



Review

NKGD2 Ligands (NKG2DLs) in Breast Cancer: In Silico Analysis and Narrative Review

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Abstract

Breast cancer (BC) is a global health problem. BC is a biologically heterogeneous disease in which novel immunotherapeutic strategies are needed, particularly in the metastatic setting. The NKG2D/NKG2D ligand (NKG2DL) axis is a key component of innate antitumor immunity and represents a potential therapeutic target, but its relevance in BC has not been fully characterized. We performed an in silico analysis of NKG2DL expression in BC cell lines, healthy breast tissue, and tumor samples using publicly available transcriptomic databases (DSMZCellDive, ShinyTHOR, GTEx, TCGA, Human Protein Atlas), complemented by survival analyses from TCGA and KMPlot and a narrative review of the literature. NKG2DL transcripts were consistently expressed in BC cell lines and tumor tissues, with higher expression observed in ductal histology, higher tumor stage, and basal molecular subtype. Survival analyses showed heterogeneous and generally weak associations between individual NKG2DLs and clinical outcomes. In silico proteomics data are scarce, but the narrative review showed that NKG2DLs are expressed by immunohistochemistry in tumor tissues but absent in surrounding healthy tissues. The literature review also revealed concomitant dysfunction of NKG2D+ effector cells due to multiple resistance mechanisms (including ligand shedding). We also review potential therapeutic approaches.

Keywords: breast cancer; NKG2D; NKG2DL; in silico



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1. Introduction

Breast cancer (BC) is a global health issue. In 2022, it was the second most frequently diagnosed cancer, accounting for around 2.3 million new cases or approximately one in nine cancer diagnoses worldwide (11.6% of all cancers). It is also the fourth largest cause of cancer-related mortality, responsible for an estimated 0.6 million deaths in 2022 (6.9% of all deaths from cancer). In women, it is the most common tumor and the leading cause of

cancer mortality [1]. BC is a biologically heterogeneous disease, with different molecular subtypes with prognostic and therapeutic implications. The triple-negative subtype (TNBC) is particularly important due to its greater aggressiveness, poorer prognosis, and fewer treatment options [2]. Perou et al. established four molecular intrinsic subtypes based on a 50-gene expression signature (PAM50): Luminal A, Luminal B, HER2-enriched and basal-like [3]. In clinical practice these subtypes are subrogated through immunohistochemistry (IHC) of four key biomarkers: estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2/ERBB2) and Ki67 (proliferation marker). Depending on the expression of these proteins, tumors are classified as: luminal or hormone receptor-positive (ER+/PR+), HER2-positive (HER2+) or triple-negative (ER-/PR-/HER2-) [2]. There is approximately 80% concordance between the basal molecular subtype and TNBC surrogate subtype [4].

There have been multiple therapeutic innovations in metastatic BC. Traditionally, there was classic chemotherapy, hormone therapy (for luminal tumors), and anti-HER2 targeted therapy (for HER2-positive tumors). However, new treatments have been introduced in recent years:

- Immune checkpoint inhibitors have been incorporated in triple-negative cases if PD-L1 (CD274) is positive (around 40% of the trial population) [5].
- Antibody–drug conjugates (ADCs). Sacituzumab–govitecan (anti-Trop2/TACSTD2) has demonstrated benefits in TNBC and luminal BC [6,7]. Trastuzumab–deruxtecan (antiHER2) has not only demonstrated benefits in HER2-positive tumors, but also in HER2-low tumors (expression present but insufficient to meet the classic criteria for positivity) [8,9]. Others, such as enfortumab–vedotin (anti-Nectin4), are currently being studied [10].

Nevertheless, metastatic BC is incurable in practically most cases and the aim of systemic cancer-specific treatment is palliative [11]. Overall survival rates in the metastatic setting achieved in the luminal and HER2 subtypes are around 5 years, while in TNBC the survival rate is around 1–2 years [2].

The Natural Killer Group 2 member D (NKG2D) receptor plays an important role in protecting the host against infections and cancer. It is constitutionally expressed in $\gamma\delta$ T lymphocytes, CD8+ $\alpha\beta$ T lymphocytes, and NK cells. It modulates the activation of these lymphocytes by recognizing ligands on target cells such as major histocompatibility complex class I-related proteins A and B (MICA and MICB) and UL16 binding proteins 1–6 (ULBP1, ULBP2, ULBP3, ULBP4/RAET1E, ULBP5/RAET1G and ULBP6/RAET1L). The expression of these ligands can be induced in cells that are infected, damaged by DNA, undergoing carcinogenesis, or stressed by other inducers. Thus, cellular immunity is promoted to eliminate cells that express the ligand [12,13]. These ligands are not widely expressed on healthy adult tissue so NKG2D ligands may present a useful target for immunotherapeutic approaches in cancer [14].

The NKG2D/NKG2DL axis is being targeted in pediatric leukemia with chimeric antigen receptor (CAR) anti-NKG2DL or with ex vivo IL-15-stimulated NK cells [15,16]. The question arises as to whether it is possible to transfer this approach to BC. Notably, early-phase clinical trials are being conducted with CAR-based adoptive cell therapy against NKG2DLs in solid tumors, including BC [17]. To assess the feasibility of this strategy, there are two aspects to consider. First, it is necessary to determine whether NKG2DL expression exists through in silico analysis in different databases. Second, the available evidence regarding BC (especially TNBC) and NKG2DLs must be analyzed.

2. In Silico Analysis

2.1. In Silico Analysis of Cell Lines

DSMZCellDive [<https://celldive.dsmz.de/>] (accessed on 23 December 2025) contains RNA sequencing (RNA-seq) transcriptome data (expressed as TPM, transcripts per million) of different immortalized human cell lines (breast cancer $n = 29$, leukemia/lymphoma $n = 101$, neuroblastoma $n = 18$ and retinoblastoma $n = 9$) (Table S1) [18]. We focused specifically on NKG2DL genes and breast cancer cell lines (Table S2) [19]. In the case of breast cancer, the values for CD274, TACSTD2, ERBB2, and NECTIN4 (genes currently being targeted) were added.

The expression of NKG2DLs in the different human cell lines (grouped by tumor) is shown in Figures A1 and A2. The data obtained are illustrated in Table 1 and Figure 1. When specifically analyzing BC cell lines, no statistically significant differences in gene expression were found when stratified by primary vs. metastatic origin or by luminal vs. basal subtype.

Table 1. RNA expression in TPM (mean $\pm$ SD) in different immortalized human cell lines. BC = breast cancer ($n = 29$). LL = leukemia/lymphoma ($n = 101$). NB = neuroblastoma ($n = 18$). RB = retinoblastoma ($n = 9$). Data obtained from <https://celldive.dsmz.de/> (accessed on 23 December 2025).

Gene (TPM)—Mean $\pm$ SD	BC	LL	NB	RB
MICA	26.1 $\pm$ 20.7	9.8 $\pm$ 6.4	2.9 $\pm$ 8.5	1.1 $\pm$ 2.7
MICB	11.0 $\pm$ 13.5	19.0 $\pm$ 13.7	0.6 $\pm$ 1.3	3.0 $\pm$ 5.7
ULBP1	2.1 $\pm$ 2.2	1.7 $\pm$ 3.6	1.3 $\pm$ 2.0	0.2 $\pm$ 0.3
ULBP2	6.8 $\pm$ 6.7	2.0 $\pm$ 4.3	1.5 $\pm$ 1.8	4.2 $\pm$ 3.9
ULBP3	3.6 $\pm$ 3.9	1.1 $\pm$ 1.9	1.0 $\pm$ 1.3	0.8 $\pm$ 0.8
RAET1E	1.5 $\pm$ 2.2	0.9 $\pm$ 3.8	1.4 $\pm$ 5.7	0.0 $\pm$ 0.0
RAET1G	1.4 $\pm$ 3.0	0.1 $\pm$ 0.3	4.1 $\pm$ 16.8	0.0 $\pm$ 0.0
RAET1L	1.4 $\pm$ 4.5	0.2 $\pm$ 1.6	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
CD274	5.6 $\pm$ 11.6			
TACSTD2	220.1 $\pm$ 213.0			
NECTIN4	21.8 $\pm$ 27.6			
ERBB2	408.2 $\pm$ 783.5			

ShinyTHOR [<https://alexismurillo.shinyapps.io/ShinyThor/>] (accessed on 6 February 2026) is a web app for access to multi-omic data from different datasets (CCLE, miRTarBase, circInteractome, and the Genomics of Drug Sensitivity in Cancer) [20]. This allowed us to create a heatmap of NKG2DL expression in 60 immortalized human BC cell lines (Figure A3).

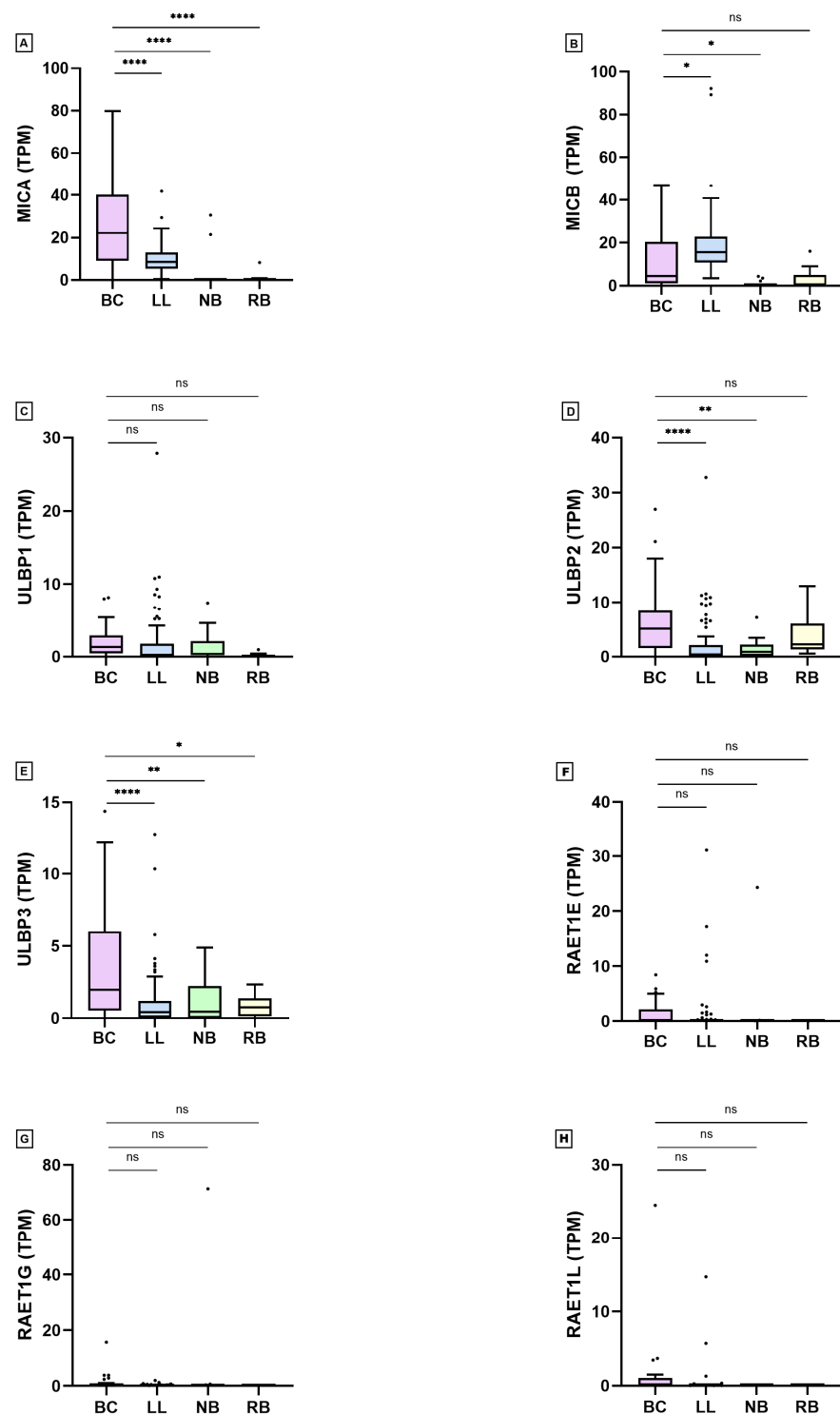


Figure 1. NKG2DL RNA expression in TPM (Tukey boxplot) in different immortalized human cell lines: MICA (A), MICB (B), ULBP1 (C), ULBP2 (D), ULBP3 (E), RAET1E (F), RAET1G (G) and RAET1L (H). BC = breast cancer ($n = 29$). LL = leukemia/lymphoma ($n = 101$). NB = neuroblastoma ($n = 18$). RB = retinoblastoma ($n = 9$). Horizontal line represents direct comparisons (Student's *t*-test). ns = non-significant. * = $p < 0.05$. ** = $p < 0.01$. *** = $p < 0.0001$. Statistical analyses were performed and figures were generated using GraphPad Prism for Windows, Version 8.4.2 (GraphPad Software, Boston, MA, USA, <http://www.graphpad.com/>).

2.2. In Silico Analysis of Healthy Tissue

The Adult Genotype Tissue Expression (GTEx) Project [<http://www.gtexportal.org/home/>] (accessed on 23 December 2025)] collected samples from up to 54 non-diseased

tissue sites across nearly 1000 deceased individuals [21]. It was combined with RNA-seq transcriptome data of NKG2DL genes (collected as nTPM, normalized transcripts per million) present in The Human Protein Atlas [<https://www.proteinatlas.org/>] (accessed on 23 December 2025)] [22]. From this, we selected the 514 breast samples (Table S3).

The expression of NKG2DLs in healthy breast tissues from the GTex database is illustrated in Table 2. The heatmap of NKG2DL expression in different healthy tissues is shown in Figure A4.

Table 2. RNA expression in nTPM (mean $\pm$ SD) in healthy breast tissue. Data obtained from <https://www.proteinatlas.org/> and <http://www.gtportal.org/home/> (both accessed on 23 December 2025).

Gene (nTPM)—Mean $\pm$ SD	Breast
MICA	29.1 $\pm$ 10.6
MICB	2.1 $\pm$ 1.2
ULBP1	0.0 $\pm$ 0.1
ULBP2	0.7 $\pm$ 0.8
ULBP3	1.5 $\pm$ 2.0
RAET1E	0.4 $\pm$ 0.3
RAET1G	0.5 $\pm$ 0.4
RAET1L	0.0 $\pm$ 0.1

The Human Protein Atlas was also used to evaluate the protein expression of ULBP1, ULBP2, and ULBP3 (the only ones for which information was available). No protein expression was detected in ULBP1 and ULBP2. “Low” protein expression of ULBP3 was detected in the glandular component.

