

Original Article



CD39 and CD73 Expression in Breast Cancer: CD73 as a Favorable Prognostic Factor in HER2-Positive Tumors

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ABSTRACT

Purpose: CD39 (*ENTPD1*, ectonucleoside triphosphate diphosphohydrolase-1) and CD73 (*NT5E*, 5'-nucleotidase) are mediators of adenosine release in the tumor microenvironment (TME), and adenosine has the effect of overcoming anti-tumor immunity. This CD39-CD73-adenosine pathway is thought that play a role in immune suppression and promotion of tumor growth and infiltration. This study investigated the clinical and prognostic significance of CD39 and CD73 in breast cancer.

Methods: This study included 472 patients with primary invasive breast cancer who received surgical treatment at the Breast Cancer Clinic at Chung-Ang University Hospital from June 2010 to May 2017. Using a tissue microarray, biomarker immunostaining was performed for CD39 and CD73. We assessed the expression ratio of CD39 and CD73 in breast cancer subgroups, and investigated the association between disease-free survival (DFS) and the expression of CD39 and CD73.

Results: The expression ratio of CD39 and CD73 in breast cancer subgroups differed according to hormone receptor status and human epidermal growth factor receptor 2 (HER2) status. In the case of CD39, the high-level rate was increased in estrogen receptor (ER)/progesterone receptor (PR) positive ($p = 0.001$, $p = 0.009$), and HER2 negative ($p < 0.001$) breast cancer. In contrast, in the case of CD73 expressed in tumor-infiltrating lymphocyte (TIL), the expression rate was increased in ER/PR negative ($p < 0.001$), and HER2 positive ($p < 0.001$), breast cancer. High CD39 expression was significantly associated with lower stage ($p < 0.001$), but there was no relationship CD39 expression and survival. In patients with HER2-positive breast cancer, CD73 expression in TIL was associated with lower stage ($p = 0.024$), and better survival in DFS analysis ($p = 0.015$).

Conclusion: In conclusion, the expression ratio of CD39 and CD73 in breast cancer subgroups differed according to hormone receptor status and HER2 status, and CD73 expression in TILs associated with a trend toward favorable prognosis in HER2 positive breast cancer.

Keywords: 5'-Nucleotidase; Breast Neoplasms; CD39 Antigen; Prognosis

Hee Sung Kim <https://orcid.org/0000-0002-8154-2391>Min Kyoan Kim <https://orcid.org/0000-0002-1848-7801>**Conflict of Interest**

The authors declare that they have no competing interests.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

Conceptualization: Kim MK; Data curation: Kang E, Kim HS, Kim MK; Formal analysis: Kang E, Suh SW, Choi YS, Kim MK; Methodology: Kim HS; Supervision: Kim HS, Kim MK; Visualization: Kang E, Kim HS, Kim MK; Writing - original draft: Kang E; Writing - review & editing: Kang E, Suh SW, Choi YS, Kim HS, Kim MK.

INTRODUCTION

In breast cancer immunotherapy, immune checkpoint inhibitors (ICIs) have been pivotal, particularly targeting the programmed cell death protein 1/programmed death-ligand 1 (PD-L1) and cytotoxic T-lymphocyte-associated protein 4 pathways to enhance antitumor immune responses by inhibiting T-cell suppressive signals. Agents such as pembrolizumab and nivolumab have demonstrated clinical efficacy, especially in immune-infiltrated subtypes like triple-negative breast cancer (TNBC), by reducing T-cell exhaustion and activating antitumor immunity [1].

Food and Drug Administration approved atezolizumab plus nab-paclitaxel and pembrolizumab plus chemotherapy for patients with early-stage and metastatic TNBC, respectively, with PD-L1 expression of 1% or more. However, the factors determining the effectiveness of immunotherapy for breast cancer is not universally established [2,3]. One of the major challenges in optimizing immunotherapy for breast cancer is the heterogeneity of the tumor microenvironment (TME). The TME consists of a complex network of metabolic pathways that supply not only the nutrients necessary for tumor cell survival and growth but also produce metabolites that influence the composition of the immune infiltrate, immune cell differentiation and effector function [4,5]. The adenosine pathway plays a crucial role in establishing an immunosuppressive tumor microenvironment in breast cancer. The enzymes CD39 (*ENTPD1*, ectonucleoside triphosphate diphosphohydrolase-1) and CD73 (*NT5E*, 5'-nucleotidase) are key regulators in this pathway, converting extracellular adenosine triphosphate (ATP) and adenosine diphosphate (ADP) into immunosuppressive adenosine. This process inhibits the functions of T cells and natural killer (NK) cells, facilitating immune evasion and promoting tumor progression [4,6]. CD39 and CD73 are ectoenzymes expressed in regulatory T cells (Tregs) and tumor cells [7]. CD39 converts ATP and ADP into adenosine monophosphate (AMP), which is subsequently converted into adenosine by CD73 [4,7]. The CD73-derived adenosine then binds to its receptor at the surface of T cells and NK cells [6,7], leading to inhibition of immune cell responses and immune escape [8]. Furthermore, adenosine enhances endothelial cell proliferation and the production of proangiogenic factors such as vascular endothelial growth factor and interleukin-8, contributing to tumor progress [9]. This mechanism can potentially undermine the efficacy of ICIs, underscoring the potential benefit of combining adenosine pathway inhibitors with existing checkpoint blockade therapies [6].

The activation of the CD39/CD73/adenosine pathway has been closely associated with poor prognosis in multiple cancers, including TNBC [10-12], however, CD39 and CD73 is also expressed in various stromal cells and immune cells. Its prognostic significance may depend on the specific cell type and immune context within the tumor microenvironment [4,12,13]. In particular, Supernat et al. [14] demonstrated that CD73 expression is associated with favorable prognosis in breast cancer, suggesting that the prognostic impact of CD39 and CD73 can vary by cancer subtype and cellular localization. Moreover, CD73 expressed on tumor-infiltrating lymphocytes (TILs) may reflect an active immune microenvironment, potentially associated with improved patient outcomes.

Therefore, our study aims to investigate the expression levels of adenosine pathway markers in breast cancer tissues and TILs. Specifically, we seek to explore the differences in expression across breast cancer subtypes and assess their correlation with patient prognosis.

