**Programmed death-ligand 1 (PD-L1) expression in tumour cell and tumour infiltrating lymphocytes of HER2-positive breast cancer and its prognostic value**

Ahrong Kim, MD, PhD[1], So Jeong Lee, MD[1], Young-Keum Kim, MD[1], Won-Young Park, MD[1], Do Youn Park, MD, PhD[1], Jee Yeon Kim, MD, PhD[2], Chang Hun Lee, MD, PhD[1], Gyungyub Gong, MD, PhD[3], Gi Young Huh, MD, PhD[1], Kyung Un Choi, MD, PhD[1]*

Author Affiliations:

[1]Department of Pathology, BioMedical Research Institute, Pusan National University Hospital, Pusan, Korea

[2]Department of Pathology, Pusan National University Yangsan Hospital, Yangsan, Korea

[3]Department of Pathology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

**Supplementary Table 1.** Basic data for 167 HER2-positive breast cancers

| Characteristic | | Number (%)* |
|---|---|---|
| Age, mean ± SD** | | 52.59 ±10.126 years |
| Size, mean ± SD** | | 3.213±1.773 cm |
| Side | | |
| | Left | 93 cases (55.7) |
| | Right | 74 cases (44.3) |
| Histologic grade | | |
| | Grade 1, 2 | 62 (37.1) |
| | Grade 3 | 105 (62.9) |
| Nuclear grade | | |
| | Grade 1, 2 | 29 (17.4) |
| | Grade 3 | 138 (82.6) |
| Necrosis | | |
| | Absent | 57 (34.1) |
| | Present | 110 (65.9) |
| Lymphovascular invasion | | |
| | Absent | 90 (53.9) |
| | Present | 77 (46.1) |
| Lymph node metastasis | | |
| | Absent | 78 (46.7) |
| | Present | 89 (53.3) |
| Stage | | |
| | Early (stage I, II) | 126 (75.4) |
| | Advanced (stage III, IV) | 41 (24.6) |
| HR expression | | |
| | Positive | 89 (53.3) |
| | Negative | 78 (46.7) |
| TILs level | | |
| | ≤10% | 61 (36.5) |
| | 20-60% | 74 (44.3) |
| | >60% | 32 (19.2) |
| TLSs around DCIS | | |
| | Low | 102 (61.1) |
| | High | 65 (38.9) |
| TLSs around invasive component | | |
| | Low | 109 (65.3) |
| | High | 58 (34.7) |

*except for age and size **SD, standard deviation

**Supplementary Table 2**. PD-L1 expression and TILs level in total 167 HER2-positive breast cancers

| Characteristic | | Number (%) |
|---|---|---|
| PD-L1 expression in tumour cells | | |
| | Immunoscore, mean±SD* | 7.03±20.853 |
| | Negative by Allred system | 86 (51.5) |
| | Positive by Allred system | 81 (48.5) |
| Immunoscore of PD-L1 in TILs | | |
| | Immunoscore, mean±SD | 15.38±23.135 |
| | Low | 116 (69.5) |
| | High | 51 (30.5) |
| Total immunoscore of PD-L1 in both tumour cells and TILs | | |
| | Immunoscore, mean±SD | 22.41±35.921 |
| | Low | 114 (68.3) |
| | High | 53 (31.7) |
| Tumour type according to Teng's classification | | |
| | Type I | 28 (16.8) |
| | Type II | 82 (49.1) |
| | Type III | 53 (31.7) |
| | Type IV | 4 (2.4) |

*SD, standard deviation

**Supplementary Table 3**. Correlation of PD-L1 expression in tumour cells and TILs*

| | PD-L1 expression in tumour cells by Allred scoring system | | p-value |
|---|---|---|---|
| | Negative (Number (%)) | Positive (Number (%)) | |
| PD-L1 expression level in TILs | | | <0.001 |
| Low | 78 (90.7) | 38 (46.9) | |
| High | 8 (9.3) | 43 (53.1) | |

*The Pearson's Chi-square test was used.