2.3. In Silico Analysis of Tumor Tissue

The Cancer Genome Atlas (TCGA) breast cancer database, contained in cBioportal [<http://www.cbioportal.org/>] (accessed on 23 December 2025)], includes clinical–pathological information from 1084 breast cancer samples [23–26]. It was combined with RNA-seq transcriptome data of NKG2DL genes (collected as pTPM, protein-transcripts per million) present in The Human Protein Atlas [<https://www.proteinatlas.org/>] (accessed on 23 December 2025)] [22]. Then, we selected the 1009 samples from patients diagnosed with invasive breast carcinoma (Table S4). In the case of breast cancer, the values for CD274, TACSTD2, ERBB2, and NECTIN4 (genes currently being targeted) were added.

Figure A4, obtained from The Human Protein Atlas, shows the expression of NKG2DLs in different tumors (using TCGA data). The baseline characteristics of the 1009 BC patients are summarized in Table 3. Figure 2 shows the differences in expression by histology (ductal vs. lobular), tumor stage code (T1 vs. T2–4), and nodal stage code (N0 vs. N+). Ductal histology is associated with higher NKG2DL expression (except MICB). T $\geq$ 2 is associated with higher ULBP1–ULBP2–ULBP3 expression. In N+, higher expression was only detected in ULBP1.

Table 3. Baseline characteristics of selected TCGA BC patients. Data obtained from <https://www.proteinatlas.org/> and <http://www.cbiportal.org/> (both accessed on 23 December 2025).

Baseline Characteristics	N = 1009
Age (years)—median (range)	58 (26–90)
Female sex—n (%)	1009 (100%)
Histology—n (%)	
- Ductal	721 (71.5%)
- Lobular	188 (18.6%)
- Mixed	28 (2.8%)
- Mucinous	17 (1.7%)
- Other	41 (4.1%)
- Unknown	14 (1.4%)
Molecular subtype (PAM50)—n (%)	
- Luminal A	473 (46.9%)
- Luminal B	180 (17.8%)
- HER2-enriched	72 (7.1%)
- Basal	162 (16.1%)
- Normal	34 (3.4%)
- Unknown	90 (8.9%)
Tumor stage code—n (%)	
- T1	263 (26.1%)
- T2	584 (57.9%)
- T3	124 (12.3%)
- T4	35 (3.5%)
Nodal stage code—n (%)	
- N0	475 (47.1%)
- N1	335 (33.2%)
- N2	114 (11.3%)
- N3	69 (6.8%)
RNA expression—mean $\pm$ SD (pTPM)	
- MICA	4.4 $\pm$ 6.3
- MICB	1.1 $\pm$ 1.9
- ULBP1	0.9 $\pm$ 1.7
- ULBP2	2.0 $\pm$ 3.6
- ULBP3	1.5 $\pm$ 2.1
- RAET1E	1.6 $\pm$ 3.3
- RAET1G	0.7 $\pm$ 1.1
- RAET1L	1.0 $\pm$ 3.1
- CD274	2.2 $\pm$ 2.6
- TACSTD2	292.2 $\pm$ 213.4
- NECTIN4	37.7 $\pm$ 27.2
- ERBB2	319.6 $\pm$ 680.9

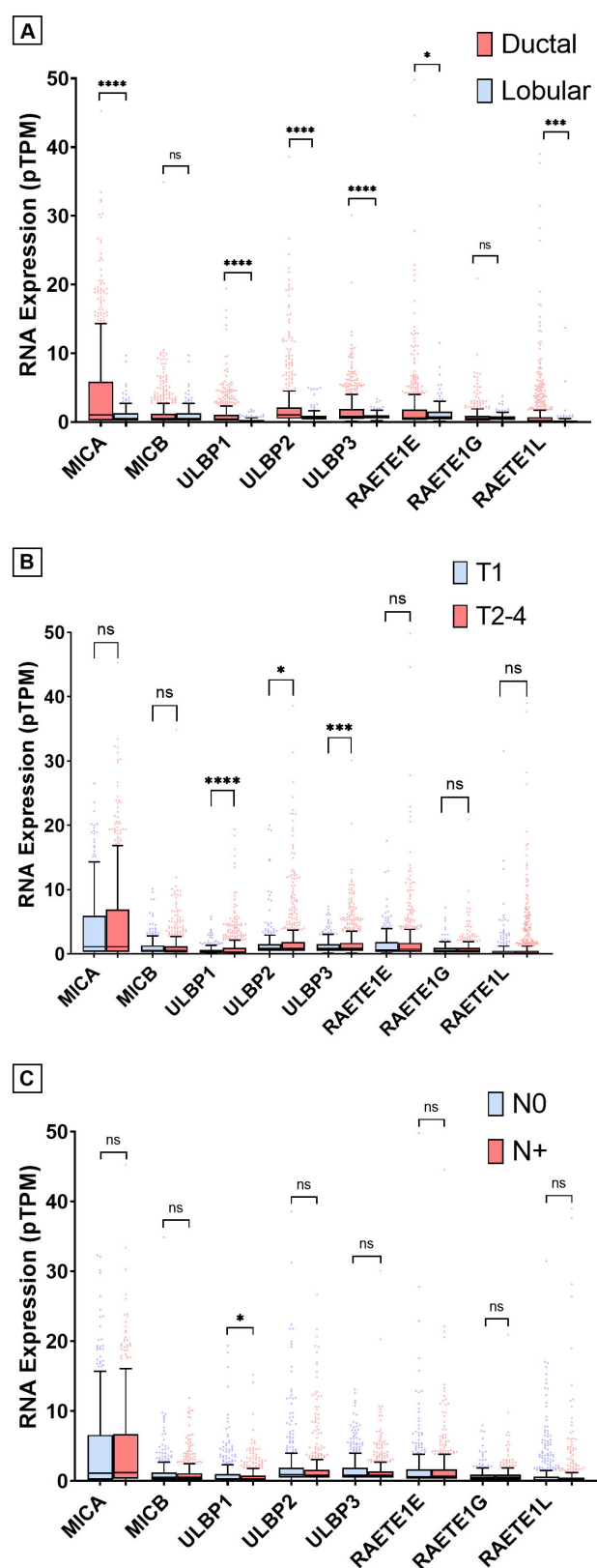


Figure 2. RNA expression in pTPM (Tukey boxplot) by histology (A), tumor stage (B) and nodal stage (C). N zigzag line represents direct comparisons (Student's *t*-test). ns = non-significant. * = $p < 0.05$. ** = $p < 0.01$. *** = $p < 0.001$. **** = $p < 0.0001$. Statistical analyses were performed and figures were generated using GraphPad Prism for Windows, Version 8.4.2 (GraphPad Software, Boston, MA, USA, <http://www.graphpad.com/>).

Figure 3 shows the differences in expression by molecular subtype. There are significant differences in ULBP1, ULBP2, ULBP3, RAET1G, and RAET1L. In these cases, the basal subtype has higher levels than the other subtypes (significance is not detected only versus HER2 for ULBP2 and RAET1G).

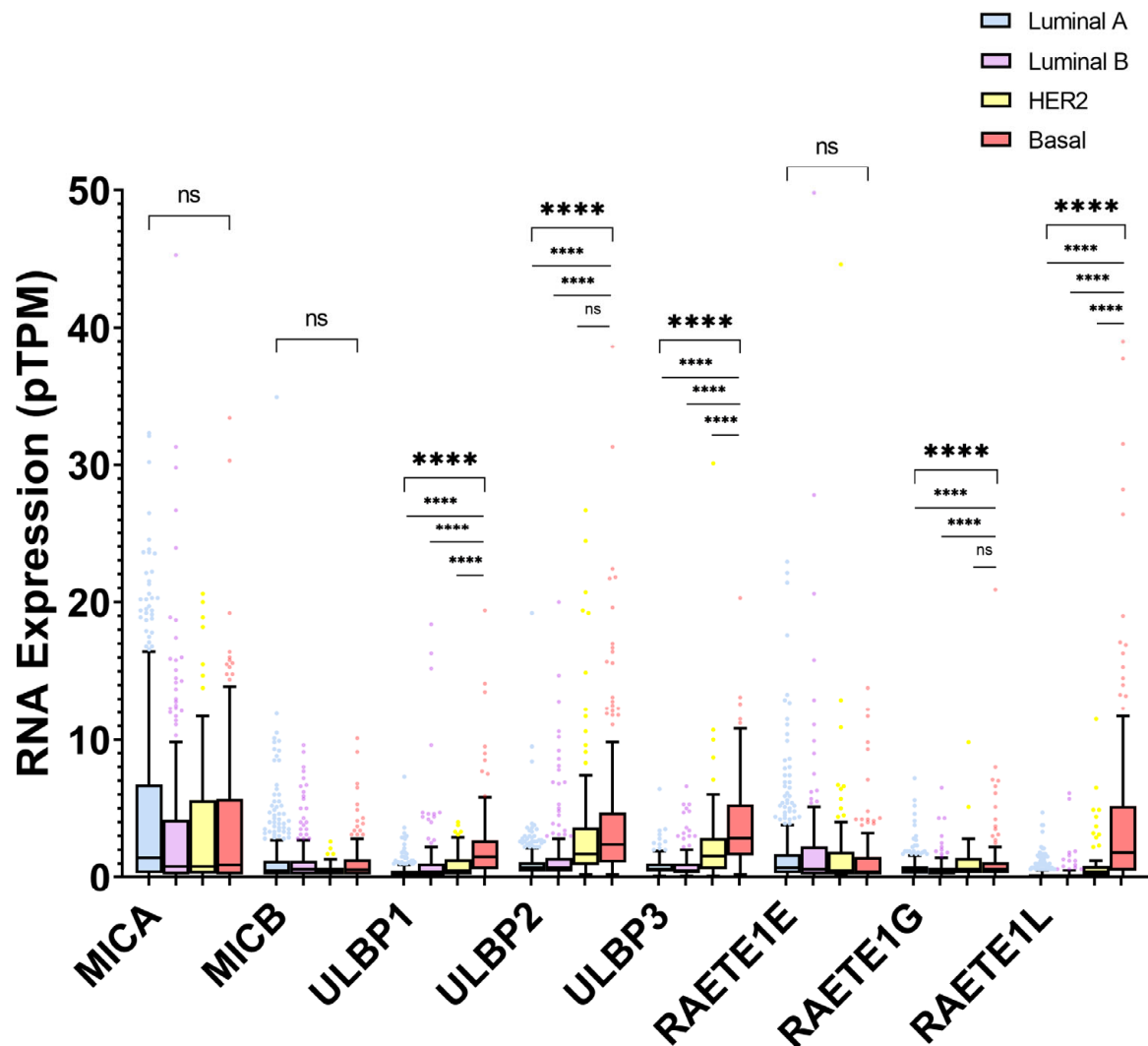


Figure 3. RNA expression in pTPM (Tukey boxplot) by molecular subtype. N zigzag line represents ANOVA test. Horizontal line represents direct comparisons versus basal subtype (Student's *t*-test). ns = non-significant. **** = $p < 0.0001$. Statistical analyses were performed and figures were generated using GraphPad Prism for Windows, Version 8.4.2 (GraphPad Software, Boston, MA, USA, <http://www.graphpad.com/>).

The Human Protein Atlas was also used to evaluate the protein expression of ULBP1, ULBP2, and ULBP3 (the only ones for which information was available). In ULBP1, “medium” protein expression was recorded in 1/11 samples. In ULBP3, “medium” protein expression was recorded in 1/11 samples. In ULBP2, “weak” staining was recorded in <25%.

With the TCGA data, a survival analysis (estimated using the Kaplan–Meier method) could be performed. The median follow-up was 31.4 months (95%CI: 28.6–34.3) and death had occurred in 141 patients (14.0%). The median overall survival (mOS) was 132.0 months (95%CI: 115.3–148.7).

A univariate analysis was conducted using the Cox regression model to correlate overall survival (OS) with NKG2DL RNA expression (“low” and “high” groups were

created using the median expression as the cut-off point) or clinical–pathological factors. No significant differences were found in mOS based on levels of MICA ($p = 0.402$), MICB ($p = 0.202$), ULBP1 ($p = 0.081$), ULBP2 ($p = 0.215$), ULBP3 ($p = 0.647$), RAET1E ($p = 0.165$), and RAET1L ($p = 0.166$). Significant differences were found in RAET1G (low vs. high; 131.5 months vs. 132.0 months; $p = 0.044$; HR = 1.4, 95%CI: 1.01–1.96; Figure 4). In relation to clinical–pathological factors, no differences were found in terms of histology (ductal vs. lobular; $p = 0.510$). Differences were found in tumor stage code (T1 vs. T2–4; $p = 0.026$), nodal stage code (N0 vs. N+; $p < 0.001$), and molecular subtype ($p = 0.008$).

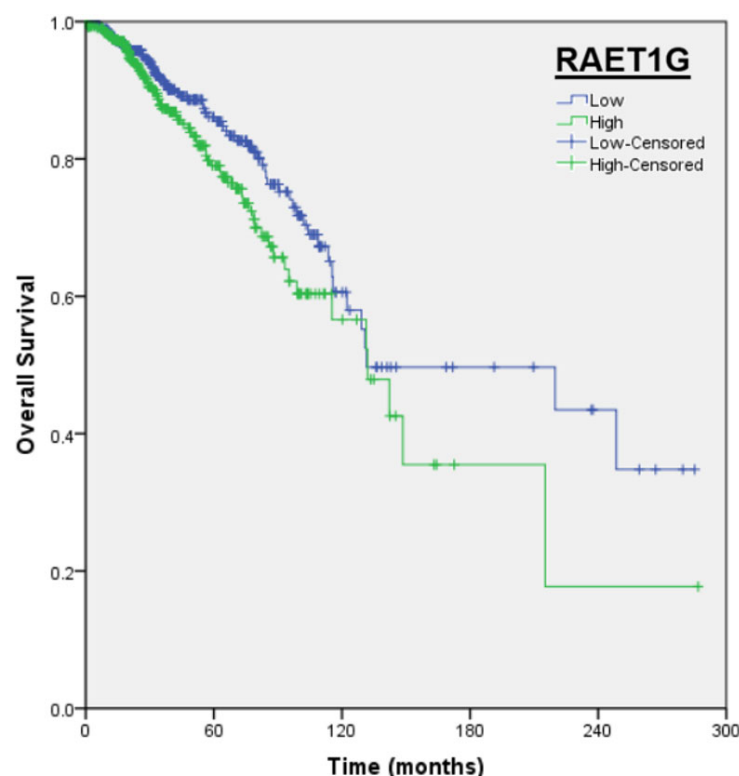


Figure 4. Survival curve for overall survival stratified by low–high levels of RAET1G. Survival analyses were performed using IBM SPSS Statistics for Windows, Version 19.0 (IBM Corporation, Armonk, NY, USA).

The significant variables in the univariate model were incorporated into a multivariate model. The independent prognostic factors identified were molecular subtype Luminal A ($p = 0.020$, aHR = 0.58, 95%CI: 0.37–0.92) and N+ ($p = 0.001$, aHR = 1.91, 95%CI: 1.31–2.80).