METHODS

Patients

This study included 472 patients with primary invasive breast cancer who received surgical treatment at the Breast Cancer Clinic at Chung-Ang University Hospital from June 2010 to May 2017. This is a retrospective study. The exclusion criteria were as follows: 1) tumor size < 0.7 cm which is inappropriate for tissue microarray analysis; 2) recurrent breast cancer; 3) distant metastasis; or, 4) insufficient data for analysis. The last follow-up date was December 2021. Recurrence was defined as locoregional recurrence or distant metastasis. Disease-free survival (DFS) was defined as the period after surgery without recurrence. The primary end point was DFS based on the expression of CD39 and CD73. This study was approved by the Institutional Review Board (IRB) of Chung-Ang University Hospital (IRB No. 2081-002-433). The need for informed consent was waived on account of the retrospective nature of the study.

Immunohistochemistry (IHC)

After a diagnosis of breast cancer was made, a tissue microarray was generated from residual tumor paraffin blocks. Biomarker immunostaining was performed on the tissue microarray for CD39 and CD73. In the case of CD73, staining for CD73 expression was analyzed separately for tumor cells and TILs. After sectioning the paraffin-embedded tissue to a thickness of 4 μ m, it was dried well in an oven at 56°C–58°C. For deparaffinization, xylene was applied five times for four minutes each, 100% ethanol was applied three times for one minute each, and 95% ethanol was applied three times for one minute each. Then the section was immersed in distilled water.

For antigen activation, the slide was immersed in 10 mM citrate buffer (pH 6.0, 0.01 M solution of citric acid, 0.01 M solution of sodium citrate) and the slide was then boiled for 10 minutes or reacted with enzyme in an oven at 37°C for 10 minutes. To wash with phosphate-buffered saline (PBS), the slide was reacted with normal serum for 30 minutes and with anti-CD39 and anti-CD73 monoclonal antibody for two hours. After washing with PBS, the slide was reacted with biotin-labeled secondary antibody for 30 minutes and then incubated with peroxidase-labeled streptavidin for 30 minutes. After staining with 3,3'-diaminobenzidine, the slide was counterstained for 10 seconds with Mayer's hematoxylin. After passing through 95% ethanol twice, 100% ethanol twice, and xylene five times, the tissue microarray was sealed with a non-aqueous sealing agent.

Biomarker analysis

For each biomarker, the intensity and percentage expression were evaluated separately and scored. Intensity was rated on a scale of 0 to 2 (0: negative, 1: weak, 2: strong). Median values were then determined for each biomarker. Based on these median values, high and low expression levels were categorized.

If the expression ratio of a biomarker was less than 50%, it was categorized simply as “expressed” or “not expressed” rather than divided into high or low levels. For CD39, intensity was rated on a scale of 0 to 2, with level 2 defined as high expression (**Figure 1**). For CD73, the expression ratio was below 50% for both tumor cells and TILs (**Figure 2**), so CD73 was classified as either expressed or not expressed.

The intensity of TILs was classified into grades 1 through 3. Grade 1 (low grade) was defined as having less than 10% lymphocyte presence in the stromal tissue surrounding the cancer nest.

Grade 2 (intermediate grade) was assigned when lymphocytes made up less than 50%, and grade 3 (high grade) was assigned when lymphocytes comprised 50% or more of the area [15,16].

Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) data set

The survival analysis of our study was verified using the METABRIC database, one of the breast cancer datasets [17]. The METABRIC database was accessed through the cBioportal website (<https://www.cbioportal.org/>). It contains normalized about 2000 RNA microarray profiling and clinical information such as hormone receptor, human epidermal growth factor receptor 2 (HER2) status, relapse, survival data and treatment. This cohort represents an entirely independent validation set with no patient overlap with our study population.

CD39 and CD73 are cell surface protein that is encoded by ectonucleoside triphosphate diphosphohydrolase 1 (*ENTPD1*) and 5'-nucleotidase ecto (*NT5E*) gene, respectively. So, we

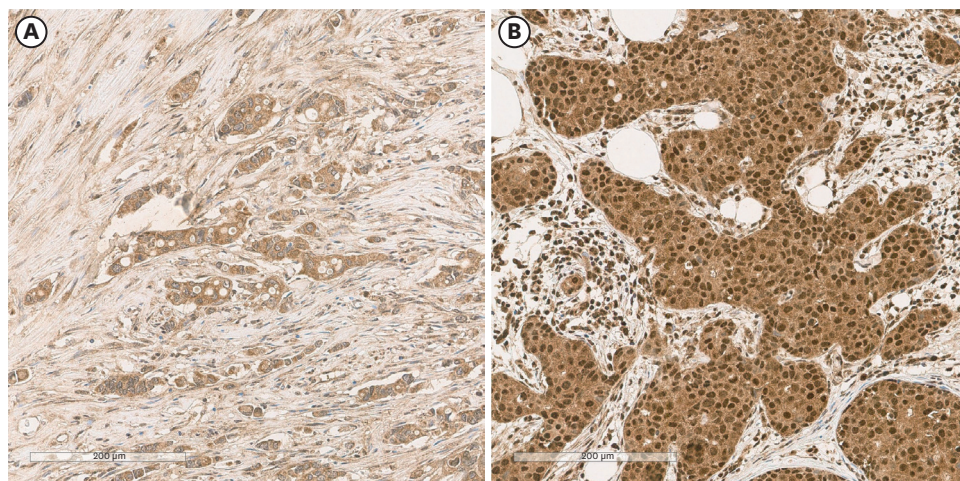


Figure 1. CD39 expression in tumor epithelial cell. Representative images of (A) low CD39 expression in tumor epithelial cell, and (B) high CD39 expression in tumor epithelial cell.

