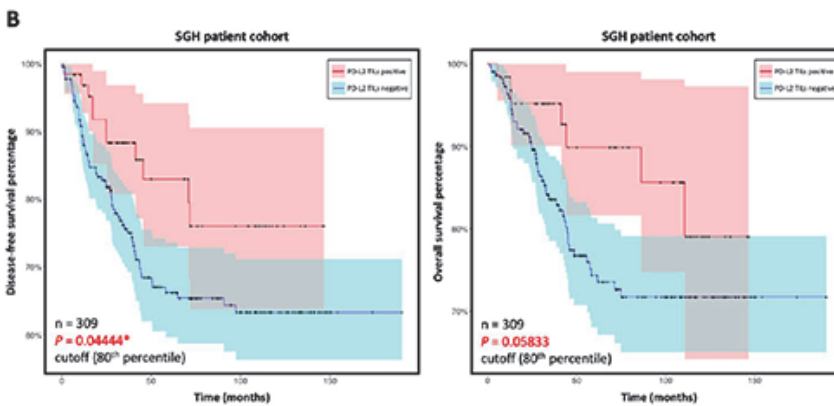
**Table 3B.** Summary of comparative survival analysis of hub genes in PDCD1LG2 expressing TNBCs

PDCD1LG2 hub genes		SGH		TCGA	FUSCC	METABRIC	
		Overall survival	Disease-free survival	Overall survival	Relapse-free survival	Overall survival	Relapse-free survival
PTPRC	P value	1.536e-03*	1.428e-03*	1.134e-01	5.859e-02	7.060e-04*	1.998e-04*
	Poorer prognosis expression	Low	Low	Low	High	Low	Low
CD4	P value	1.056e-03*	7.553e-05*	4.292e-02*	1.953e-01	1.516e-01	7.600e-02
	Poorer prognosis expression	Low	Low	High	High	Low	Low
CD8B	P value	2.085e-03*	1.662e-04*	5.110e-02	2.460e-02*	5.345e-02	8.566e-02
	Poorer prognosis expression	Low	Low	High	High	Low	Low
IL10RA	P value	2.374e-07*	1.820e-06*	5.550e-01	1.920e-01	1.051e-03*	5.247e-04*
	Poorer prognosis expression	Low	Low	High	High	Low	Low
IL1B	P value	1.573e-01	1.472e-01	5.396e-01	3.248e-01	2.177e-02*	3.531e-02*
	Poorer prognosis expression	Low	Low	High	High	Low	Low
ITGB2	P value	2.065e-03*	5.518e-05*	2.485e-01	1.084e-01	2.992e-02*	1.449e-02*
	Poorer prognosis expression	Low	Low	Low	High	Low	Low
C0B0	P value	5.412e-04*	2.289e-03*	1.454e-01	3.320e-01	2.851e-02*	1.966e-01
	Poorer prognosis expression	Low	Low	High	High	Low	Low
IL10	P value	1.739e-03*	9.570e-03*	1.530e-01	2.525e-02*	2.380e-01	2.687e-01
	Poorer prognosis expression	Low	Low	High	High	Low	High
IFNG	P value	7.153e-04*	1.029e-02*	1.131e-01	2.697e-01	1.125e-03*	7.155e-04*
	Poorer prognosis expression	Low	Low	Low	Low	Low	Low
CCL5	P value	2.435e-03*	2.239e-03*	2.116e-02*	3.412e-02*	2.054e-03*	2.510e-02*
	Poorer prognosis expression	Low	Low	Low	Low	Low	Low

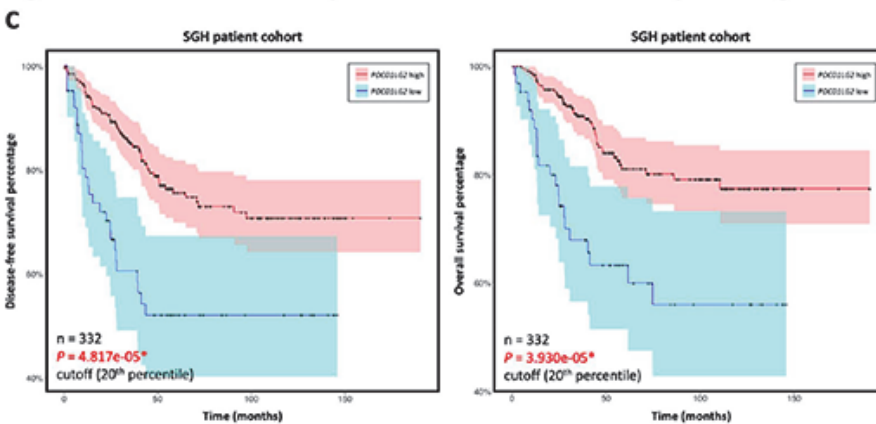
**Figure 1A.** Tissue microarray sections with PDL2 TILs staining