A similar analysis was performed using another database, The Kaplan–Meier (KM) Plotter Online Tool [<https://www.kmplot.com/analysis/>] (accessed on 23 December 2025). It has RNA-seq data on 2976 breast cancer patients obtained from the GEO and EGA repositories [27]. It allowed us to analyze the relationship between the relevance of different NKG2DL RNA expressions (“low” and “high” groups were created using the median expression as the cut-off point) and patients’ clinical outcomes (OS). No significant differences were found in mOS when stratified by low–high levels of MICA ($p = 0.48$), MICB ($p = 0.70$), ULBP3 ($p = 0.62$), RAET1G ($p = 0.29$) and RAET1L ($p = 0.75$). Significant differences were found in ULBP1 ($p = 0.001$; HR = 1.9, 95%CI: 1.5–2.4), ULBP2 ($p = 0.013$; HR = 1.3, 95%CI: 1.1–1.7) and RAET1E ($p = 0.044$; HR = 1.26, 95%CI: 1.01–1.6) (Figure 5). When specifically analyzing the TNBC subtype ($n = 126$) and the basal subtype ($n = 309$), no significant differences in mOS were found based on NKG2DL expression.

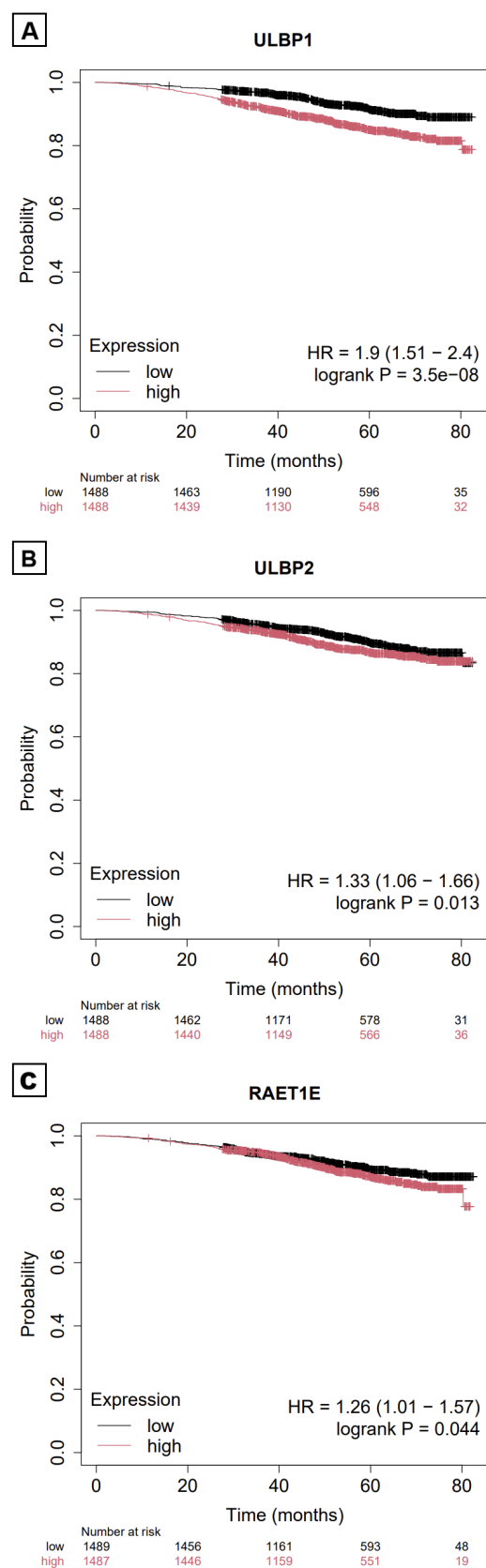


Figure 5. Survival curves for overall survival stratified by low–high levels of ULBP1 (A), ULBP2 (B) and RAET1E (C). Obtained from <https://www.kmplot.com/analysis/> (accessed on 23 December 2025). Parameters: mRNA > RNA-seq > breast cancer. Patients were split by median. Survival: OS ($n = 2976$).

3. Narrative Review

We conducted a keyword-search-based literature review using PubMed. The following output was introduced in December 2025: (“breast neoplasms”[MeSH Terms] OR BREAST CANCER[Text Word] AND NKG2D) OR (“breast neoplasms”[MeSH Terms] OR BREAST CANCER[Text Word] AND NKG2DL). A total of 114 studies were obtained and their distribution over time was recorded (Table S5).

3.1. Potential Resistance Mechanisms

The mechanisms by which each cancer (not only BC) can evade the NKG2D system are numerous and complex. Table 4 summarizes the available evidence.

Table 4. Evidence of resistance mechanisms.

Resistance Mechanisms	Evidence
Soluble forms of NKG2DL (They can block NKG2D+ lymphocytes, preventing their action in the tumor microenvironment and facilitating immune evasion)	- Exosomes released by BC cell lines express NKG2DLs and reduce the proportion of NKG2D+ lymphocytes [28,29]. - NK cells incubated in soluble MICA (sMICA) demonstrate reduced cytotoxicity [30]. - The “a disintegrin and metalloproteases” (ADAMs) 10 and 17 are largely responsible for the generation of sMICA-MICB [31]. - Senescent cancer cells (SCCs) treated with antineoplastic agents upregulate NKG2DLs but also metalloproteases (MMPs), which causes their shedding [32].
Anesthetics	- Serum from patients undergoing breast tumorectomy anesthetized with sevoflurane–opioid (versus paravertebral block and propofol) was associated with NK cell dysfunction. However, it was not associated with a deficit in NKG2D [33]. - Sevoflurane attenuates NK cell-mediated cancer cell lysis. It downregulates the mRNA/protein expression of the NKG2DLs in cancer cell lines without affecting MMP or soluble NKG2D levels [34]. - Propofol modifies NKG2DL levels (increasing them in some cell lines and decreasing them in others) without altering MMP levels [35].
Metabolic syndrome	- Streptozotocin-induced diabetes accelerates tumor growth, which is associated with decreased NK cell cytotoxicity (reduced NKG2D) [36]. - LDL cholesterol inhibits NKG2D and the cytotoxicity of phosphoantigen-expanded Vγ9Vδ2 T cells [37]. - A mouse model of obese postmenopausal breast cancer shows increased tumor growth and decreased levels of circulating NK cells with increased expression of NKG2DLs in adipose tissue [38].
Hypoxia	- Hypoxia did not alter the expression of MICA/MICB but did increase their extracellular shedding, potentially explaining resistance to γδTc cytotoxicity [39]. - Extracellular lactate flux and acidified tumor microenvironment may disrupt NK cell function [40].
H ₂ S (synthesized by enzymes cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE))	- Silencing CBS/CBE increases MICA and ULBP2 RNA transcripts [41]. - Ectopic expression of miR-939-5p represses CBS/CSE transcript and protein levels, diminishes H₂S production, upregulates NKG2DLs and attenuates triple-negative hallmarks [42].
IL-33	- Especially in the presence of ST2/IL1R-L1, promotes a reduction in intratumoral NKG2D+ cells and favors tumor progression [43]. - Blocking PD-1 with an antibody in ST2 knockout mice increases NKG2D+ T cells in the tumor microenvironment [44].
P53	- Murine p53 missense mutation G242A (corresponding to human G245A) suppresses the activation of host NK cells. p53 can modulate expression by cancer cells of Mult-1 (murine NKG2DL) and H60a [45].

Table 4. *Cont.*

Resistance Mechanisms	Evidence
PI3K/AKT/GSK-3 β	- Inactivated GSK-3 β downregulates the expression of NKG2DLs via ROS and eIF2B [46].
EHMT2	- EHMT2 loss increases AZGP1 and decreases TGF- β 1 levels, resulting in elevation of NKG2DLs (MICB and ULBP3), chemokines in cancer cells, and the paracrine stimulation of NK cell function [47].
ATM	- Associated with MICA upregulation. In triple-negative breast cancer, there is ATM downregulation, which may influence its immunoreactive profile [48].
Heat shock factor 1 (HSF1)	- HSF1 upregulates MICA/MICB expression. NZ28 completely inhibits HSF1 blocking the MICA/MICB membrane expression on tumor cells and thereby strongly inhibits NK cell-mediated cytotoxicity [49].
Podocalyxin-like protein 1 (PCLP1)	- CD34-related sialomucin. Overexpressed in cancer cells. Associated with decreased NKG2D levels in NK cells [50].
IGFBP-3	- May promote breast tumor growth through T-cell dysfunction, but does not alter NKG2D levels [51].
Breast cancer stem-like cells (BCSCs)	- Aberrantly express oncogenic miR20a, which inhibits MICA/MICB, protecting them from NK cell-mediated cytotoxicity [52]. - Activate platelets inducing TGF β production and inhibiting NK cells (reducing NKG2D expression) [53].
Adipose-derived stem cells (ASCs)	- ASCs from breast tumors exert an immunosuppressive effect on NK cells, showing reduced NKG2D levels [54]. - MICB expression in ASCs from healthy subjects and those with stage II breast cancer is higher than in those with stage III cancer. However, this is reversed with the administration of INF γ [55].
“Central” markers of dormancy/quiescence	- Breast cancer brain metastasis cells NKG2DL+ express H2BK, IGFBP5 and EphA5 [56].
Ectopic expression of NKG2D	- Induced expression of NKG2D in cancer cell lines may paradoxically have a pro-tumor effect [57].
DNA methylation	- Can contribute to the absence of NKG2DL expression during tumor progression [58].
RNA	- miR-519a-3p interferes with tumor-cell-killing NK cells via downregulation of MICA and ULBP2 [59]. - UCA1 (an lncRNA) is upregulated in NK-resistant cell lines. UCA1 upregulates ULBP2 via the transcription factor CREB1, while it upregulates ADAM17 by “sponging” the miR-26b-5p. ADAM17 facilitates the shedding of soluble ULBP2 [60].
Xanthine oxidoreductase (XOR)	- Inhibition of XOR blocks the expression of MICA/MICB [61,62].

3.2. Findings in Breast Cancer Patients

Over the past 20 years, data on NKG2DL in patients with BC has been collected. Table 5 summarizes the available evidence.

Table 5. Available evidence for NKG2DL in BC.

NKG2DL in BC	Evidence
NKG2DL is expressed in BC. NKG2DL expression is low in surrounding non-tumor tissues. NKG2DL expression could be associated with a worse prognosis.	- Madjd et al. studied 530 BC patients. MICA staining was detected in 97% of blocks. It was not detected in the extracellular stroma but was detected in adjacent normal epithelial cells, endothelial cells, and some tumor-infiltrating leukocytes. MICA expression was associated with worse grade and nodal stage indicating a worse prognosis [63]. - Shen et al. studied 92 BC patients. 92.2% of the samples tested positive for MICA/MICB by immunohistochemistry (compared to only 15.6% of healthy tissues). MICA/MICB expression was inversely correlated with TNM staging (this was not detected with ULBP1, ULBP2, and ULBP3) [64]. - de Kruijf et al. studied 677 primary BC tumors. They detected NKG2DL expression (MICA/MICB 50%, ULBP1 90%, ULBP2 99%, ULBP3 100%, ULBP4 26% and ULBP5 90%). Low levels of MICA/MICB and ULBP-2 were associated with a longer relapse-free period (RFP) [65]. - BC cells show upregulated NKG2DLs compared with primary breast epithelial cells. NKG2DLs activate mucosal-associated invariant T innate cells [66].
However, there is a dysfunction in NKG2DL expression and NKG2D+ cell function in BC. The recovery of NK function may be a good prognostic sign.	- Elsabbagh et al. detected higher m⁶A levels in the 3' untranslated region (3'UTR) accompanied by a marked reduction in their corresponding mRNA levels in BC patients compared to controls [67]. - BC patients have decreased NK cell cytotoxicity and lower NKG2D expression [68]. Malignant mammary-tumor-infiltrating NK cells show reduced expression of activating receptors such as NKG2D compared to circulating NK cells of healthy donors [69]. Arianfar et al. showed that NK subtypes showed lower NKG2D expression in breast cancer (<i>n</i> = 26) patients than healthy subjects (<i>n</i> = 12) [70]. - However, Kawaguchi et al. detected no differences in NKG2D levels between patients with primary tumors (<i>n</i> = 12), metastases (<i>n</i> = 30), and healthy subjects (<i>n</i> = 6) [71]. Darvishvand et al. analyzed 30 patients with breast cancer and 20 healthy subjects. Around 15% of lymphocytes in peripheral blood had NK cell characteristics. No differences were found between the two groups [72]. - Wu et al. detected Vδ1⁺ T cells (which could be activated innately via the NKG2D receptor) in human BC tumors. When paired tumor and nonmalignant samples from 11 patients with TNBC were analyzed, PFS (progression-free survival) and OS correlated with Vδ1⁺ cell representation [73]. - In TNBC, there are more NKG2D+ cells in tumors without nodal involvement (N0) [74]. - Verma et al. showed that, with regard to controls (<i>n</i> = 10), patients with locoregional BC (<i>n</i> = 25) had a lower absolute number of NK cells and lower cytotoxicity. This decrease was corrected in patients who had a complete pathological response with neoadjuvant chemotherapy and/or after surgery [75]. - Lobo-Martins et al. studied 44 BC patients treated with hormone therapy and cyclin inhibitors. The treatment decreased Vδ2⁺ T cells expressing NKG2D at 3 months. There was an increase in CD8⁺ T cells expressing NKG2D in responders (PFS > 6 months) [76].
There is expression of soluble forms of NKG2DL in BC. sNKG2DL could also be associated with a worse prognosis.	- sMICA/sMICB levels are higher in breast cancer patients than in healthy individuals [77]. sMICA levels correlate with tumor staging [30,78]. This is also detected in saliva and urine [79]. - Seller et al. collected serum from 140 BC patients, 20 ductal carcinoma in situ patients and 20 healthy volunteers. Higher sNKG2DL serum levels were associated with TNM stage and grading. Low sMICA serum levels were associated with significantly longer PFS and OS [80].
Germinal alterations in NKG2DL could predispose to BC	- Lavado-Valenzuela et al. studied 110 BC cases and 121 controls in Spain. They found that the MICA-A5 allele appears less frequently in BC, while the MICA-A5.1 allele appears more frequently in HLA-B7-positive BC [81]. - Ouni et al. detected that MICA-129 Met/Val polymorphism is associated with higher breast cancer risk in Tunisian women <40 years old [82]. Ouni et al. also showed that MICA-129 Met/Val polymorphism is associated with BC risk in Tunisian women [83].

3.3. Activating Mechanisms

Various mechanisms are being studied to improve the function of the NKG2D system. Table 6 shows the available evidence.

Table 6. Evidence of activating mechanisms.