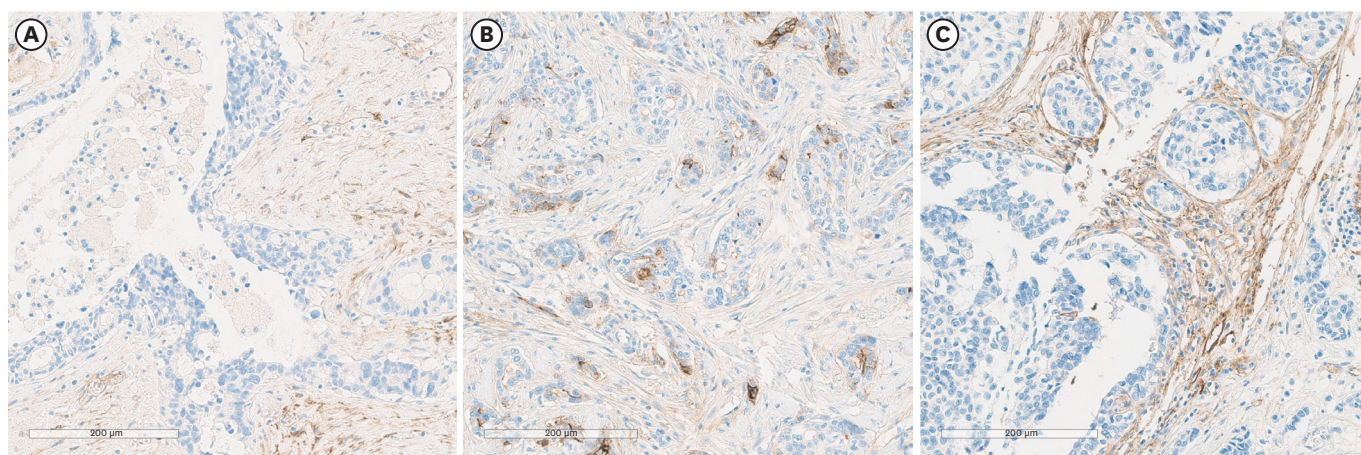


Figure 2. CD73 expression in tumor epithelial cell in invasive breast cancer.

(A) No CD73 expression in either epithelial cells or TILs, (B) representative images of CD73 expression in tumor epithelial cell. CD73 is positive in 5%–10% of tumor epithelial cells with intensity 2, (C) representative images of CD73 expression in TILs, CD73 is positive in 60% of TILs. TIL = tumor-infiltrating lymphocyte.

analyzed patients with stage 1 to 3, excluding ductal carcinoma *in situ* and metastasis. Survival analysis was performed according to the expression of *ENTPD1* and *NTSE* gene. For each gene expression, high and low level cut-off points were determined based on the median value of RNA microarray expression level within the METABRIC dataset. The subtypes of breast cancer were divided based on Prediction Analysis of Microarray 50 (PAM50). It is molecular subtyping, performed using gene expression derived from microarray, and it consisted of luminal A, luminal B, HER2-enriched, basal-like, normal-like subtypes [18].

Statistical analysis

For intergroup comparisons, Student's *t*-test or the Wilcoxon rank sum test was used for continuous variables, and the χ^2 test was using for descriptive data. Kaplan-Meier curves and log-rank tests were used to assess DFS and overall survival (OS). A *p*-value < 0.05 was considered statistically significant. Statistical analyses were conducted using R version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patient characteristics

The total number of patients was 472, and the median age was 53.0 years (range: 24–86). A total of 194 patients (41.1%) were pre-menopause, and 273 patients (57.8%) were post-menopause. According to stage, the number of patients with T1, T2, T3 and T4 stage disease was 260 (55.1%), 186 (39.4%), 19 (4.0%), and 7 (1.5%), respectively. A total of 303 patients had no axillary node metastasis (**Supplementary Table 1**).

According to breast cancer subtype, there were 355 patients (75.2%) with luminal type (estrogen receptor [ER]-positive or progesterone receptor [PR]-positive) and 45 patients (9.5%) with HER2 type (hormone receptor [HR]-negative, HER2-positive). There were 71 patients (15.0%) with the triple negative type (HR-negative, HER2-negative).

Clinicopathologic features according to ectoenzyme expression

For IHC, the number of cases with a TIL intensity of grade 1, 2 and 3 was 284 (60.2%), 129 (27.3%), and 59 (12.5%), respectively. For CD39, approximately 60.7% of cases showed a high expression level. CD73 was expressed in both tumor cells and TILs; there were 131 patients (27.8%) with CD73 expression in TILs, and 25 patients (5.3%) with CD73 expression in tumor cells (**Supplementary Table 2**).

The clinicopathologic features according to CD39/CD73 expression are shown in **Table 1**. High CD39 expression was significantly associated with lower stage ($p < 0.001$), but there was no relationship between CD73 expression and stage. With regard to the relationship between TILs and CD39/CD73, high CD39 expression was associated with the higher TIL grade, but CD73 expression in TIL was associated with lower TIL grade ($p = 0.007$, $p < 0.001$, respectively).

Although the expression of CD73 was not related to the pathologic stage in all breast cancer patient, CD73 expression in TIL was associated with stage in subgroup analysis divided by HR status and HER2 status. CD73 expression in TIL was not associated with stage in HR-positive and triple negative breast cancer. However, in HER2-positive breast cancer, CD73 expression in TIL was more common in N0 group, and it was significantly associated with lower stage (**Table 2**).

CD73 as a Good Prognostic Factor in Breast Cancer

Table 1. Clinicopathologic features according to ectoenzyme expression related to adenosine pathway