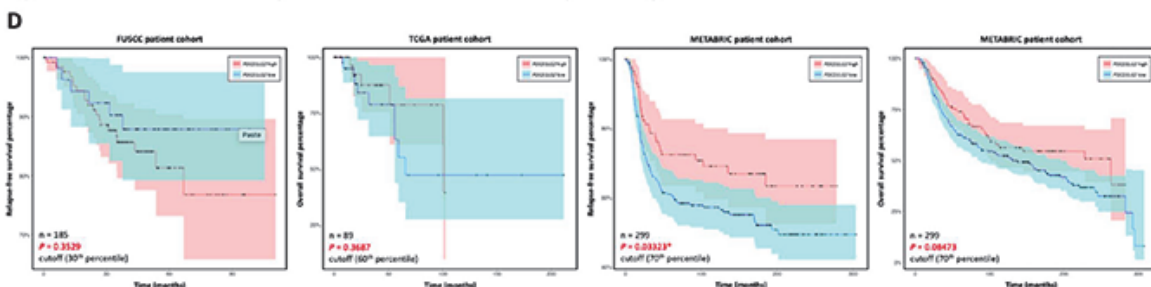
**Figure 1B.** Survival analysis of PDL2 TILs expressing TNBCs



**Figure 1C.** Survival analysis of *PDCD1LG2* TILs expressing TNBCs



**Figure 1D.** Survival analysis of *PDCD1LG2* TILs expressing TNBCs in other cohorts

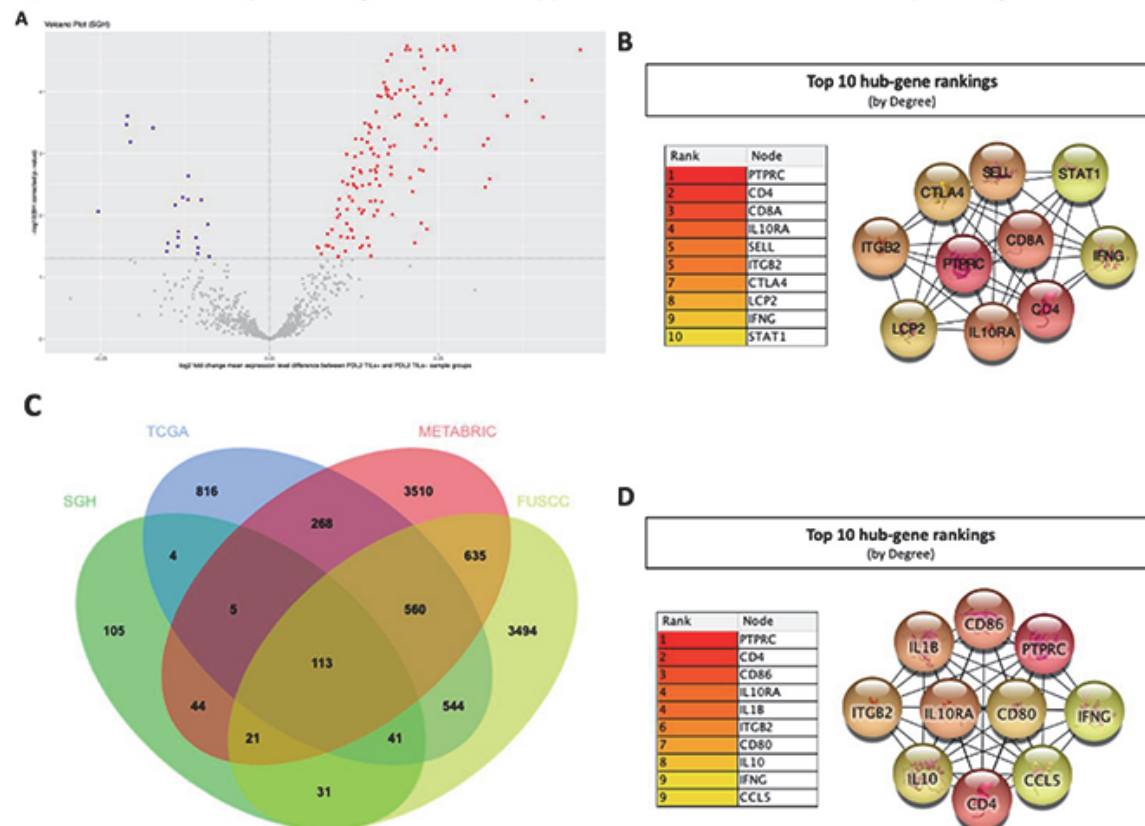


Abstract 167 Figure 1

**Figure 2. Identification of hub genes in PDL2 TILs and *PDCD1LG2* sample groups**

A) Volcano plot on differentially expressed genes of PDL2 TILs expressing TNBCs

B) PPI network of top 10 hub genes in PDL2 TILs expressing TNBCs

C) Venn diagram on overlapping DEGs of *PDCD1LG2* expressing TNBCsD) PPI network of top 10 hub genes in overlapped 113 DEGs of *PDCD1LG2* expressing TNBCs

Abstract 167 Figure 2

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