Activating Mechanisms	Evidence
Chemotherapy	- <i>Arsenic trioxide</i> increases NKG2DL expression (especially ULBP1), promoting cytotoxicity [84]. - <i>Docetaxel</i> promotes NKG2D expression and enhances trastuzumab-mediated ADCC [85]. - <i>Gemcitabine</i> induces MAP kinase activation and MICA/B expression [62]. - <i>Epirubicin</i> increases NKG2DL expression (MICA, ULBP1, and ULBP2) in tumor cells and promotes NK cell-mediated cytotoxicity [86]. - <i>Doxorubicin</i> plus TAK-981 (SUMOylation inhibitor) downregulates ULBP2 through suppression of the NF-κB pathway inhibiting tumor growth and enhancing NK cell activity [87].
Cytokines	- <i>IL-15</i> upregulates NKG2D expression in NK cells previously inhibited by sMICA and increases NK cell cytotoxicity toward breast cancer cells [30]. <i>IL-15</i> increases NKG2D+ cell levels in BC metastases (the addition of everolimus has no synergistic effect) [88]. - <i>IL-2</i> and <i>IL-15</i> lead to greater sensitivity to cytotoxicity mediated by NK cells to BSCS (which express higher levels of MICA, ULBP1, and ULBP2) [89]. - <i>Bryostatins</i> 1 and <i>ionomycin</i> combined with <i>IL-2</i>, <i>IL-7</i>, and <i>IL-15</i> can reactivate peripheral blood mononuclear cells (including NK cells) of patients with BC inhibited by myeloid-derived suppressor cells [90]. - <i>IFNγ</i> and <i>TNFα</i> enhanced NK cell infiltration with increased expression of activating receptors (e.g., NKG2D) in BC spheroids. However, regardless of treatment, markers of NK cell exhaustion, such as PD-1 and CTLA-4, were also upregulated [91].
Antibodies	- <i>Anti-TGFβ</i> increases NKG2D/NKG2DL expression, promoting tumor lysis [92]. - <i>Anti-EGFR</i> (cetuximab) promotes antibody-dependent cellular cytotoxicity (ADCC), especially when administered with <i>IL15</i> (which increases NKG2D expression) [93]. - <i>Anti-CD6</i> (UMCD6) augments killing of breast cancer cells through direct effects on both CD8+ T cells and NK cells (upregulating NKG2D) [94]. - <i>Anti-CD6</i> (itolizumab) increases the cytotoxicity of CD8 T and NK cells over CD318+ tumor lines, reverses the NKG2A/NKG2D ratio, and increases granzyme B and <i>IFNγ</i> production [95]. - <i>Anti-CD137</i> agonist (urelumab) can overcome TGFβ-mediated inhibition of human NK cell proliferation, NKG2D expression and antitumor function [96].
Fusion proteins	- <i>Anti-HER2:IgG3-Rae-1β</i> (murine NKG2DL) promotes cytotoxicity [97]. - <i>Anti-NKG2D:IgG1 with amino acid exchange S239D/I332E in their Fc part</i> improves degranulation, ADCC, and <i>IFN-γ</i> production of NK cells in response to breast cancer cells. In addition, it increases sensitivity to trastuzumab in HER2-low targets [98]. - <i>NKG2D:anti-HER2 acts synergistically with B7-H6(NKp30):anti-HER2 and AIICL(NKp80):anti-HER2</i> to produce ADCC [99]. - <i>mAb04(anti-VEGF):MICA</i> demonstrate superior antitumor efficacy compared to a combination therapy of mAb04 + docetaxel or bevacizumab + docetaxel [100]. - <i>IL-15SA:IL-15RαSu</i> enhances subpopulations of NK and has an antitumor effect [101]. - <i>ULBP2:anti-HER2</i> triggers NK cell-mediated killing of HER2-positive BC cells [102]. - <i>NKG2D:CD3 and NKG2D:CD16</i> efficiently activate both NK and T cells against TNBC [103]. - <i>NKG2D:anti-HER2</i> enhances NK cell activation and cytokine production [104]. - <i>Anti-Fzd7:MICA</i> significantly enhances the cytotoxicity of NK cells against hypoxic TNBC cells [105].
Adoptive T-cell therapy	- <i>CAR anti-NKG2D</i> exhibits antitumor activity against TNBC cell lines ex vivo and in vivo in a mouse model [106]. - <i>Cytokine-induced killer (CIK)</i> cells in combination with cetuximab showed an antitumor effect in TNBC lines ex vivo and in vivo in a mouse model [107]. - <i>NK-92 cell line</i> has an antitumor effect on spheroids of cell lines [108]. - <i>Intratumoral injection of allogeneic NK cell</i>: Chemotherapy was found to upregulate the expression of NKG2D ligands at both mRNA and protein levels on cancer cells, increasing their susceptibility to NK cell-mediated cytotoxicity [109]. - <i>Bispecific T-cell engager CAR-T (BiTE CAR-T) cells targeting mesothelin (MSLN) and secreting NKG2D-Bispecific T-cell Engagers (BiTEs) to engage NKG2DL</i>: BiTE CAR-T cells exhibit superior cytotoxicity, T-cell activation, and cytokine production against heterogeneous target cells compared to MSLN CAR-T [110]. Their use is being studied with closed-loop sonothermogenetics for spatial and temporal control of CAR-T cells [111].
Other types of immunotherapy	- <i>Adenovirus E1A</i>: Infection of cancerous cell lines increases NKG2DL expression and facilitates cell lysis [112]. - <i>Antitumor vaccines</i>: Inclusion of NKG2DLs as adjuvants is being evaluated [113].

Table 6. Cont.

Activating Mechanisms	Evidence
Other compounds	- <i>Resveratrol</i> upregulates the protein and mRNA expression of MICA and MICB [114]. <i>Resveratrol</i> potentiates ULBP2-mediated immune eradication of BC cells by NK cells through the downregulation of miR-17-5p and concurrent activation of the MINK1/JNK/c-Jun cascade [115]. Nevertheless, ectopic expression of miR-17-5p represses oncogenic mediator STAT3 and oncogenic lncRNA H19, inducing ULBP2 expression [116]. - <i>Inhibition of MCT4</i> (monocarboxylate transporter-4) upregulates NKG2D/NKG2DL, enhancing the cytotoxicity of NK cells [40]. - <i>P. ostreatus</i> polysaccharides induced NK cell cytotoxic effects against BC (correlated with NKG2D upregulation). These cytotoxic effects were enhanced in the presence of IL-2 [117]. - <i>3'-O-acetylavitexin</i> (3OA) produced a dose-dependent repression of TNBC viability, colonogenicity and migration capacity. It produced a marked dose-dependent repression of miR-20a with a concomitant dose-dependent increase in MICA/MICB expression [118]. - <i>Tanshinol</i> can interfere with the negative regulation of NK cell functions by TGF-β1, mainly through promoting the expression of NKG2D and its molecular chaperone DAP10 [119]. - <i>Amygdalin-folic acid nanoparticles</i>, as radiosensitizers, interfere with TGFβ, INFγ, IL2, IL6 and VEGF, upregulating NKG2D [120]. - <i>Ginsenoside Rh2</i> retards the growth and metastasis of BC through boosting the cytotoxic function of NK cells. It prevents the formation of sMICA and upregulates MICA in vitro and in vivo [121]. - <i>Cordycepin</i> and <i>C. militaris ethanolic extract</i> increases NKG2D/NKG2DL and elevated cancer cell death [122]. - <i>Dichloroacetate</i> significantly increases the mRNA of MICA/B and ULBP1 exclusively in cells that express normal p53 [123].
Bisphosphonates	- Promote cell lysis mediated by Vγ9Vδ2 T cells via $\gamma\delta$ TCR, perforin–granzyme pathway, and NKG2D (although they do not modify MICA expression) [124]. - Zolendronic acid increases NKG2D in peripheral blood mononuclear cells from BC patients [125].
Demethylating agents	- <i>5-azacytidine</i> and <i>5-aza-2'-deoxycytidine</i> can restore the expression of NKG2DL [58].
HER2/HER3 pathway	- Promotes NKG2D-MICA/B recognition by NK cells and T cells [126].
Nanotechnology	- <i>Biopolymer</i> implants can enhance the efficacy of adoptive NK-CAR cell therapy [127]. - <i>Nanoemulsion system</i> to co-deliver TGF-β inhibitor and selenocysteine enhances the lytic potency of NK92 cells (via enhanced NKG2DL expression on tumor cells and stimulated NKG2D expression on NK92 cell) [128]. - <i>Selenadiazole-derivative-loaded metal azolate framework</i> (PSeD@MAF-4(R)) exhibits synergistic effects with NK cells in inhibiting tumor cell growth by upregulating NKG2D/NKG2DL [129]. - <i>Ruthenium polypyridyl complex pretreatment</i> together with NK cells can maximize the interactions between NK and tumor cells via upregulating NKG2D and its multiple ligands [100]. - <i>Nanoparticle@K3-E3@V_HHs</i> (nanoparticle platforms in which nanobodies are non-covalently displayed on the surface of nanoparticles through a specific heterotetrameric peptide assembly K3–E3) can target multiple cell surface receptors (e.g., NKG2DL) [130].
Exercise	- <i>Acute exercise</i> does not alter NKG2D levels. However, different <i>chronic exercise</i> techniques (standard endurance training vs. polarized endurance training after a cardiopulmonary exercise test) do modify it [131].
Radiation	- <i>Low doses of chronic internal radiation</i> in mice produce a decrease in NKG2D expression and an increase in NKG2DL expression at 3.5 months, which subsequently normalizes [132].

4. Discussion

In this study, we reviewed the published evidence and in silico data available on NKG2DL in BC. At the transcriptional level, NKG2DL is expressed in BC cell lines and tumor samples. This expression is robust compared to other tumor types. However, expression levels are lower than those of some genes whose targets are currently used in the treatment of metastatic BC, such as TACSTD2 or ERBB2. Nevertheless, we have the case of CD274 (which also participates in the immune response) that resembles that of NKG2DL. It should be noted, however, that in metastatic TNBC, immune checkpoint inhibitors only work in a selected PD-L1-positive population [5], so we wonder whether future therapies against the NKG2D/NKG2DL axis will also need to be selected based on expression. One

way to stratify indirectly is to see which subpopulations have higher levels of NKG2DL. Using TCGA data, we see that expression is generally higher in ductal histology, $T \geq 2$, and basal molecular subtype. This last molecular aspect could support the idea that the NKG2D/NKG2DL axis could be a target in TNBC.

An important aspect is that *in silico* studies of healthy breast tissue also show NKG2DL mRNA expression. This could lead to problems in terms of “on target, off tumor” toxicities. However, the expression of RNA transcripts does not necessarily imply effective protein expression. There is evidence that healthy tissues can inhibit NKG2DL protein expression despite mRNA transcripts existence through post-transcriptional mechanisms (e.g., miRNA) or post-translational mechanisms (e.g., preventing protein translocation from the endoplasmic reticulum to the cell surface) [133]. However, the *in silico* data we have on protein expression to clarify this is scarce. Therefore, to determine the evidence of NKG2DL protein expression in BC, we conducted a narrative review in PubMed. As previously mentioned, there is NKG2DL protein expression in BC tumor cells, while immunohistochemical staining in non-tumor border tissues is low. This supports the use of NKG2DL as a tumor target.

There is evidence that NKG2DL protein expression may be associated with aggressiveness (higher TNM stage, higher histological grade, etc.) [64]. In fact, this is what we have seen at the transcription level with TCGA data. However, this also seems to be accompanied by greater dysfunction of NKG2D+ effector cells. This is where the resistance mechanisms described above come into play and form part of the complex process of immune editing that occurs in the tumor to evade the immune system. Among these, the secretion of soluble factors appears to be important. It has been observed that soluble NKG2DL levels are increased in patients with BC and that these levels are associated with aggressiveness [30,78]. In the proposed model, more aggressive tumors would produce more NKG2DL transcripts, but part of the translated proteins would be shed into the microenvironment and bloodstream. This shedding, among other mechanisms, would cause dysfunction of NKG2D+ effector cells (Figure 6).

The data on prognostic value are mixed. With TCGA data, only high RAET1G expression is associated with a worse prognosis. However, the absolute difference between the two subgroups is not clinically significant (0.5 months), with a modest HR (HR 1.4) and no significance in the multivariate analysis. With KMPlot data, high expression of ULBP1, ULBP2, and RAET1E is associated with a worse prognosis. The study by Madjd et al. finds that high expression of MICA/MICB and ULBP-2 is associated with a worse prognosis [63]. However, this is not unique to BC. Similar studies in colorectal or ovarian cancer have identified specific NKG2DLs as prognostic factors [134,135]. One challenge in analyzing NKG2DL expression is that at least eight different ligands are being evaluated, which may lead to concordance issues across datasets. In addition, the distribution of patients into “low” and “high” expression subgroups depends on the cut-off values chosen, influencing subgroup size and survival analyses. In future studies, it may be valuable to develop a composite score integrating expression across multiple NKG2DLs to provide a more robust and reproducible assessment of their prognostic relevance.

Finally, we reviewed potential therapeutic strategies currently being developed to target the NKG2D/NKG2DL axis in BC. On the one hand, it is interesting to note how classic chemotherapy or monoclonal antibodies used in routine clinical practice are capable of overcoming tumor immune-editing mechanisms and promoting NK-mediated cytotoxicity. Looking ahead, clinical studies in humans appear to be focused on adoptive T-cell therapy (especially CAR technology). A search on www.clinicaltrials.gov (accessed on 27 December 2025) using NKG2D as the “intervention/treatment” yields 44 studies. In 41 studies, the target population is cancer patients (12 studies may have included breast cancer patients).

Most studies are phase I (with some phase I/II). The experimental treatment is adoptive cell therapy in 39 studies (35 of which are CAR-based) [136].

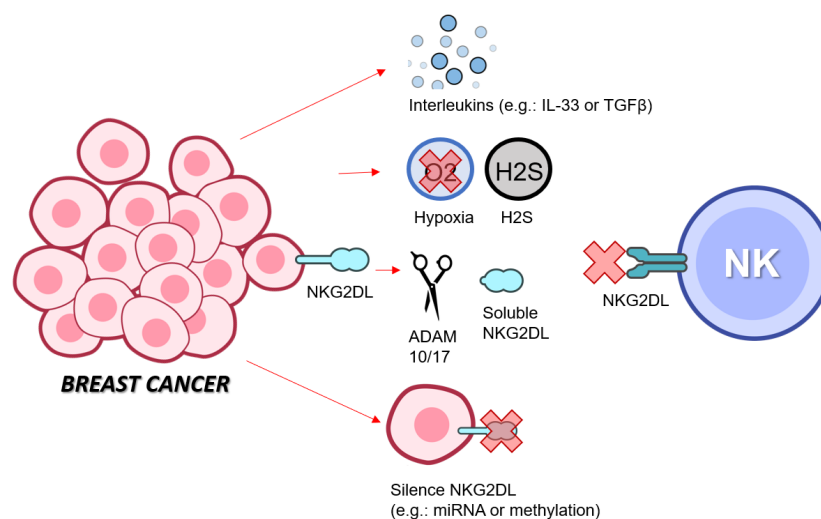


Figure 6. Potential mechanisms by which BC tumor cells can evade NK cell cytotoxicity mediated by NKG2D/NKG2DL. Illustrations obtained from <https://bioart.niaid.nih.gov/> (accessed on 5 February 2026) [137].