Features	CD39			CD73 in TC			CD73 in TIL		
	Low (n = 185)	High (n = 287)	p-value	Low (n = 447)	High (n = 25)	p-value	Low (n = 341)	High (n = 131)	p-value
Age (yr)	54 [47–65]	53 [46–62]	0.093	53 [47–63]	47 [42–58]	0.030	54 [47–64]	52 [46–59]	0.045
Menopause			0.013			< 0.001			0.653
Premenopause	70 (37.8%)	124 (43.2%)		179 (40.0%)	15 (60.0%)		136 (39.9%)	58 (44.3%)	
Postmenopause	110 (59.5%)	163 (56.8%)		265 (59.3%)	8 (32.0%)		201 (58.9%)	72 (55.0%)	
Unknown	5 (2.7%)	0 (0.0%)		3 (0.7%)	2 (8.0%)		4 (1.2%)	1 (0.8%)	
T stage			0.001			0.771			0.537
1	81 (43.8%)	179 (62.4%)		248 (55.5%)	12 (48.0%)		181 (53.1%)	79 (60.3%)	
2	91 (49.2%)	95 (33.1%)		174 (38.9%)	12 (48.0%)		140 (41.1%)	46 (35.1%)	
3	11 (5.9%)	8 (2.8%)		18 (4.0%)	1 (4.0%)		15 (4.4%)	4 (3.1%)	
4	2 (1.1%)	5 (1.7%)		7 (1.6%)	0 (0.0%)		5 (1.5%)	2 (1.5%)	
N stage			0.034			0.653			0.931
NO	108 (58.4%)	195 (67.9%)		288 (64.4%)	15 (61.5%)		218 (63.9%)	85 (64.9%)	
Node +	77 (41.6%)	92 (32.1%)		159 (35.6%)	10 (38.5%)		123 (36.1%)	46 (35.1%)	
ER			0.001			< 0.001			< 0.001
Negative	64 (34.6%)	58 (20.2%)		102 (22.8%)	20 (80.0%)		71 (20.8%)	51 (38.9%)	
Positive	121 (65.4%)	229 (79.8%)		345 (77.2%)	5 (20.0%)		270 (79.2%)	80 (61.1%)	
PR			0.009			< 0.001			< 0.001
Negative	76 (41.1%)	83 (28.9%)		139 (31.1%)	20 (80.0%)		98 (28.7%)	61 (46.6%)	
Positive	109 (58.9%)	204 (71.1%)		308 (68.9%)	5 (20.0%)		243 (71.3%)	70 (53.4%)	
HER2			< 0.001			0.362			< 0.001
Negative	122 (65.9%)	235 (81.9%)		341 (76.3%)	16 (64.0%)		272 (79.8%)	85 (64.9%)	
Positive	56 (30.3%)	43 (15%)		91 (20.4%)	8 (32.0%)		55 (16.1%)	44 (33.6%)	
Unknown	7 (3.8%)	9 (3.1%)		15 (3.4%)	1 (4.0%)		14 (4.1%)	2 (1.5%)	
TIL			0.007			0.413			< 0.001
1	95 (51.4%)	189 (65.9%)		267 (59.7%)	17 (68.0%)		259 (76.0%)	25 (19.1%)	
2	62 (33.5%)	67 (23.3%)		125 (28.0%)	4 (16.0%)		69 (20.2%)	60 (45.8%)	
3	28 (15.1%)	31 (10.8%)		55 (12.3%)	4 (16.0%)		13 (3.8%)	46 (35.1%)	
Ki-67			< 0.001			0.276			0.079
< 10%	32 (17.3%)	78 (27.2%)		107 (23.9%)	3 (12.0%)		86 (25.2%)	24 (18.3%)	
≥ 10%	100 (54.1%)	171 (59.6%)		253 (56.6%)	18 (72.0%)		185 (54.3%)	86 (65.6%)	
Unknown	53 (28.6%)	38 (13.2%)		87 (19.5%)	4 (16.0%)		70 (20.5%)	21 (16.0%)	
HG			0.001			0.013			0.017
1	29 (15.7%)	57 (19.9%)		85 (19.0%)	1 (4.0%)		67 (19.6%)	19 (14.5%)	
2	62 (33.5%)	139 (48.4%)		194 (43.4%)	7 (28.0%)		155 (45.5%)	46 (35.1%)	
3	76 (41.1%)	75 (26.1%)		136 (30.4%)	15 (60.0%)		99 (29.0%)	52 (39.7%)	
Unknown	18 (9.7%)	16 (5.6%)		32 (7.2%)	2 (8.0%)		20 (5.9%)	14 (10.7%)	

Values are presented as median [interquartile range] or number of patients (%).

TC = tumor cell; TIL = tumor-infiltrating lymphocyte; ER = estrogen receptor; PR = progesterone receptor; HER2 = human epidermal growth factor receptor; HG = histologic grade.

Table 2. Subgroup analysis of pathologic stage according to CD73 expression

Variables	CD73 in TIL with HR+			CD73 in TIL with HER2+			CD73 in TIL with TN		
	Low (n = 273)	High (n = 82)	p-value	Low (n = 55)	High (n = 44)	p-value	Low (n = 41)	High (n = 30)	p-value
T stage			0.327			0.093			0.115
1	156 (57.1%)	53 (64.6%)		22 (40.0%)	27 (61.4%)		14 (34.1%)	14 (46.7%)	
2	102 (37.4%)	28 (34.1%)		26 (47.3%)	16 (36.4%)		26 (63.4%)	12 (40.0%)	
3	11 (4.0%)	1 (1.2%)		5 (9.1%)	1 (2.3%)		1 (2.4%)	2 (6.7%)	
4	4 (1.5%)	0 (0.0%)		2 (3.6%)	0 (0.0%)		0 (0.0%)	2 (6.7%)	
N stage			0.999			0.004			0.846
NO	182 (66.7%)	55 (67.1%)		27 (49.1%)	35 (79.5%)		24 (58.5%)	16 (53.3%)	
Node +	91 (33.3%)	27 (32.9%)		28 (50.9%)	9 (20.5%)		17 (41.5%)	14 (46.7%)	
HG			0.228			0.422			0.625
1	65 (23.8%)	17 (20.7%)		5 (9.1%)	3 (6.8%)		0 (0.0%)	0 (0.0%)	
2	139 (50.9%)	36 (43.9%)		19 (34.5%)	15 (34.1%)		6 (14.6%)	3 (10.0%)	
3	54 (19.8%)	20 (24.4%)		26 (47.3%)	17 (38.6%)		32 (78.0%)	26 (86.7%)	
Unknown	15 (5.5%)	9 (11.0%)		5 (9.1%)	9 (20.5%)		3 (7.3%)	1 (3.3%)	

Values are presented as number of patients (%).

TIL = tumor infiltrating lymphocyte; HR+ = hormone receptor-positive; HER2+ = human epidermal growth factor receptor 2-positive; TN = triple negative; HG = histologic grade.

The expression levels of CD39 and CD73 in breast cancer subgroups differed significantly according to HR status and HER2 status. High expression of CD39 was more frequently observed in ER-positive breast cancer (65.4% in ER-positive vs. 47.5% in ER-negative, $p = 0.001$) and PR-positive breast cancer (65.2% in PR-positive vs. 52.2% in PR-negative, $p = 0.009$). Similarly, HER2-negative breast cancer showed higher CD39 expression rates compared to HER2-positive cases (65.8% vs. 43.4%, $p < 0.001$).

In contrast, CD73 expression in tumor cells was also significantly more frequent in ER-negative (16.4% vs. 1.4%, $p < 0.001$) and PR-negative breast cancer (41.8% vs. 22.9%, $p < 0.001$). CD73 expression in TILs was higher in ER-negative breast cancer (41.8% vs. 22.9%, $p < 0.001$) and PR-negative breast cancer (38.4% vs. 22.4%, $p < 0.001$). Detailed subgroup expression rates are shown in **Figure 3**.