**Supplementary Table 4.** Disease-free survival based on various clinicopathologic parameters in HR-/HER2+ breast cancers*

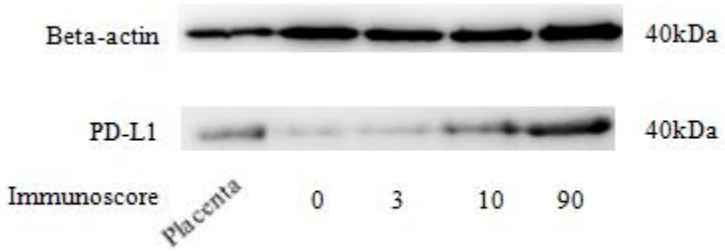
| | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | HR** | 95% CI*** | *p*-value | HR | 95% CI | *p*-value |
| Stage (I,II vs III, IV) | 15.453 | 3.370-70.849 | *P*<0.001 | 9.760 | 2.152-44.263 | *P*=0.013 |
| T stage (1,2 vs 3,4) | 6.091 | 1.955-18.979 | *P*=0.002 | 1.626 | 0.566-4.671 | *P*=0.449 |
| Lymph node metastasis | 63.050 | 0.605-6570.270 | *P*=0.080 | | | |
| Lymphovascular invasion | 6.774 | 1.482-30.956 | *P*=0.014 | 1.692 | 0.395-7.245 | *P*=0.552 |
| TILs (0-60% vs >60%) | 0.027 | 0.000-4.536 | *P*=0.167 | | | |
| PD-L1 | 0.277 | 0.075-1.022 | *P*=0.054 | 0.336 | 0.105-1.076 | *P*=0.123 |

*Cox proportional hazard regression model was used.