5. Future Directions

However, as we have seen, the tumor develops different strategies to evade NKG2D+ cells. Therefore, it seems necessary to develop new approaches to overcome the resistance in the NKG2D/NKG2DL axis [138]. One option would be to combine adoptive cell therapy with other treatment modalities (e.g., concomitant/sequential use of chemotherapy, immune checkpoint inhibitors, ADAM inhibitors, deacetylating agents, etc.). Another option is to optimize adoptive cell therapy with technologies such as CIK, CAR-NK or TRUCK (T cells redirected for antigen-unrestricted cytokine-initiated killing) [139]. In Figure 7, we propose a potential phase Ib/II clinical trial that could be considered after positive preclinical studies have been conducted.

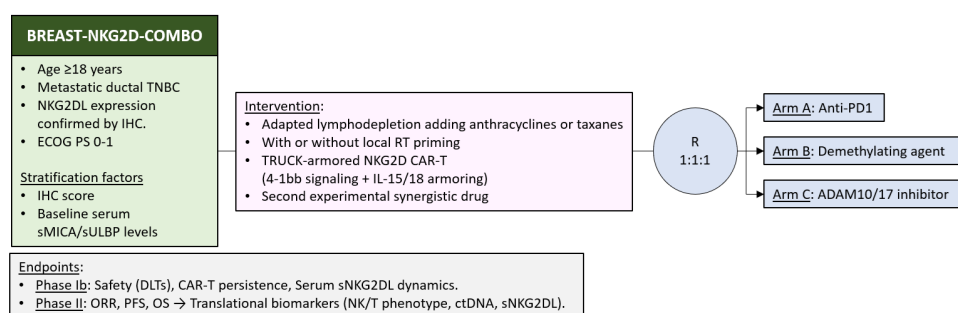


Figure 7. Potential phase Ib/II clinical trial combining CAR-T therapy with other modalities in metastatic TNBC NKG2DL+. ECOG PS = European Eastern Cooperative Oncology Group performance status, DLTs = dose-limiting toxicities, ctDNA = circulating tumor DNA.

An important consideration is CAR-T cell dosing. Based on the CAR4SAR study in sarcoma, weekly intravenous doses of 3×10^6 cells/kg (up to three doses, with discontinuation in case of $\geq$ grade 3 toxicity) are proposed as a rational reference framework [140].

Anthracyclines and taxanes are two chemotherapy drugs widely used in BC, and there is also evidence that they increase NKG2DL expression [85,86]. However, the classic conditioning regimen for CAR-T therapy uses other chemotherapy modalities (cyclophos-

phamide and fludarabine) and does not use anthracyclines or taxanes. In fact, the concomitant use of anthracyclines with CAR-T therapy is not recommended [141]. Therefore, the question arises as to how these cytostatic drugs could be incorporated (added to the lymphodepletion regimen or as bridge therapy prior to CAR-T infusion). The potential toxicity associated with the addition of these cytostatic agents must be carefully considered. Importantly, it remains to be determined whether lower, subtherapeutic doses sufficient to induce NKG2DL expression could be employed, potentially limiting treatment-related toxicity.

Another drug that is effective in TNBC is pembrolizumab (anti-PD1) [5]. A study has previously been mentioned in which the administration of anti-PD1 could increase NKG2D+ effector cells [42]. Another interesting target is the methylation of NKG2DL genes. This methylation appears to be described as one of those responsible for immune evasion in acute myeloid leukemia and agents like 5-aza-cytidine could reverse this epigenetic silencing [58]. Old drugs with newly discovered functions are also being studied, such as spironolactone, a diuretic that could increase the expression of NKG2DL in tumor cells [142]. Other drugs of interest currently in preclinical research are ADAM10/17 inhibitors and monoclonal antibodies against sNKG2DL [138].

6. Conclusions

In summary, available *in silico* data and published evidence indicate that NKG2DL ligands are transcriptionally expressed in BC across cell lines and tumor samples, with higher levels generally observed in biologically aggressive subtypes (especially TNBC). The frequent coexistence of NKG2DL expression with impaired NKG2D-positive effector cell function, together with multiple tumor-driven resistance mechanisms—particularly ligand shedding—highlights the complexity of immune surveillance in BC. However, this raises a starting point for the development of new therapies addressing this NKG2D/NKG2DL axis (with adoptive cell therapy taking on particular importance).

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/ijms27041848/s1>.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

3'UTR	3' untranslated region
95%CI	95% confidence interval
ADAM	A disintegrin and metalloprotease
ADC	Antibody–drug conjugate
aHR	Adjusted hazard ratio
ASC	Adipose-derived stem cell
ATM	Ataxia-telangiectasia mutated
BC	Breast cancer
BCSC	Breast cancer stem-like cells
BiTE	Bispecific T-cell engager
CAR	Chimeric antigen receptor
CBS	Cystathionine β -synthase
CIK	Cytokine-induced killer
CSE	Cystathionine γ -lyase
ctDNA	Circulating tumor DNA
CTLA4	Cytotoxic T-lymphocyte-associated protein 4
DLTs	Dose-limiting toxicities
ECOG PS	European Eastern Cooperative Oncology Group performance status
EGFR	Epidermal growth factor receptor
EHMT2	Euchromatic histone-lysine N-methyltransferase 2
EphA5	Ephrin type-A receptor 5
ER	Estrogen receptor
GSK-3 β	Glycogen synthase kinase-3 beta
H2BK	H2B.K variant histone
H2S	Hydrogen sulfide
HER2	Human epidermal growth factor receptor 2
HR	Hazard ratio
HSF1	Heat shock factor 1
IGFBP	Insulin-like growth factor binding protein
IHC	Immunohistochemistry
IL	Interleukin
INF γ	Interferon gamma
lncRNA	Long non-coding RNA
MCT4	Monocarboxylate transporter-4
Met	Methionine
MICA	Major histocompatibility complex class I-related protein A
MICB	Major histocompatibility complex class I-related protein B
miR	Micro RNA
MMP	Metalloprotease
mOS	Median overall survival
mPFS	Median progression-free survival
NK	Natural killer
NKG2D	Natural Killer group 2 member D receptor
NKG2DL	NKG2D ligand
nTPM	Normalized transcripts per million
ORR	Overall response rate
OS	Overall survival
PAM50	Predictor analysis of microarray 50
PCLP1	Podocalyxin-like protein 1
PFS	Progression-free survival
PD-L1	Protein death ligand 1

PI3K	Phosphoinositide 3-kinase
PR	Progesterone receptor
RFS	Relapse-free survival
RNA-seq	RNA sequencing
SCC	Senescent cancer cells
sMICA	Soluble MICA
sNKG2DL	Soluble NKG2D ligand
STAT3	Signal transducer and activator of transcription 3
TCGA	The Cancer Genome Atlas
TFGβ	Transforming growth factor beta
TNFα	Tumor necrosis factor alpha
TNBC	Triple-negative breast cancer
TPM	Transcripts per million
TRUCK	T cells redirected for antigen-unrestricted cytokine-initiated killing
ULBP1	UL16 binding protein 1
ULBP2	UL16 binding protein 2
ULBP3	UL16 binding protein 3
ULBP4	UL16 binding protein 4. Also: RAET1E (Retinoic Acid Early Transcript 1E)
ULBP5	UL16 binding protein 5. Also: RAET1G (Retinoic Acid Early Transcript 1G)
ULBP6	UL16 binding protein 6. Also: RAET1L (Retinoic Acid Early Transcript 1L)
Val	Valine
XOR	Xanthine oxidoreductase

Appendix A

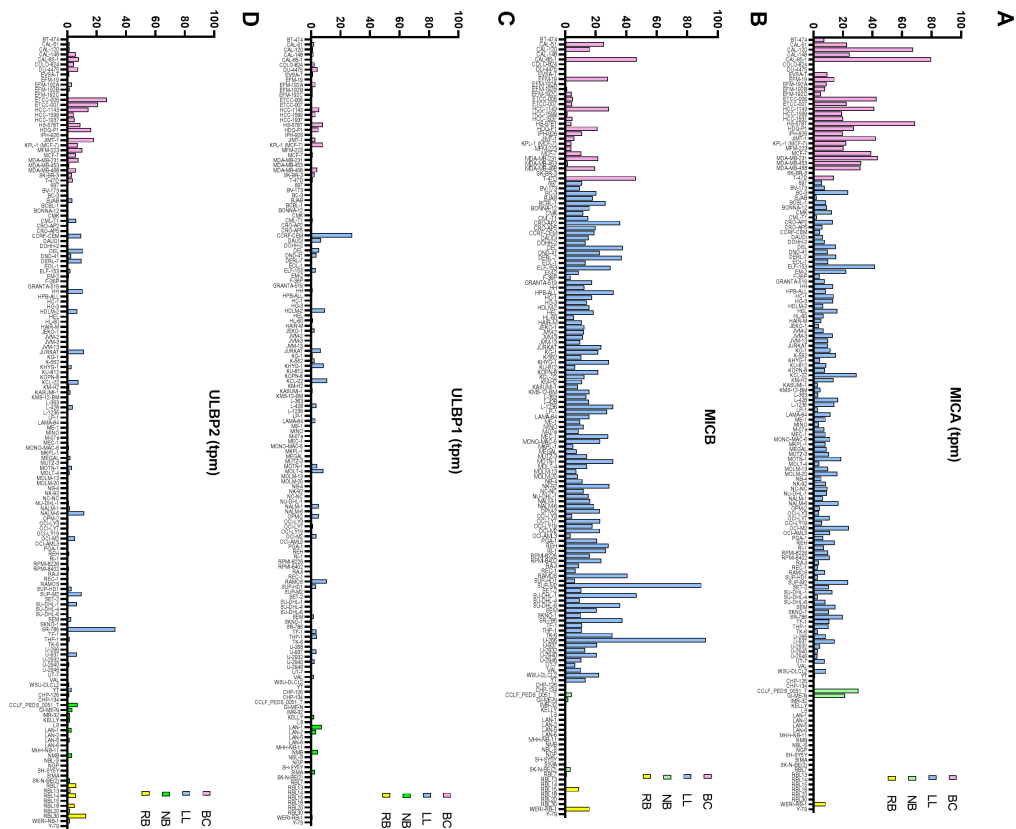
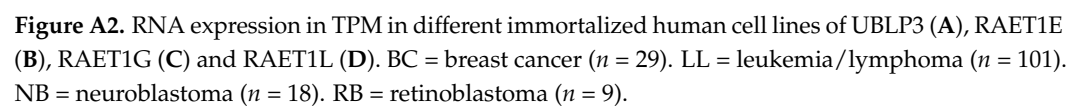


Figure A1. RNA expression in TPM in different immortalized human cell lines of MICA (A), MICB (B), ULBP1 (C) and ULBP2 (D). BC = breast cancer (*n* = 29). LL = leukemia/lymphoma (*n* = 101). NB = neuroblastoma (*n* = 18). RB = retinoblastoma (*n* = 9).



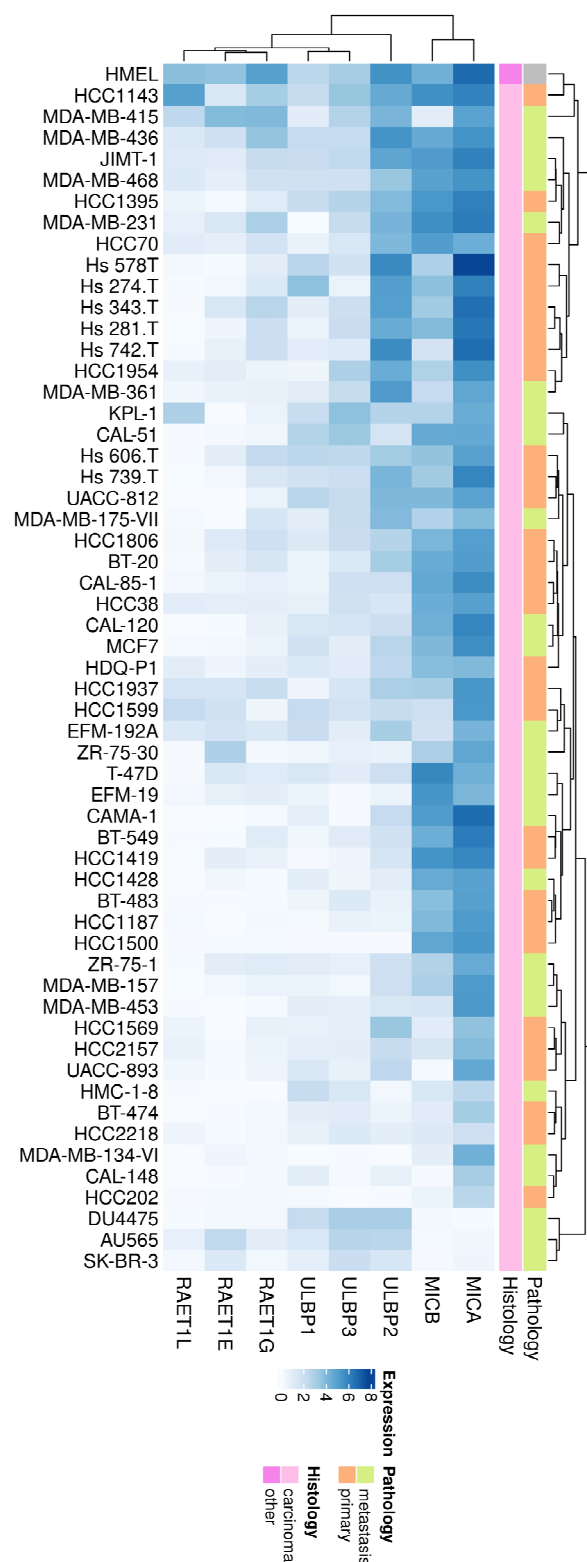


Figure A3. Heatmap of RNA expression of NK2DL in 60 immortalized human BC cell lines [20]. Obtained from <https://alexismurillo.shinyapps.io/ShinyThor/> (accessed on 6 February 2026). Parameters: Multiple Analytes > Choose your module of interest: Gene Expression > Write your analytes of interest (one analyte per line): MICA//MICB//ULBP1//ULBP2//ULBP3//RAET1E//RAET1G//RAET1L > Choose a specific group of cell lines: Breast.