Survival analysis based on breast cancer subgroups

Since the CD39/CD73 expression rates were different according to the subgroups, and CD39/CD73 expression was associated with lower stage, a survival analysis was performed based on these subgroups. In patients with HER2-positive breast cancer, the DFS was better when CD73 was expressed in TILs ($p = 0.015$; **Figure 4B**). In HR positive breast cancer, triple negative breast cancer and in the overall cohort, DFS was not associated with CD73 expression in TIL ($p = 0.267$, $p = 0.810$ and $p = 0.49$; **Figure 4A, C and D**). CD39 high expression was associated with lower stage, but it showed no significant association with DFS or OS. Similarly, CD73 expression in tumor cells was not associated with differences in DFS or OS. These results remained consistent across all subgroups (**Supplementary Figures 1 and 2**).

We found that CD73 expression in TIL was good prognostic factor in HER2-positive breast cancer using Kaplan-Meier analysis of DFS. CD73 expression in TIL was correlated with good survival in univariate Cox regression models for disease free survival (hazard ratio, 0.12; $p = 0.043$; **Table 3**). In multivariate Cox regression model, adjusting T stage, N stage, histologic grade, hormone receptor, CD73 expression in TIL was close to significance correlated with good survival for DFS (hazard ratio, 0.13; $p = 0.062$; **Table 3**).

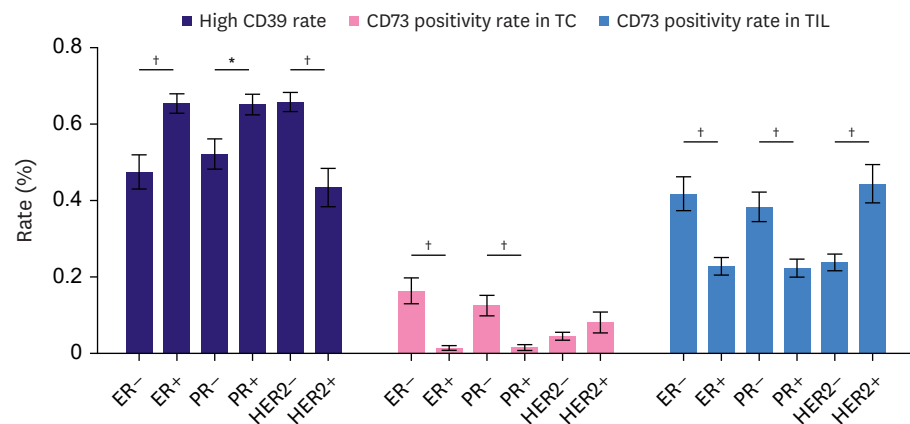


Figure 3. CD39/CD73 expression rate for each subgroup.

TC = tumor cell; TIL = tumor-infiltrating lymphocyte; ER- = estrogen receptor-negative; ER+ = estrogen receptor-positive; PR- = progesterone receptor-negative; PR+ = progesterone receptor-positive; HER2- = human epidermal growth factor receptor 2-negative; HER2+ = human epidermal growth factor receptor 2-positive.

* $p < 0.01$, † $p < 0.001$.

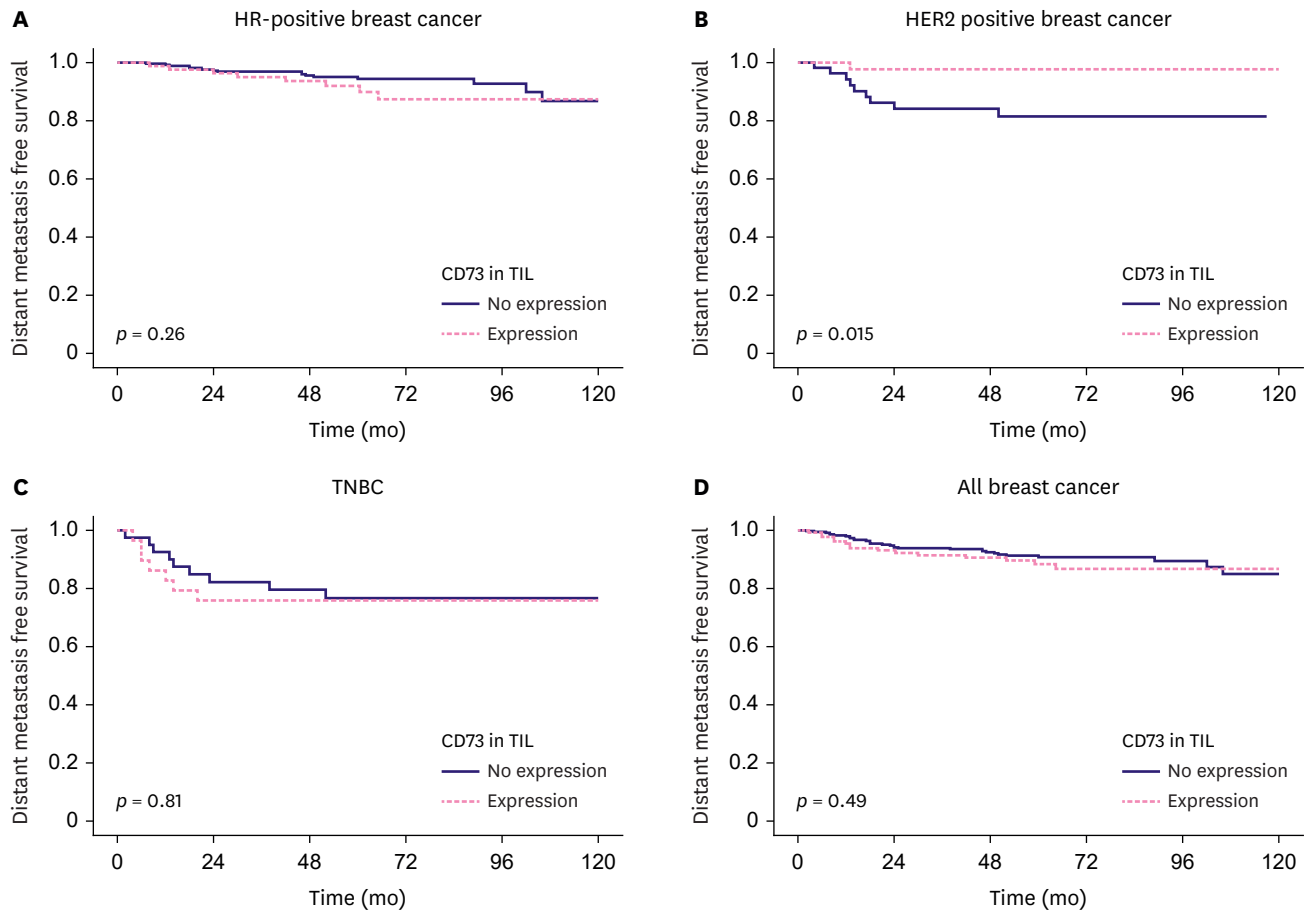


Figure 4. Disease-free survival curves stratified by CD73 expression in tumor-infiltrating lymphocytes.