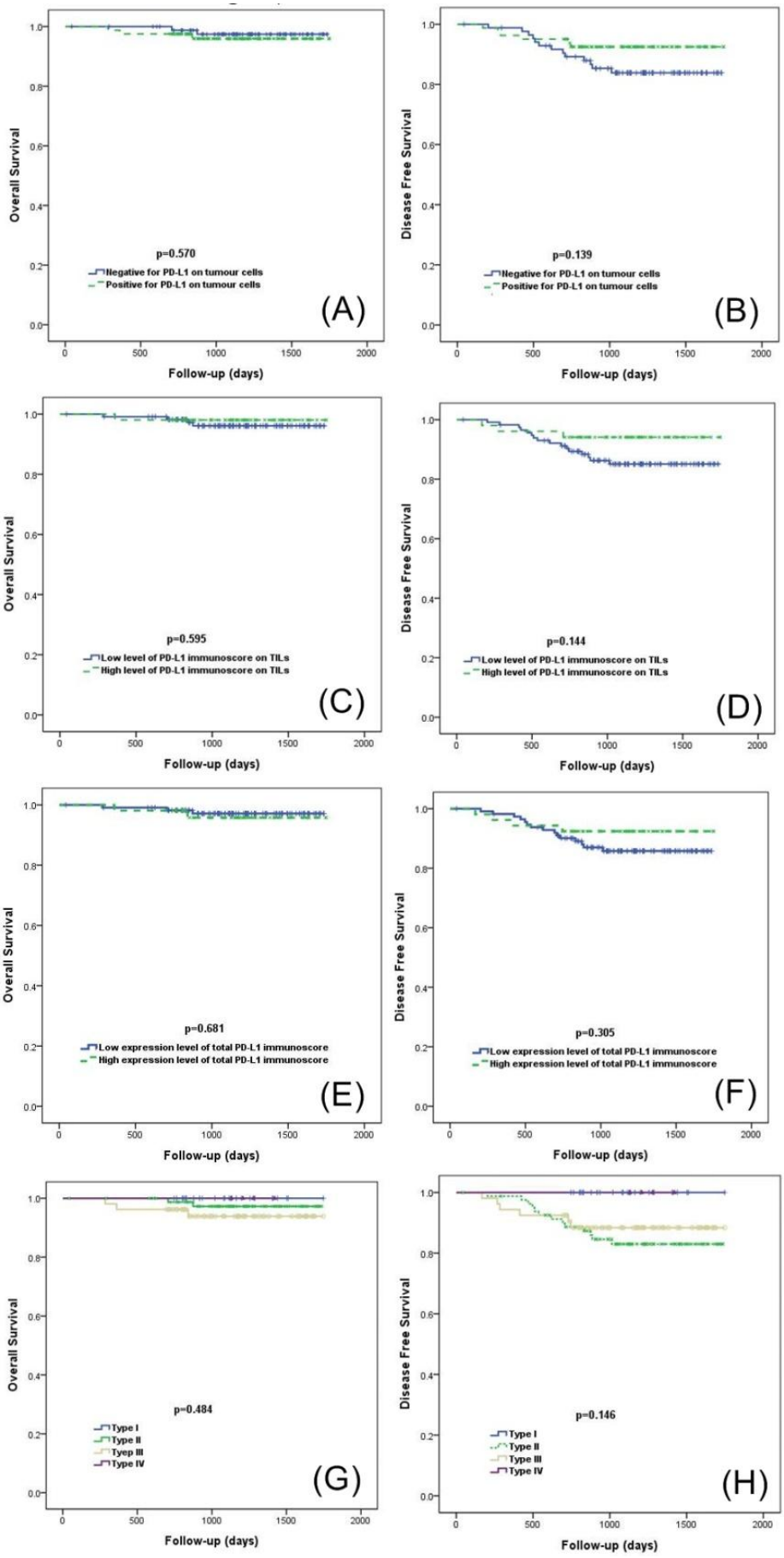
**HR, hazard ratio

***CI, confidence interval

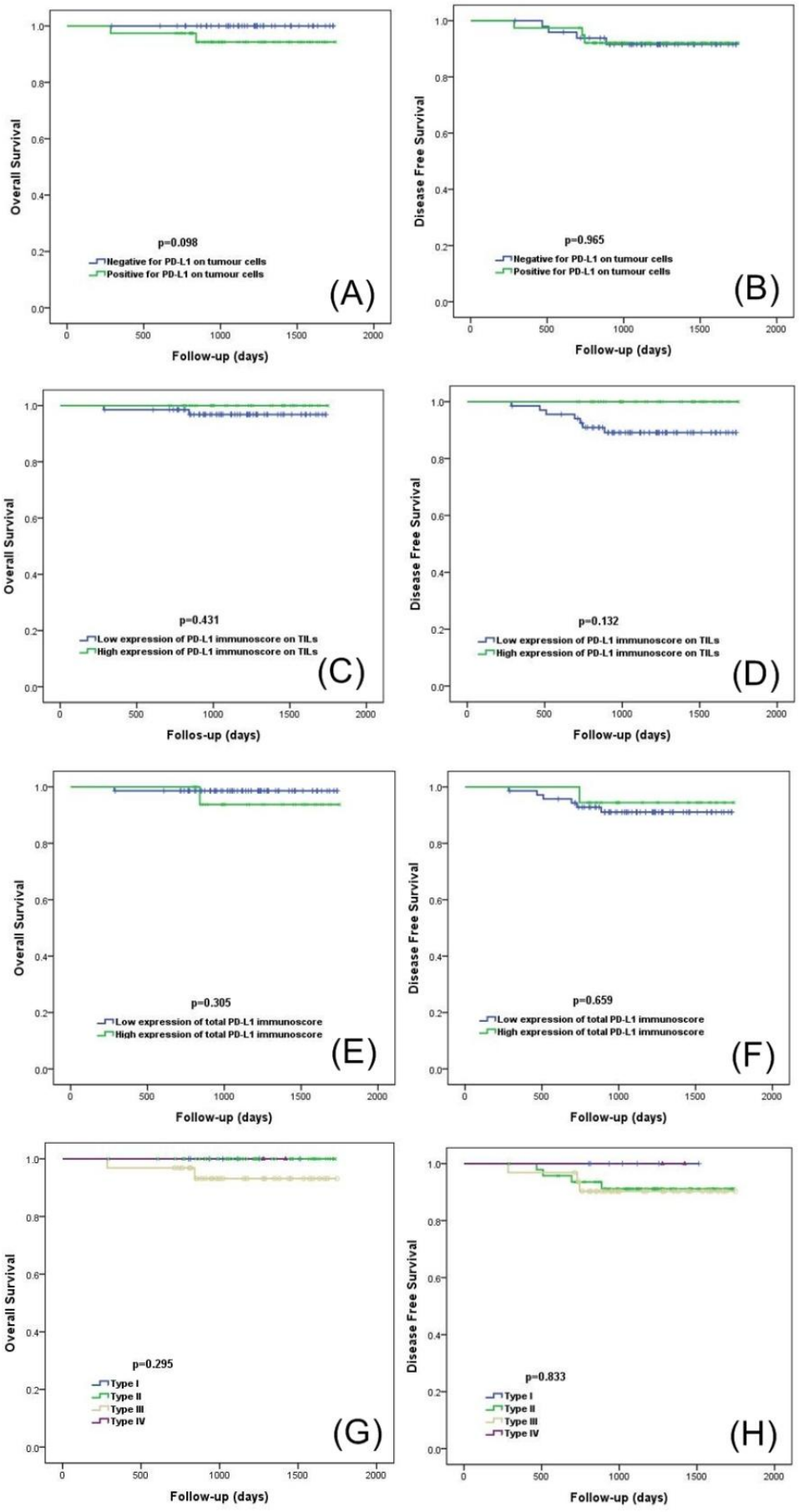
**Supplementary Figure 1.** Western blot analysis of 4 fresh tissue samples with HER2-positive breast cancers