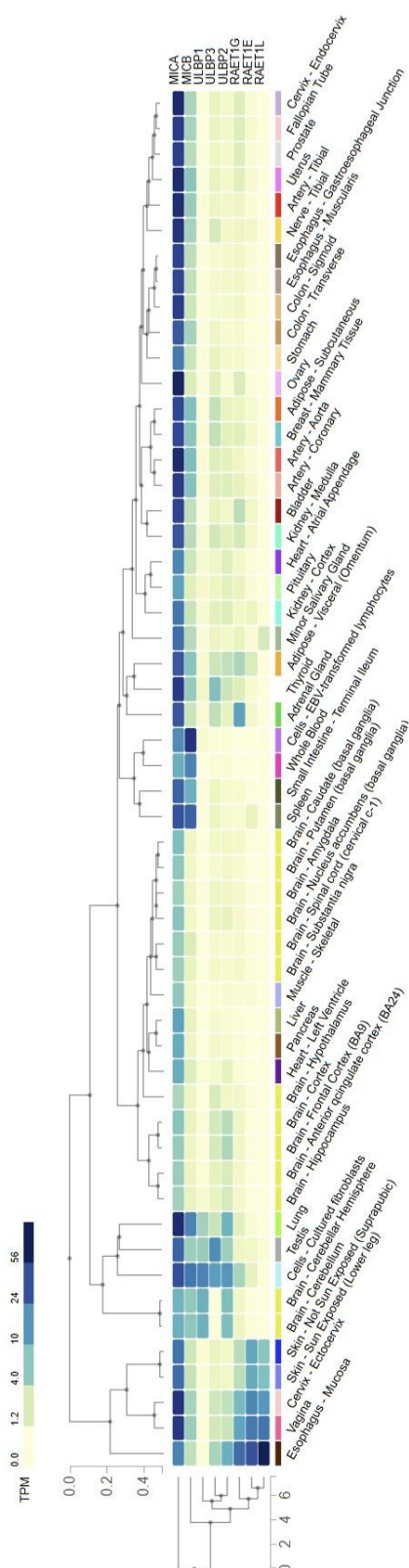


Figure A4. Heatmap of RNA expression in TPM of NKG2DL in different healthy tissues. Obtained from <http://www.gtexportal.org/> (accessed on 23 December 2025). Parameters: Expression > Multi-Gene Query > Enter Gencode IDs or Gene Names: MICA//MICB//ULBP1//ULBP2//ULBP3//RAET1E//RAET1G//RAET1L > Select Tissues: All.

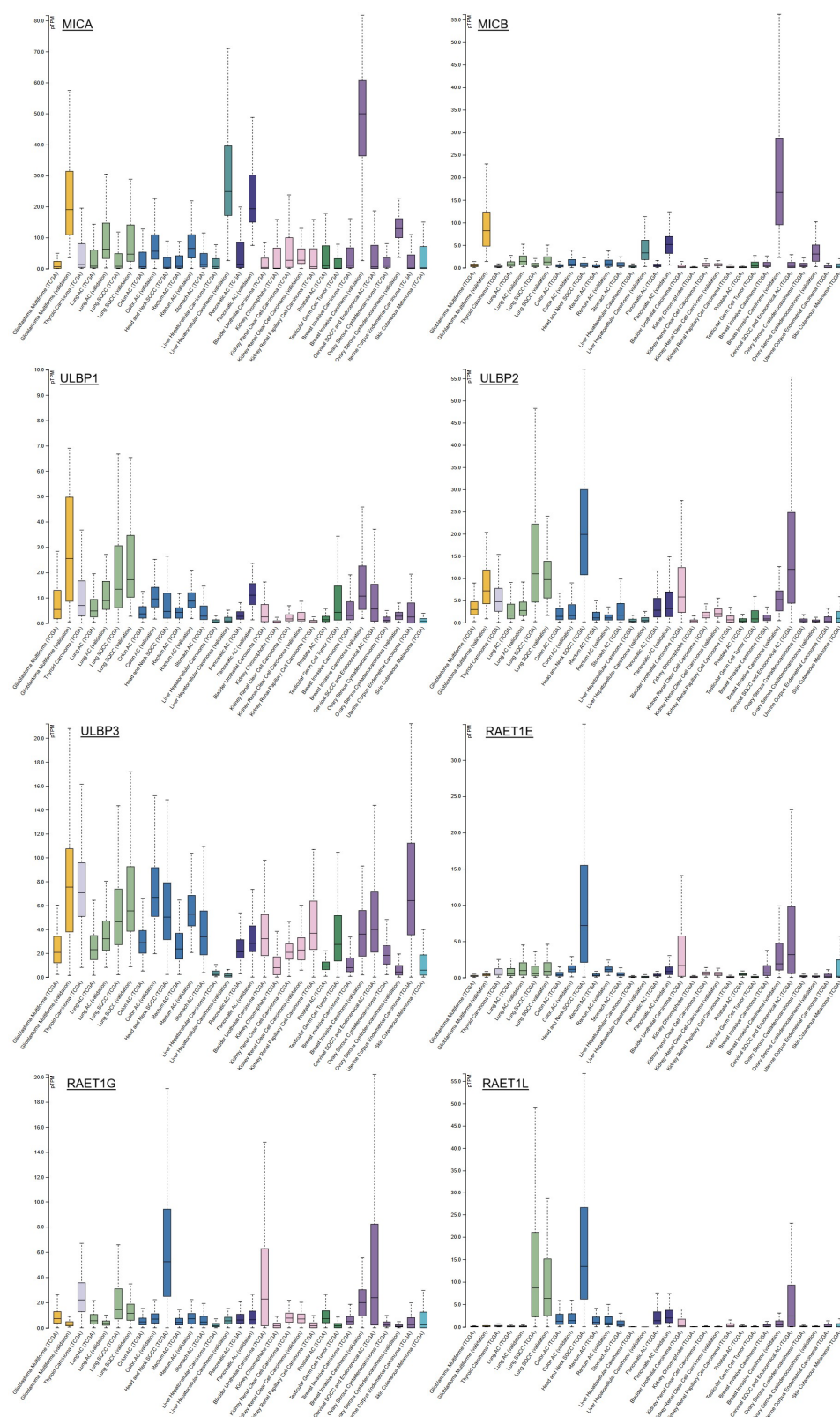


Figure A5. RNA expression in pTPM of NKG2DL in different tumors. Obtained from <https://www.proteinatlas.org/> (accessed on 20 January 2026) [143–150].

References

- Bray, F.; Laversanne, M.; Sung, H.; Ferlay, J.; Siegel, R.L.; Soerjomataram, I.; Jemal, A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* **2024**, *74*, 229–263. [\[CrossRef\]](#) [\[PubMed\]](#)
- Harbeck, N.; Penault-Llorca, F.; Cortes, J.; Gnant, M.; Houssami, N.; Poortmans, P.; Ruddy, K.; Tsang, J.; Cardoso, F. Breast cancer. *Nat. Rev. Dis. Primer* **2019**, *5*, 66. [\[CrossRef\]](#) [\[PubMed\]](#)
- Perou, C.M.; Sørlie, T.; Eisen, M.B.; van de Rijn, M.; Jeffrey, S.S.; Rees, C.A.; Pollack, J.R.; Ross, D.T.; Johnsen, H.; Akslen, L.A.; et al. Molecular portraits of human breast tumours. *Nature* **2000**, *406*, 747–752. [\[CrossRef\]](#) [\[PubMed\]](#)
- Prat, A.; Adamo, B.; Cheang, M.C.U.; Anders, C.K.; Carey, L.A.; Perou, C.M. Molecular characterization of basal-like and non-basal-like triple-negative breast cancer. *Oncologist* **2013**, *18*, 123–133. [\[CrossRef\]](#) [\[PubMed\]](#)
- Cortes, J.; Rugo, H.S.; Cescon, D.W.; Im, S.-A.; Yusof, M.M.; Gallardo, C.; Lipatov, O.; Barrios, C.H.; Perez-Garcia, J.; Iwata, H.; et al. Pembrolizumab plus Chemotherapy in Advanced Triple-Negative Breast Cancer. *N. Engl. J. Med.* **2022**, *387*, 217–226. [\[CrossRef\]](#)
- Bardia, A.; Hurvitz, S.A.; Tolaney, S.M.; Loirat, D.; Punie, K.; Oliveira, M.; Brufsky, A.; Sardesai, S.D.; Kalinsky, K.; Zelnak, A.B.; et al. Sacituzumab Govitecan in Metastatic Triple-Negative Breast Cancer. *N. Engl. J. Med.* **2021**, *384*, 1529–1541. [\[CrossRef\]](#)
- Rugo, H.S.; Bardia, A.; Marmé, F.; Cortés, J.; Schmid, P.; Loirat, D.; Trédan, O.; Ciruelos, E.; Dalenc, F.; Gómez Pardo, P.; et al. Overall survival with sacituzumab govitecan in hormone receptor-positive and human epidermal growth factor receptor 2-negative metastatic breast cancer (TROPiCS-02): A randomised, open-label, multicentre, phase 3 trial. *Lancet Lond. Engl.* **2023**, *402*, 1423–1433. [\[CrossRef\]](#)
- Cortés, J.; Kim, S.-B.; Chung, W.-P.; Im, S.-A.; Park, Y.H.; Hegg, R.; Kim, M.H.; Tseng, L.-M.; Petry, V.; Chung, C.-F.; et al. Trastuzumab Deruxtecan versus Trastuzumab Emtansine for Breast Cancer. *N. Engl. J. Med.* **2022**, *386*, 1143–1154. [\[CrossRef\]](#)
- Modi, S.; Jacot, W.; Yamashita, T.; Sohn, J.; Vidal, M.; Tokunaga, E.; Tsurutani, J.; Ueno, N.T.; Prat, A.; Chae, Y.S.; et al. Trastuzumab Deruxtecan in Previously Treated HER2-Low Advanced Breast Cancer. *N. Engl. J. Med.* **2022**, *387*, 9–20. [\[CrossRef\]](#)
- Giordano, A.; Awan, A.A.; Bruce, J.Y.; Rugo, H.S.; Diamond, J.R.; Novik, Y.; Baranda, J.; Muro, K.; Ono, M.; Nanda, R.; et al. Enfortumab Vedotin in Patients with Previously Treated Advanced Breast Cancer in the Phase II EV-202 Study. *JCO Oncol. Adv.* **2025**, *2*, e2500053. [\[CrossRef\]](#)
- Pagani, O.; Senkus, E.; Wood, W.; Colleoni, M.; Cufer, T.; Kyriakides, S.; Costa, A.; Winer, E.P.; Cardoso, F.; ESO-MBC Task Force. International guidelines for management of metastatic breast cancer: Can metastatic breast cancer be cured? *J. Natl. Cancer Inst.* **2010**, *102*, 456–463. [\[CrossRef\]](#) [\[PubMed\]](#)
- Bauer, S.; Groh, V.; Wu, J.; Steinle, A.; Phillips, J.H.; Lanier, L.L.; Spies, T. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science* **1999**, *285*, 727–729. [\[CrossRef\]](#) [\[PubMed\]](#)
- Cosman, D.; Müllberg, J.; Sutherland, C.L.; Chin, W.; Armitage, R.; Fanslow, W.; Kubin, M.; Chalupny, N.J. ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor. *Immunity* **2001**, *14*, 123–133. [\[CrossRef\]](#) [\[PubMed\]](#)
- Spear, P.; Wu, M.-R.; Sentman, M.-L.; Sentman, C.L. NKG2D ligands as therapeutic targets. *Cancer Immun.* **2013**, *13*, 8.
- Fernández, A.; Pérez-Martínez, A.; Escudero, A.; Mirones, I.; González, B.; de Paz, R.; Matamala, N.; Clares, L.; Navarro, A.; Galán, V.; et al. Infusion of haploidentical NKG2D-CAR-TCD45RA- cells in two pediatric patients with advanced relapsed and refractory acute leukemia was safe but achieved no clinical benefits. *Leuk. Lymphoma* **2022**, *63*, 1970–1974. [\[CrossRef\]](#)
- Mestre-Durán, C.; León-Triana, O.; Bareke, H.; Pertíñez, L.; Clares-Villa, L.; Bueno, D.; Lozano-Ojalvo, D.; Navarro-Zapata, A.; Sisinni, L.; Badell-Serra, I.; et al. Ex Vivo IL-15-Stimulated NK Cells as Adoptive Cell Therapy in Haploidentical Transplantation for Pediatric Leukemia. *Am. J. Hematol.* **2025**, *100*, 1898–1901. [\[CrossRef\]](#)
- Kristeleit, R.; Maher, J.; Davies, M.; Mitra, A.; Beehag, R.; Carlsen, B.; Yong, C.H.; Gomm, L.; Josephs, D.; Bashir, Z.; et al. 578 A First-in-human phase I/IIa dose escalation trial evaluating the safety and preliminary efficacy of LEU011, a novel CAR-T, in subjects with relapsed/refractory solid tumors (AERIAL). *J. Immunother. Cancer* **2025**, *13*, A657. [\[CrossRef\]](#)
- Koblitz, J.; Dirks, W.G.; Eberth, S.; Nagel, S.; Steenpass, L.; Pommerenke, C. DSMZCellDive: Diving into high-throughput cell line data. *F1000Research* **2022**, *11*, 420. [\[CrossRef\]](#)
- Pommerenke, C.; Nagel, S.; Haake, J.; Koelz, A.L.; Christgen, M.; Steenpass, L.; Eberth, S. Molecular Characterization and Subtyping of Breast Cancer Cell Lines Provide Novel Insights into Cancer Relevant Genes. *Cells* **2024**, *13*, 301. [\[CrossRef\]](#)
- Navarrete-Bencomo, E.; Campos Segura, A.V.; Sevillano, O.R.; Mayanga, A.; Buleje Sono, J.L.; Ortiz Rojas, C.A.; Murillo Carrasco, A.G. ShinyTHOR app: Shiny-built tumor high-throughput omics-based roadmap. *Bioinforma. Adv.* **2025**, *5*, vbaf061. [\[CrossRef\]](#)
- The GTEx Consortium. The GTEx Consortium atlas of genetic regulatory effects across human tissues. *Science* **2020**, *369*, 1318–1330. [\[CrossRef\]](#)
- Uhlen, M.; Zhang, C.; Lee, S.; Sjöstedt, E.; Fagerberg, L.; Bidkhori, G.; Benfeitas, R.; Arif, M.; Liu, Z.; Edfors, F.; et al. A pathology atlas of the human cancer transcriptome. *Science* **2017**, *357*, eaan2507. [\[CrossRef\]](#)