Kaplan-Meier analysis, log rank p -value of DFS for each subgroup; (A) HR-positive breast cancer, (B) HER2-positive breast cancer, (C) TNBC, and (D) all breast cancer. DFS = disease-free survival; HR = hormone receptor; HER2 = human epidermal growth factor receptor 2; TNBC = triple-negative breast cancer; TIL = tumor-infiltrating lymphocyte.

Table 3. Univariate and multivariate analysis of prognostic factors in HER2 positive breast cancer

Variables	Disease-free survival					
	Univariate			Multivariate		
	Hazard ratio	95% CI	p -value	Hazard ratio	95% CI	p -value
CD73 expression in TIL						
No expression	1			1		
Expression	0.12	0.02–0.94	0.043	0.13	0.02–1.10	0.062
T stage						
T1	1			1		
T2	2.61	0.65–10.42	0.176	2.25	0.46–10.92	0.316
T3/T4	2.48	0.26–23.89	0.432	1.28	0.10–15.71	0.848
N stage						
Node negative	1			1		
Node positive	2.85	0.81–10.12	0.104	1.42	0.33–6.14	0.637
HG						
HG1, 2, unknown	1			1		
HG3	0.85	0.24–3.02	0.803	0.59	0.16–2.22	0.432
HR						
Negative	1			1		
Positive	0.57	0.16–2.01	0.380	0.68	0.19–2.48	0.563

HER2 = human epidermal growth factor receptor; CI = confidence interval; TIL = tumor-infiltrating lymphocyte; HG = histologic grade; HR = hormone receptor.

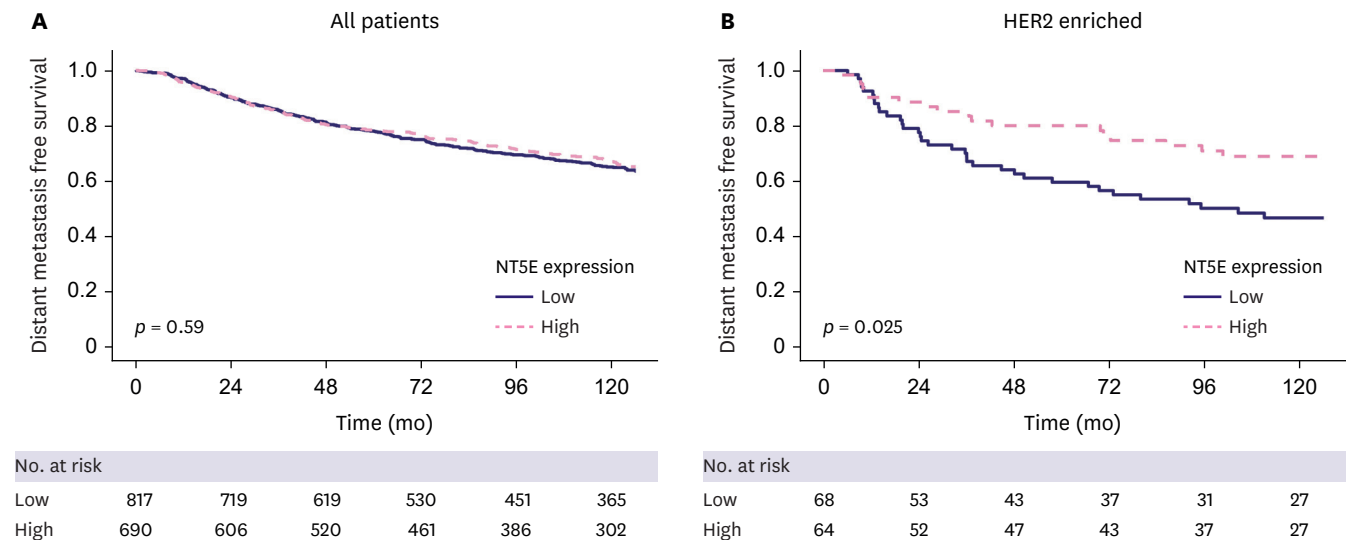


Figure 5. Disease-free survival stratified *NT5E* expression of Molecular Taxonomy of Breast Cancer International Consortium database. Analyze DFS of breast cancer patients who have not been treated adjuvant therapy with METABRIC database. (A) DFS according to *NT5E* expression level in total group, (B) DFS according to *NT5E* expression level in HER2-enriched group. DFS = disease-free survival; METABRIC = Molecular Taxonomy of Breast Cancer International Consortium; HER2 = human epidermal growth factor receptor 2.

To confirm the prognostic value of CD73, survival analysis was performed using METABRIC database. We analyzed DFS of breast cancer patients who have not been treated chemotherapy. For all untreated patients, *NT5E* (CD73) expression was not associated with DFS, but in HER2-enriched group, higher *NT5E* (CD73) was good prognostic factor in DFS same as our results ($p = 0.019$; **Figure 5**).

DISCUSSION

In this study, we showed the levels of CD39 and CD73 were different according to the expression of HR and HER2 in breast cancer. In the case of CD39, high level of expression was more common in HR-positive and HER2-negative. In contrast, in the case of CD73, the expression rate was increased in HR-negative and HER2-positive breast cancer. The pathologic stage and patients' survival analysis also showed different results according to CD39/CD73 expression and breast cancer subgroups. High CD39 expression was significantly associated with lower stage, but there was no relationship CD39 expression and survival. In patients with HER2-positive breast cancer, CD73 expression in TIL was associated with lower stage, and better survival in DFS analysis.

There are several studies showing the relationship between CD39/CD73 and ER status. Ni et al. [19] showed that CD39 was overexpressed in luminal breast cancer using RNA sequencing expression data. Using clinical samples, Martin et al. [20] revealed that a significant inverse correlation between ER status and CD73 expression, and Sychala et al. [21] revealed that CD73 mRNA, CD73 protein expression, and adenosine generation are negatively regulated through ER-positive cells and found that administration of tamoxifen as an ER antagonist increased the mRNA expression level of CD73 *in vitro*. Similarly, in our study, CD39 expression was high in ER-positive breast cancer, and CD73 expression was high in ER-negative breast cancer.

Although the adenosine pathway has the effect of overcoming anti-tumor immunity, in our study, CD39 was not associated with survival, and CD73 was a good prognostic factor in some subgroup.