**Supplementary Figure 2.** Overall survival and disease-free survival in 167 HER2-positive breast cancers (A) Overall survival according to PD-L1 expression in tumour cells (B) Disease-free survival according to PD-L1 expression in tumour cells (C) Overall survival according to level of PD-L1 immunoscore in TILs (D) Disease-free survival according to level of PD-L1 immunoscore in TILs (E) Overall survival according to level of total PD-L1 immunoscore (F) Disease-free survival according to level of total PD-L1 immunoscore (G) Overall survival according to tumour types (H) Disease-free survival according to tumour types


Overall Survival
p=0.570
Negative for PD-L1 on tumour cells
Positive for PD-L1 on tumour cells
(A)
Follow-up (days)
Disease Free Survival
p=0.139
Negative for PD-L1 on tumour cells
Positive for PD-L1 on tumour cells
(B)
Follow-up (days)
Overall Survival
p=0.595
Low level of PD-L1 immunoscore on TILs
High level of PD-L1 immunoscore on TILs
(C)
Follow-up (days)
Disease Free Survival
p=0.144
Low level of PD-L1 immunoscore on TILs
High level of PD-L1 immunoscore on TILs
(D)
Follow-up (days)
Overall Survival
p=0.681
Low expression level of total PD-L1 immunoscore
High expression level of total PD-L1 immunoscore
(E)
Follow-up (days)
Disease Free Survival
p=0.305
Low expression level of total PD-L1 immunoscore
High expression level of total PD-L1 immunoscore
(F)
Follow-up (days)
Overall Survival
p=0.484
Type I
Type II
Tyep III
Type IV
(G)
Follow-up (days)
Disease Free Survival
p=0.146
Type I
Type II
Type III
Type IV
(H)
Follow-up (days)

**Supplementary Figure 3.** Overall survival and disease-free survival in HR+/HER2+ breast cancers (A) Overall survival according to PD-L1 expression in tumour cells (B) Disease-free survival according to PD-L1 expression in tumour cells (C) Overall survival according to level of PD-L1 immunoscore in TILs (D) Disease-free survival according to level of PD-L1 immunoscore in TILs (E) Overall survival according to level of total PD-L1 immunoscore (F) Disease-free survival according to level of total PD-L1 immunoscore (G) Overall survival according to tumour types (H) Disease-free survival according to tumour types


Overall Survival
p=0.098
Negative for PD-L1 on tumour cells
Positive for PD-L1 on tumour cells
(A)
Follow-up (days)
Disease Free Survival
p=0.965
Negative for PD-L1 on tumour cells
Positive for PD-L1 on tumour cells
(B)
Follow-up (days)
Overall Survival
p=0.431
Low expression of PD-L1 immunoscore on TILs
High expression of PD-L1 immunoscore on TILs
(C)
Follos-up (days)
Disease Free Survival
p=0.132
Low expression of PD-L1 immunoscore on TILs
High expression of PD-L1 immunoscore on TILs
(D)
Follow-up (days)
Overall Survival
p=0.305
Low expression of total PD-L1 immunoscore
High expression of total PD-L1 immunoscore
(E)
Follow-up (days)
Disease Free Survival
p=0.659
Low expression of total PD-L1 immunoscore
High expression of total PD-L1 immunoscore
(F)
Follow-up (days)
Overall Survival
p=0.295
Type I
Type II
Type III
Type IV
(G)
Follow-up (days)
Disease Free Survival
p=0.833
Type I
Type II
Type III
Type IV
(H)
Follow-up (days)