23. Cerami, E.; Gao, J.; Dogrusoz, U.; Gross, B.E.; Sumer, S.O.; Aksoy, B.A.; Jacobsen, A.; Byrne, C.J.; Heuer, M.L.; Larsson, E.; et al. The cBio cancer genomics portal: An open platform for exploring multidimensional cancer genomics data. *Cancer Discov.* **2012**, *2*, 401–404, Erratum in *Cancer Discov.* **2012**, *2*, 960. <https://doi.org/10.1158/2159-8290.CD-12-0326>. [\[CrossRef\]](#)
24. Gao, J.; Aksoy, B.A.; Dogrusoz, U.; Dresdner, G.; Gross, B.; Sumer, S.O.; Sun, Y.; Jacobsen, A.; Sinha, R.; Larsson, E.; et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci. Signal.* **2013**, *6*, pl1. [\[CrossRef\]](#) [\[PubMed\]](#)
25. de Bruijn, I.; Kundra, R.; Mastrogiacomio, B.; Tran, T.N.; Sikina, L.; Mazor, T.; Li, X.; Ochoa, A.; Zhao, G.; Lai, B.; et al. Analysis and Visualization of Longitudinal Genomic and Clinical Data from the AACR Project GENIE Biopharma Collaborative in cBioPortal. *Cancer Res.* **2023**, *83*, 3861–3867. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Berger, A.C.; Korkut, A.; Kanchi, R.S.; Hegde, A.M.; Lenoir, W.; Liu, W.; Liu, Y.; Fan, H.; Shen, H.; Ravikumar, V.; et al. A Comprehensive Pan-Cancer Molecular Study of Gynecologic and Breast Cancers. *Cancer Cell* **2018**, *33*, 690–705.e9. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Györfy, B. Survival analysis across the entire transcriptome identifies biomarkers with the highest prognostic power in breast cancer. *Comput. Struct. Biotechnol. J.* **2021**, *19*, 4101–4109. [\[CrossRef\]](#)
28. Clayton, A.; Tabi, Z. Exosomes and the MICA-NKG2D system in cancer. *Blood Cells. Mol. Dis.* **2005**, *34*, 206–213. [\[CrossRef\]](#)
29. Mamessier, E.; Sylvain, A.; Bertucci, F.; Castellano, R.; Finetti, P.; Houvenaeghel, G.; Charaffe-Jaufret, E.; Birnbaum, D.; Moretta, A.; Olive, D. Human breast tumor cells induce self-tolerance mechanisms to avoid NKG2D-mediated and DNAM-mediated NK cell recognition. *Cancer Res.* **2011**, *71*, 6621–6632. [\[CrossRef\]](#)
30. Ye, Y.; Zhou, Z.; Chen, Q.; Li, J.; Chen, X.; Huang, W. [The roles of soluble MICA in immune escape of breast tumor]. *Chin. J. Cell. Mol. Immunol.* **2008**, *24*, 904–907.
31. Chitadze, G.; Lettau, M.; Bhat, J.; Wesch, D.; Steinle, A.; Fürst, D.; Mytilineos, J.; Kalthoff, H.; Janssen, O.; Oberg, H.-H.; et al. Shedding of endogenous MHC class I-related chain molecules A and B from different human tumor entities: Heterogeneous involvement of the “a disintegrin and metalloproteases” 10 and 17. *Int. J. Cancer* **2013**, *133*, 1557–1566. [\[CrossRef\]](#)
32. Muñoz, D.P.; Yannoni, S.M.; Daemen, A.; Sun, Y.; Vakar-Lopez, F.; Kawahara, M.; Freund, A.M.; Rodier, F.; Wu, J.D.; Desprez, P.-Y.; et al. Targetable mechanisms driving immuno-evasion of persistent senescent cells link chemotherapy-resistant cancer to aging. *JCI Insight* **2019**, *5*, e124716. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Buckley, A.; McQuaid, S.; Johnson, P.; Buggy, D.J. Effect of anaesthetic technique on the natural killer cell anti-tumour activity of serum from women undergoing breast cancer surgery: A pilot study. *Br. J. Anaesth.* **2014**, *113*, i56–i62. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Kim, H.J.; Jeon, S.; Lee, H.J.; Bae, J.; Ri, H.-S.; Hong, J.-M.; Paek, S.I.; Kwon, S.K.; Kim, J.-R.; Park, S.; et al. Effects of sevoflurane on metalloproteinase and natural killer group 2, member D (NKG2D) ligand expression and natural killer cell-mediated cytotoxicity in breast cancer: An in vitro study. *Korean J. Anesthesiol.* **2023**, *76*, 627–639. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Ri, H.-S.; Lee, H.J.; Bae, J.; Cho, A.-R.; Kim, J.R.; Park, S.; Lee, K.Y.; Jeon, S. Receptor subtype-dependent effects of propofol on metalloproteinase activity, NKG2D ligand expression, and NK cell-mediated cytotoxicity in breast cancer: An in vitro study. *Korean J. Anesthesiol.* **2025**; online ahead of printing. [\[CrossRef\]](#)
36. Gajovic, N.; Jurisevic, M.; Pantic, J.; Radosavljevic, G.; Arsenijevic, N.; Lukic, M.L.; Jovanovic, I. Attenuation of NK cells facilitates mammary tumor growth in streptozotocin-induced diabetes in mice. *Endocr. Relat. Cancer* **2018**, *25*, 493–507. [\[CrossRef\]](#)
37. Rodrigues, N.V.; Correia, D.V.; Mensurado, S.; Nóbrega-Pereira, S.; deBarros, A.; Kyle-Cezar, F.; Tutt, A.; Hayday, A.C.; Norell, H.; Silva-Santos, B.; et al. Low-Density Lipoprotein Uptake Inhibits the Activation and Antitumor Functions of Human Vγ9Vδ2 T Cells. *Cancer Immunol. Res.* **2018**, *6*, 448–457. [\[CrossRef\]](#)
38. Spielmann, J.; Mattheis, L.; Jung, J.-S.; Rauße, H.; Glaß, M.; Bähr, I.; Quandt, D.; Oswald, J.; Kielstein, H. Effects of obesity on NK cells in a mouse model of postmenopausal breast cancer. *Sci. Rep.* **2020**, *10*, 20606. [\[CrossRef\]](#)
39. Siegers, G.M.; Dutta, I.; Lai, R.; Postovit, L.-M. Functional Plasticity of Gamma Delta T Cells and Breast Tumor Targets in Hypoxia. *Front. Immunol.* **2018**, *9*, 1367. [\[CrossRef\]](#)
40. Long, Y.; Gao, Z.; Hu, X.; Xiang, F.; Wu, Z.; Zhang, J.; Han, X.; Yin, L.; Qin, J.; Lan, L.; et al. Downregulation of MCT4 for lactate exchange promotes the cytotoxicity of NK cells in breast carcinoma. *Cancer Med.* **2018**, *7*, 4690–4700. [\[CrossRef\]](#)
41. Youness, R.A.; Gad, A.Z.; Sanber, K.; Ahn, Y.J.; Lee, G.-J.; Khallaf, E.; Hafez, H.M.; Motaal, A.A.; Ahmed, N.; Gad, M.Z. Targeting hydrogen sulphide signaling in breast cancer. *J. Adv. Res.* **2021**, *27*, 177–190. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Nafea, H.; Youness, R.A.; Dawoud, A.; Khater, N.; Manie, T.; Abdel-Kader, R.; Bourquin, C.; Szabo, C.; Gad, M.Z. Dual targeting of H₂S synthesizing enzymes; cystathionine β-synthase and cystathionine γ-lyase by miR-939-5p effectively curbs triple negative breast cancer. *Heliyon* **2023**, *9*, e21063. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Jovanovic, I.P.; Pejnovic, N.N.; Radosavljevic, G.D.; Pantic, J.M.; Milovanovic, M.Z.; Arsenijevic, N.N.; Lukic, M.L. Interleukin-33/ST2 axis promotes breast cancer growth and metastases by facilitating intratumoral accumulation of immunosuppressive and innate lymphoid cells. *Int. J. Cancer* **2014**, *134*, 1669–1682. [\[CrossRef\]](#) [\[PubMed\]](#)

44. Jovanović, M.Z.; Jurišević, M.; Jovanović, M.; Gajović, N.; Jocić, M.; Jovanović, M.M.; Milev, B.; Vasiljev, K.D.; Jovanović, I. Co-Targeting PD-1 and IL-33/ST2 Pathways for Enhanced Acquired Anti-Tumor Immunity in Breast Cancer. *Int. J. Mol. Sci.* **2025**, *26*, 9600. [\[CrossRef\]](#)
45. Uddin, M.B.; Roy, K.R.; Hill, R.A.; Roy, S.C.; Gu, X.; Li, L.; Zhang, Q.-J.; You, Z.; Liu, Y.-Y. p53 missense mutant G242A subverts natural killer cells in sheltering mouse breast cancer cells against immune rejection. *Exp. Cell Res.* **2022**, *417*, 113210. [\[CrossRef\]](#)
46. Jin, F.; Wu, Z.; Hu, X.; Zhang, J.; Gao, Z.; Han, X.; Qin, J.; Li, C.; Wang, Y. The PI3K/Akt/GSK-3 β /ROS/eIF2B pathway promotes breast cancer growth and metastasis via suppression of NK cell cytotoxicity and tumor cell susceptibility. *Cancer Biol. Med.* **2019**, *16*, 38–54. [\[CrossRef\]](#)
47. Chava, S.; Bugide, S.; Malvi, P.; DeMarco, K.D.; Ma, B.; Parikh, C.N.; Ruscetti, M.; Zajac, A.; Cai, G.; Gupta, R.; et al. Loss of EHMT2 enhances NK cell-driven anti-tumor immunity through TGF- β 1 suppression. *EMBO Mol. Med.* **2025**, *18*, 232–274. [\[CrossRef\]](#)
48. Xu, P.; Sun, Y.; Song, Y.; Jiao, J.; Shen, B.; Li, W.; Jiang, C.; Li, Y.; Zhang, X.; Yu, J.; et al. ATM kinase regulates tumor immunoreactions in lymphocyte-predominant breast cancer through modulation of NKG2D ligand and TNF cytokines on tumor cells. *Med. Mol. Morphol.* **2020**, *53*, 210–220. [\[CrossRef\]](#)
49. Schilling, D.; Kühnel, A.; Tetzlaff, F.; Konrad, S.; Multhoff, G. NZ28-induced inhibition of HSF1, SP1 and NF- κ B triggers the loss of the natural killer cell-activating ligands MICA/B on human tumor cells. *Cancer Immunol. Immunother. CII* **2015**, *64*, 599–608. [\[CrossRef\]](#)
50. Amo, L.; Tamayo-Orbegozo, E.; Maruri, N.; Buqué, A.; Solaun, M.; Riñón, M.; Arrieta, A.; Larrucea, S. Podocalyxin-like protein 1 functions as an immunomodulatory molecule in breast cancer cells. *Cancer Lett.* **2015**, *368*, 26–35. [\[CrossRef\]](#)
51. Scully, T.; Scott, C.D.; Firth, S.M.; Sedger, L.M.; Pintar, J.E.; Twigg, S.M.; Baxter, R.C. Enhancement of mammary tumour growth by IGFBP-3 involves impaired T cell accumulation. *Endocr. Relat. Cancer* **2018**, *25*, 111–122. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Wang, B.; Wang, Q.; Wang, Z.; Jiang, J.; Yu, S.-C.; Ping, Y.-F.; Yang, J.; Xu, S.-L.; Ye, X.-Z.; Xu, C.; et al. Metastatic consequences of immune escape from NK cell cytotoxicity by human breast cancer stem cells. *Cancer Res.* **2014**, *74*, 5746–5757. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Wang, S.; Zhang, Y.; Cong, W.; Liu, J.; Zhang, Y.; Fan, H.; Xu, Y.; Lin, H. Breast cancer stem-like cells can promote metastasis by activating platelets and down-regulating antitumor activity of natural killer cells. *J. Tradit. Chin. Med.* **2016**, *36*, 530–537. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Bahrami, B.; Hosseini, A.; Talei, A.-R.; Ghaderi, A.; Razmkhah, M. Adipose Derived Stem Cells Exert Immunomodulatory Effects on Natural Killer Cells in Breast Cancer. *Cell J. Yakhteh* **2017**, *19*, 137–145.
55. Mansourabadi, Z.; Razmkhah, M.; Mohtasebi, M.S.; Talei, A.-R.; Ghaderi, A. Expression of major histocompatibility complex class I polypeptide-related sequence B in adipose-derived stem cells from breast cancer patients and normal individuals. *J. Cancer Res. Ther.* **2019**, *15*, 1067–1072. [\[CrossRef\]](#)
56. Flüh, C.; Mafael, V.; Adamski, V.; Synowitz, M.; Held-Feindt, J. Dormancy and NKG2D system in brain metastases: Analysis of immunogenicity. *Int. J. Mol. Med.* **2020**, *45*, 298–314. [\[CrossRef\]](#)
57. El-Gazzar, A.; Cai, X.; Reeves, R.S.; Dai, Z.; Caballero-Benitez, A.; McDonald, D.L.; Vazquez, J.; Gooley, T.A.; Sale, G.E.; Spies, T.; et al. Effects on tumor development and metastatic dissemination by the NKG2D lymphocyte receptor expressed on cancer cells. *Oncogene* **2014**, *33*, 4932–4940. [\[CrossRef\]](#)
58. Baragaño Raneros, A.; Martín-Palanco, V.; Fernandez, A.F.; Rodriguez, R.M.; Fraga, M.F.; Lopez-Larrea, C.; Suarez-Alvarez, B. Methylation of NKG2D ligands contributes to immune system evasion in acute myeloid leukemia. *Genes Immun.* **2015**, *16*, 71–82. [\[CrossRef\]](#)
59. Breunig, C.; Pahl, J.; Küblbeck, M.; Miller, M.; Antonelli, D.; Erdem, N.; Wirth, C.; Will, R.; Bott, A.; Cerwenka, A.; et al. MicroRNA-519a-3p mediates apoptosis resistance in breast cancer cells and their escape from recognition by natural killer cells. *Cell Death Dis.* **2017**, *8*, e2973. [\[CrossRef\]](#)
60. Yin, J.-Y.; Zhou, Y.; Ding, X.-M.; Gong, R.-Z.; Zhou, Y.; Hu, H.-Y.; Liu, Y.; Lv, X.-B.; Zhang, B. UCA1 Inhibits NKG2D-mediated Cytotoxicity of NK Cells to Breast Cancer. *Curr. Cancer Drug Targets* **2024**, *24*, 204–219. [\[CrossRef\]](#)
61. Xu, X.; Rao, G.; Li, Y. Xanthine oxidoreductase is required for genotoxic stress-induced NKG2D ligand expression and gemcitabine-mediated antitumor activity. *Oncotarget* **2016**, *7*, 59220–59235. [\[CrossRef\]](#)
62. Wang, J.; Liu, K.; Xiao, T.; Liu, P.; Prinz, R.A.; Xu, X. Uric acid accumulation in DNA-damaged tumor cells induces NKG2D ligand expression and antitumor immunity by activating TGF- β -activated kinase 1. *Oncoimmunology* **2022**, *11*, 2016159. [\[CrossRef\]](#) [\[PubMed\]](#)
63. Madjd, Z.; Spendlove, I.; Moss, R.; Bevin, S.; Pinder, S.E.; Watson, N.F.S.; Ellis, I.; Durrant, L.G. Upregulation of MICA on high-grade invasive operable breast carcinoma. *Cancer Immun.* **2007**, *7*, 17. [\[PubMed\]](#)
64. Shen, J.; Pan, J.; Du, C.; Si, W.; Yao, M.; Xu, L.; Zheng, H.; Xu, M.; Chen, D.; Wang, S.; et al. Silencing NKG2D ligand-targeting miRNAs enhances natural killer cell-mediated cytotoxicity in breast cancer. *Cell Death Dis.* **2017**, *8*, e2740. [\[CrossRef\]](#) [\[PubMed\]](#)