Several reasons can be considered for these results. First, the adenosine effect may be different depending on subtype of breast cancer. There are several papers that have previously revealed that relation with CD39/CD73 over expression and prognosis in breast cancer. One multivariate analysis suggested that CD39 expression was an independent poor prognostic factor for luminal breast cancer [19]. Loi et al. [11] revealed that high CD73 expression was associated with a poor prognosis TNBC using gene expression data from more than 6,000 cases. Not all previous studies revealed poor prognostic results for CD73 expression. Supernat et al. [14] showed that CD73 expression was associated with a good prognosis in 136 patients with breast cancer. This paper analyzed all types of breast cancer without distinguishing HR and HER2 expression. This suggests that the relationship between CD39/CD73 expression and survival may vary depending on the HR and HER2 status.

Second, it is thought that CD73 expressing cells may have different effects on survival. Many other studies, survival analyses were performed based on the expression of CD73 in tumor cells, but in our study, the survival analysis was performed based on the expression of CD73 in TILs. In triple negative breast cancer, Buisseret et al. [10] revealed that high level of CD73 expression in tumor cell were significantly associated with reduced DFS/OS but, no clear association CD73 expression in TIL with clinical outcome were found.

Several studies have shown that CD73 expression is a good prognostic factor in other cancers. In urothelial bladder cancer, Wettstein et al. [22] revealed that high CD73 expression was related with lower stage, lower grade, and high CD73 expression predicts better outcome in the subgroup of pTa and pT1. In addition, Oh et al. [23] revealed that CD73 overexpression in epithelial ovarian carcinoma was more frequently detected in patients with known good prognostic factors such as low stage and well differentiation, and it showed better overall survival compared to the CD73 negative group.

There are few studies about the value of CD73 expression in HER2 type breast cancer. Turcotte et al. [24] showed that high CD73 expression is associated with resistance to trastuzumab therapy using mouse models of HER2 positive breast cancer. However, CD73 expression was not associated with survival in HER2 type breast cancer [24]. Considering that CD73 expression is increased in HER2 positive breast cancer, and it is associated with trastuzumab therapy, CD73 expression is thought to be regulated by HER2 status.

CD39 and CD73 are expressed in various cells such as tumor cells, immune cells, and non-immune cells in the TME. CD39 and CD73 suppress antitumor immunity by multiple mechanisms in the TME including through, Treg differentiation, macrophage differentiation, regulatory inflammatory cytokine secretion [25-27], thus regulating the balance of immunosuppressive activity and the inflammatory response [25]. In addition, there are many factors that interact with each other, such as estrogen signaling, HER2 status and TILs.

As a results, although the CD39-CD73-adenosine pathway is thought that play a role in immune suppression and promotion of tumor growth and infiltration, it is difficult to conclude that adenosine pathway factor such as CD39 and CD73 are directly related to survival.

Considering these results, the clinical application of adenosine pathway-targeted immunotherapy in breast cancer requires a subtype-specific approach. Our findings suggest that CD73-high TILs are associated with improved DFS specifically in HER2-positive breast cancer, presenting several potential clinical applications. This marker could serve as a prognostic tool for risk stratification, potentially identifying HER2-positive patients with better outcomes who might be candidates for de-escalation strategies in future trials. Additionally, given CD73's role in immune regulation, its expression on TILs might help select patients who could benefit from combination approaches of anti-HER2 therapies with immunotherapeutic agents. The varying expression levels of CD39 and CD73 across subtypes suggest that drugs targeting the adenosine pathway may have differential effects in each subgroup. Furthermore, the observed relationships between CD73 expression, tamoxifen treatment, and TIL intensity suggest potential interactions that should be considered when developing therapeutic strategies targeting this pathway.

One limitation of our study is that it was difficult to accurately correct for other factors affecting survival due to the small number of people in each subgroup. Furthermore, the rate of CD73 expression in tumor cells was low (5.3%); thus, the relation between this and survival remains unclear. Therefore, further study of survival in a large number of patients is needed. Another limitation of our study is the inability to identify which specific immune cell subsets within the TIL population express CD73 using conventional IHC methods. Given that CD73 is known to be expressed by various immune cells with distinct functions—including immunosuppressive Tregs and effector CD8⁺ T cells—this limitation impacts our ability to fully interpret the biological significance of our findings. Future studies employing single-cell RNA sequencing or multiplex immunofluorescence approaches will be necessary to characterize the specific immune cell subsets expressing CD73 within the breast tumor microenvironment and to better understand their functional roles across different breast cancer subtypes. Our external validation used PAM50-derived HER2-enriched subtypes from METABRIC database, which is not directly equivalent to the IHC-based HER2 classification used in our primary cohort. This methodological difference should be considered when interpreting these validation results.

Our study demonstrates that the expression of CD39 and CD73 varies across different breast cancer subtypes based on HR and HER2 status. Notably, CD73 expression in TILs is associated with lower stage and a trend toward favorable prognosis in HER2-positive breast cancer, suggesting its potential as a prognostic biomarker. Conversely, CD39 expression, while linked to a lower stage, does not show significant correlation with survival outcomes. These findings underscore the complexity of the adenosine pathway's role in breast cancer and highlight the need for tailored therapeutic strategies that consider subtype-specific expression patterns. Targeting CD73 in HER2-positive breast cancer could provide therapeutic benefit by enhancing anti-tumor immunity while serving as a predictive biomarker for treatment response. Further studies with larger patient cohorts are warranted to validate these findings and explore the integration of adenosine pathway inhibitors into existing treatment regimens to improve patient outcomes.

SUPPLEMENTARY MATERIALS

Supplementary Table 1

Patient characteristics (total n = 472)

Supplementary Table 2

Result of immunohistochemistry

Supplementary Figure 1

Disease-free survival stratified by CD39 expression.

Supplementary Figure 2

Disease-free survival stratified by CD73 expression in tumor cell.

REFERENCES

- Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. *N Engl J Med* 2018;379:2108-21. [PUBMED](#) | [CROSSREF](#)
- Miles D, Gligorov J, André F, Cameron D, Schneeweiss A, Barrios C, et al. Primary results from IMpassion131, a double-blind, placebo-controlled, randomised phase III trial of first-line paclitaxel with or without atezolizumab for unresectable locally advanced/metastatic triple-negative breast cancer. *Ann Oncol* 2021;32:994-1004. [PUBMED](#) | [CROSSREF](#)
- Yang Y, Li H, Yang W, Shi Y. Improving efficacy of TNBC immunotherapy: based on analysis and subtyping of immune microenvironment. *Front Immunol* 2024;15:1441667. [PUBMED](#) | [CROSSREF](#)
- Allard B, Allard D, Buisseret L, Stagg J. The adenosine pathway in immuno-oncology. *Nat Rev Clin Oncol* 2020;17:611-29. [PUBMED](#) | [CROSSREF](#)
- Antonioni L, Fornai M, Pellegrini C, D'Antongiovanni V, Turiello R, Morello S, et al. Adenosine signaling in the tumor microenvironment. *Adv Exp Med Biol* 2021;1270:145-67. [PUBMED](#) | [CROSSREF](#)
- Leone RD, Emens LA. Targeting adenosine for cancer immunotherapy. *J Immunother Cancer* 2018;6:57. [PUBMED](#) | [CROSSREF](#)
- Bastid J, Cottalorda-Regairaz A, Alberici G, Bonnefoy N, Eliaou JF, Bensussan A. ENTPD1/CD39 is a promising therapeutic target in oncology. *Oncogene* 2013;32:1743-51. [PUBMED](#) | [CROSSREF](#)
- Allard B, Longhi MS, Robson SC, Stagg J. The ectonucleotidases CD39 and CD73: novel checkpoint inhibitor targets. *Immunol Rev* 2017;276:121-44. [PUBMED](#) | [CROSSREF](#)
- Stagg J, Smyth MJ. Extracellular adenosine triphosphate and adenosine in cancer. *Oncogene* 2010;29:5346-58. [PUBMED](#) | [CROSSREF](#)
- Buisseret L, Pommey S, Allard B, Garaud S, Bergeron M, Cousineau I, et al. Clinical significance of CD73 in triple-negative breast cancer: multiplex analysis of a phase III clinical trial. *Ann Oncol* 2018;29:1056-62. [PUBMED](#) | [CROSSREF](#)
- Loi S, Pommey S, Haibe-Kains B, Beavis PA, Darcy PK, Smyth MJ, et al. CD73 promotes anthracycline resistance and poor prognosis in triple negative breast cancer. *Proc Natl Acad Sci U S A* 2013;110:11091-6. [PUBMED](#) | [CROSSREF](#)
- Xia C, Yin S, To KKW, Fu L. CD39/CD73/A2AR pathway and cancer immunotherapy. *Mol Cancer* 2023;22:44. [PUBMED](#) | [CROSSREF](#)
- Tang K, Zhang J, Cao H, Xiao G, Wang Z, Zhang X, et al. Identification of CD73 as a novel biomarker encompassing the tumor microenvironment, prognosis, and therapeutic responses in various cancers. *Cancers (Basel)* 2022;14:5663. [PUBMED](#) | [CROSSREF](#)
- Supernat A, Markiewicz A, Welnicka-Jaskiewicz M, Seroczynska B, Skokowski J, Sejda A, et al. CD73 expression as a potential marker of good prognosis in breast carcinoma. *Appl Immunohistochem Mol Morphol* 2012;20:103-7. [PUBMED](#) | [CROSSREF](#)
- Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. *Ann Oncol* 2015;26:259-71. [PUBMED](#) | [CROSSREF](#)
- Cho SY, Park SY, Bae YK, Kim JY, Kim EK, Kim WG, et al. Standardized pathology report for breast cancer. *J Breast Cancer* 2021;24:1-21. [PUBMED](#) | [CROSSREF](#)
- Curtis C, Shah SP, Chin SF, Turashvili G, Rueda OM, Dunning MJ, et al. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature* 2012;486:346-52. [PUBMED](#) | [CROSSREF](#)

18. Kensler KH, Sankar VN, Wang J, Zhang X, Rubadue CA, Baker GM, et al. PAM50 molecular intrinsic subtypes in the nurses' health study cohorts. *Cancer Epidemiol Biomarkers Prev* 2019;28:798-806. [PUBMED](#) | [CROSSREF](#)
19. Ni X, Wan W, Ma J, Liu X, Zheng B, He Z, et al. A novel prognostic biomarker of luminal breast cancer: high CD39 expression is related to poor survival. *Front Genet* 2021;12:682503. [PUBMED](#) | [CROSSREF](#)
20. Martin KJ, Kritzman BM, Price LM, Koh B, Kwan CP, Zhang X, et al. Linking gene expression patterns to therapeutic groups in breast cancer. *Cancer Res* 2000;60:2232-8. [PUBMED](#)
21. Spychala J, Lazarowski E, Ostapkowicz A, Ayscue LH, Jin A, Mitchell BS. Role of estrogen receptor in the regulation of ecto-5'-nucleotidase and adenosine in breast cancer. *Clin Cancer Res* 2004;10:708-17. [PUBMED](#) | [CROSSREF](#)
22. Wettstein MS, Buser L, Hermanns T, Roudnicki F, Eberli D, Baumeister P, et al. CD73 predicts favorable prognosis in patients with nonmuscle-invasive urothelial bladder cancer. *Dis Markers* 2015;2015:785461. [PUBMED](#) | [CROSSREF](#)
23. Oh HK, Sin JI, Choi J, Park SH, Lee TS, Choi YS. Overexpression of CD73 in epithelial ovarian carcinoma is associated with better prognosis, lower stage, better differentiation and lower regulatory T cell infiltration. *J Gynecol Oncol* 2012;23:274-81. [PUBMED](#) | [CROSSREF](#)
24. Turcotte M, Allard D, Mittal D, Bareche Y, Buisseret L, José V, et al. CD73 promotes resistance to HER2/ ErbB2 antibody therapy. *Cancer Res* 2017;77:5652-63. [PUBMED](#) | [CROSSREF](#)
25. Bono MR, Fernández D, Flores-Santibáñez F, Roseblatt M, Sauma D. CD73 and CD39 ectonucleotidases in T cell differentiation: beyond immunosuppression. *FEBS Lett* 2015;589:3454-60. [PUBMED](#) | [CROSSREF](#)
26. Cohen HB, Briggs KT, Marino JP, Ravid K, Robson SC, Mosser DM. TLR stimulation initiates a CD39-based autoregulatory mechanism that limits macrophage inflammatory responses. *Blood* 2013;122:1935-45. [PUBMED](#) | [CROSSREF](#)
27. Moesta AK, Li XY, Smyth MJ. Targeting CD39 in cancer. *Nat Rev Immunol* 2020;20:739-55. [PUBMED](#) | [CROSSREF](#)