65. de Kruijf, E.M.; Sajet, A.; van Nes, J.G.H.; Putter, H.; Smit, V.T.H.B.M.; Eagle, R.A.; Jafferji, I.; Trowsdale, J.; Liefers, G.J.; van de Velde, C.J.H.; et al. NKG2D ligand tumor expression and association with clinical outcome in early breast cancer patients: An observational study. *BMC Cancer* **2012**, *12*, 24. [\[CrossRef\]](#)
66. Zumwalde, N.A.; Haag, J.D.; Gould, M.N.; Gumperz, J.E. Mucosal associated invariant T cells from human breast ducts mediate a Th17-skewed response to bacterially exposed breast carcinoma cells. *Breast Cancer Res. BCR* **2018**, *20*, 111. [\[CrossRef\]](#)
67. Elsabbagh, R.A.; Abdelhady, G.; Urlaub, D.; Sandusky, M.; Khorshid, O.; Gad, M.Z.; Abou-Aisha, K.; Watzl, C.; Rady, M. N6-methyladenosine RNA base modification regulates NKG2D-dependent and cytotoxic genes expression in natural killer cells. *BMC Med. Genom.* **2025**, *18*, 91. [\[CrossRef\]](#)
68. Nieto-Velázquez, N.G.; Torres-Ramos, Y.D.; Muñoz-Sánchez, J.L.; Espinosa-Godoy, L.; Gómez-Cortés, S.; Moreno, J.; Moreno-Eutimio, M.A. Altered Expression of Natural Cytotoxicity Receptors and NKG2D on Peripheral Blood NK Cell Subsets in Breast Cancer Patients. *Transl. Oncol.* **2016**, *9*, 384–391. [\[CrossRef\]](#)
69. Mamessier, E.; Sylvain, A.; Thibault, M.-L.; Houvenaeghel, G.; Jacquemier, J.; Castellano, R.; Gonçalves, A.; André, P.; Romagné, F.; Thibault, G.; et al. Human breast cancer cells enhance self tolerance by promoting evasion from NK cell antitumor immunity. *J. Clin. Investig.* **2011**, *121*, 3609–3622. [\[CrossRef\]](#)
70. Arianfar, E.; Khandoozi, S.R.; Mohammadi, S.; Memarian, A. Suppression of CD56bright NK cells in breast cancer patients is associated with the PD-1 and TGF- β RII expression. *Clin. Transl. Oncol.* **2023**, *25*, 841–851. [\[CrossRef\]](#)
71. Kawaguchi, K.; Suzuki, E.; Yamaguchi, A.; Yamamoto, M.; Morita, S.; Toi, M. Altered expression of major immune regulatory molecules in peripheral blood immune cells associated with breast cancer. *Breast Cancer Tokyo Jpn.* **2017**, *24*, 111–120. [\[CrossRef\]](#) [\[PubMed\]](#)
72. Darvishvand, R.; Rezaeifard, S.; Kiani, R.; Tahmasebi, S.; Faghih, Z.; Erfani, N. Natural killer cell subsets and their functional molecules in peripheral blood of the patients with breast cancer. *Immun. Inflamm. Dis.* **2024**, *12*, e1255. [\[CrossRef\]](#) [\[PubMed\]](#)
73. Wu, Y.; Kyle-Cezar, F.; Woolf, R.T.; Naceur-Lombardelli, C.; Owen, J.; Biswas, D.; Lorenc, A.; Vantourout, P.; Gazinska, P.; Grigoriadis, A.; et al. An innate-like V δ 1+ $\gamma\delta$ T cell compartment in the human breast is associated with remission in triple-negative breast cancer. *Sci. Transl. Med.* **2019**, *11*, eaax9364. [\[CrossRef\]](#) [\[PubMed\]](#)
74. Rezaeifard, S.; Talei, A.; Shariat, M.; Erfani, N. Tumor infiltrating NK cell (TINK) subsets and functional molecules in patients with breast cancer. *Mol. Immunol.* **2021**, *136*, 161–167. [\[CrossRef\]](#)
75. Verma, C.; Kaewkangsadan, V.; Eremin, J.M.; Cowley, G.P.; Ilyas, M.; El-Sheemy, M.A.; Eremin, O. Natural killer (NK) cell profiles in blood and tumour in women with large and locally advanced breast cancer (LLABC) and their contribution to a pathological complete response (PCR) in the tumour following neoadjuvant chemotherapy (NAC): Differential restoration of blood profiles by NAC and surgery. *J. Transl. Med.* **2015**, *13*, 180. [\[CrossRef\]](#)
76. Lobo-Martins, S.; Corredeira, P.; Cavaco, A.; Rodrigues, C.; Piai, P.; Lopes, C.; Fraga, J.; Silva, M.; Alves, P.; Wachholz Szeneszi, L.; et al. Effect of Cyclin-Dependent Kinase 4/6 Inhibitors on Circulating Cells in Patients with Metastatic Breast Cancer. *Cells* **2024**, *13*, 1391. [\[CrossRef\]](#)
77. Bargostavan, M.H.; Eslami, G.; Esfandiari, N.; Shams Shahemabadi, A. MMP9 Promoter Polymorphism (–1562 C/T) Does not Affect the Serum Levels of Soluble MICB and MICA in Breast Cancer. *Iran. J. Immunol. IJI* **2016**, *13*, 45–53.
78. Roshani, R.; Boroujerdnia, M.G.; Talaiezhadeh, A.H.; Khodadadi, A. Assessment of changes in expression and presentation of NKG2D under influence of MICA serum factor in different stages of breast cancer. *Tumour Biol. J. Int. Soc. Oncodev. Biol. Med.* **2016**, *37*, 6953–6962. [\[CrossRef\]](#)
79. Kshersagar, J.; Damle, M.N.; Bedge, P.; Jagdale, R.; Tardalkar, K.; Jadhav, D.; Jagadale, S.; Toro, Y.; Sharma, R.; Joshi, M.G. Downregulation of MICA/B tumor surface expressions and augmented soluble MICA serum levels correlate with disease stage in breast cancer. *Breast Dis.* **2022**, *41*, 471–480. [\[CrossRef\]](#)
80. Seller, A.; Tegeler, C.M.; Mauermann, J.; Schreiber, T.; Hagelstein, I.; Liebel, K.; Koch, A.; Heitmann, J.S.; Greiner, S.M.; Hayn, C.; et al. Soluble NKG2DLs Are Elevated in Breast Cancer Patients and Associate with Disease Outcome. *Int. J. Mol. Sci.* **2024**, *25*, 4126. [\[CrossRef\]](#)
81. Lavado-Valenzuela, R.; Benavides, M.; Carabantes, F.; Alonso, A.; Caballero, A. MHC class I chain-related gene A transmembrane polymorphism in Spanish women with breast cancer. *Tissue Antigens* **2009**, *74*, 46–49. [\[CrossRef\]](#)
82. Ouni, N.; Ben Chaaben, A.; Kablouti, G.; Lajnef, M.; Ayari, F.; Abaza, H.; Damak, T.; Harzallah, L.; Benammar-Elgaaied, A.; Guemira, F.; et al. MICA-129Met/Val Polymorphism Is Associated with Early-Onset Breast Cancer Risk. *Immunol. Investig.* **2017**, *46*, 603–614. [\[CrossRef\]](#)
83. Ouni, N.; Ben Chaaben, A.; Ayari, F.; Douik, H.; Guizani, I.; Benammar-Elgaaied, A.; Guemira, F.; Tamouza, R. MICA-129 Met/Val polymorphism could be a genetic biomarker for Familial Breast Cancer in the Tunisian population. *Int. J. Immunogenet.* **2020**, *47*, 406–413. [\[CrossRef\]](#) [\[PubMed\]](#)
84. Kim, J.-Y.; Bae, J.-H.; Lee, S.-H.; Lee, E.-Y.; Chung, B.-S.; Kim, S.-H.; Kang, C.-D. Induction of NKG2D ligands and subsequent enhancement of NK cell-mediated lysis of cancer cells by arsenic trioxide. *J. Immunother.* **2008**, *31*, 475–486. [\[CrossRef\]](#) [\[PubMed\]](#)

85. Di Modica, M.; Sfondrini, L.; Regondi, V.; Varchetta, S.; Oliviero, B.; Mariani, G.; Bianchi, G.V.; Generali, D.; Balsari, A.; Triulzi, T.; et al. Taxanes enhance trastuzumab-mediated ADCC on tumor cells through NKG2D-mediated NK cell recognition. *Oncotarget* **2015**, *7*, 255–265. [\[CrossRef\]](#) [\[PubMed\]](#)
86. Feng, H.; Dong, Y.; Wu, J.; Qiao, Y.; Zhu, G.; Jin, H.; Cui, J.; Li, W.; Liu, Y.-J.; Chen, J.; et al. Epirubicin pretreatment enhances NK cell-mediated cytotoxicity against breast cancer cells in vitro. *Am. J. Transl. Res.* **2016**, *8*, 473–484.
87. Liu, J.; Wang, Y.; Wei, X.; Liu, S.; Hu, C.; Peng, P.; Wu, W.; Cai, J.; Sun, H. TAK-981 potentiates doxorubicin immunocidal in triple-negative breast cancer by IFN I-dependent NK cell stimulation. *Cell. Oncol. Dordr. Neth.* **2025**, *48*, 1871–1887. [\[CrossRef\]](#)
88. Zhao, N.; Li, X.; He, X.; Qiu, Y.; Zhu, L.; Qi, F. Interleukin-15 gene therapy and the mammalian target of rapamycin inhibitor everolimus inhibit the growth of metastatic breast cancer. *J. Gene Med.* **2013**, *15*, 366–374. [\[CrossRef\]](#)
89. Yin, T.; Wang, G.; He, S.; Liu, Q.; Sun, J.; Wang, Y. Human cancer cells with stem cell-like phenotype exhibit enhanced sensitivity to the cytotoxicity of IL-2 and IL-15 activated natural killer cells. *Cell. Immunol.* **2016**, *300*, 41–45. [\[CrossRef\]](#)
90. Payne, K.K.; Zoon, C.K.; Wan, W.; Marlar, K.; Keim, R.C.; Kenari, M.N.; Kazim, A.L.; Bear, H.D.; Manjili, M.H. Peripheral blood mononuclear cells of patients with breast cancer can be reprogrammed to enhance anti-HER-2/neu reactivity and overcome myeloid-derived suppressor cells. *Breast Cancer Res. Treat.* **2013**, *142*, 45–57. [\[CrossRef\]](#)
91. Barberini, F.; Pietroni, R.; Ielpo, S.; Lucarini, V.; Nardozi, D.; Melaiu, O.; Benvenuto, M.; Focaccetti, C.; Palumbo, C.; Rossin, F.; et al. Combined IFN- γ and TNF- α treatment enhances the susceptibility of breast cancer cells and spheroids to Natural Killer cell-mediated killing. *Cell Death Dis.* **2025**, *16*, 729. [\[CrossRef\]](#) [\[PubMed\]](#)
92. Nam, J.-S.; Terabe, M.; Mamura, M.; Kang, M.-J.; Chae, H.; Stuelten, C.; Kohn, E.; Tang, B.; Sabzevari, H.; Anver, M.R.; et al. An anti-transforming growth factor beta antibody suppresses metastasis via cooperative effects on multiple cell compartments. *Cancer Res.* **2008**, *68*, 3835–3843. [\[CrossRef\]](#) [\[PubMed\]](#)
93. Roberti, M.P.; Barrio, M.M.; Bravo, A.I.; Rocca, Y.S.; Arriaga, J.M.; Bianchini, M.; Mordoh, J.; Levy, E.M. IL-15 and IL-2 increase Cetuximab-mediated cellular cytotoxicity against triple negative breast cancer cell lines expressing EGFR. *Breast Cancer Res. Treat.* **2011**, *130*, 465–475. [\[CrossRef\]](#) [\[PubMed\]](#)
94. Ruth, J.H.; Gurrea-Rubio, M.; Athukorala, K.S.; Rasmussen, S.M.; Weber, D.P.; Randon, P.M.; Gedert, R.J.; Lind, M.E.; Amin, M.A.; Campbell, P.L.; et al. CD6 is a target for cancer immunotherapy. *JCI Insight* **2021**, *6*, e145662. [\[CrossRef\]](#)
95. González Muñoz, C.; Álvarez Arzola, R.; Calvo Pérez, A.; Frometa Campanón, M.d.l.C.; Hernández Casaña, P.; Fernández-Calienes Valdés, A.; Lorenzo-Luaces, P.; Mazorra Herrera, Z.; Crombet Ramos, T.; Labrada Mon, M. Itolizumab regulates activating and inhibitory signals on effector cells, improving their cytotoxicity against CD318+ tumor cell lines. *Front. Immunol.* **2025**, *16*, 1585597. [\[CrossRef\]](#)
96. Cabo, M.; Santana-Hernández, S.; Costa-Garcia, M.; Rea, A.; Lozano-Rodríguez, R.; Ataya, M.; Balaguer, F.; Juan, M.; Ochoa, M.C.; Menéndez, S.; et al. CD137 Costimulation Counteracts TGF β Inhibition of NK-cell Antitumor Function. *Cancer Immunol. Res.* **2021**, *9*, 1476–1490. [\[CrossRef\]](#)
97. Cho, H.-M.; Rosenblatt, J.D.; Tolba, K.; Shin, S.J.; Shin, D.S.; Calfa, C.; Zhang, Y.; Shin, S.-U. Delivery of NKG2D ligand using an anti-HER2 antibody-NKG2D ligand fusion protein results in an enhanced innate and adaptive antitumor response. *Cancer Res.* **2010**, *70*, 10121–10130. [\[CrossRef\]](#)
98. Raab, S.; Steinbacher, J.; Schmiedel, B.J.; Kousis, P.C.; Steinle, A.; Jung, G.; Grosse-Hovest, L.; Salih, H.R. Fc-optimized NKG2D-Fc constructs induce NK cell antibody-dependent cellular cytotoxicity against breast cancer cells independently of HER2/neu expression status. *J. Immunol.* **2014**, *193*, 4261–4272. [\[CrossRef\]](#)
99. Peipp, M.; Derer, S.; Lohse, S.; Staudinger, M.; Klausz, K.; Valerius, T.; Gramatzki, M.; Kellner, C. HER2-specific immunoligands engaging NKp30 or NKp80 trigger NK-cell-mediated lysis of tumor cells and enhance antibody-dependent cell-mediated cytotoxicity. *Oncotarget* **2015**, *6*, 32075–32088. [\[CrossRef\]](#)
100. Xie, W.; Liu, F.; Wang, Y.; Ren, X.; Wang, T.; Chen, Z.; Tang, M.; Sun, F.; Li, Z.; Wang, M.; et al. VEGFR2 targeted antibody fused with MICA stimulates NKG2D mediated immunosurveillance and exhibits potent anti-tumor activity against breast cancer. *Oncotarget* **2016**, *7*, 16445–16461. [\[CrossRef\]](#)
101. Kim, P.S.; Kwilas, A.R.; Xu, W.; Alter, S.; Jeng, E.K.; Wong, H.C.; Schlom, J.; Hodge, J.W. IL-15 superagonist/IL-15R α Sushi-Fc fusion complex (IL-15SA/IL-15R α Su-Fc; ALT-803) markedly enhances specific subpopulations of NK and memory CD8+ T cells, and mediates potent anti-tumor activity against murine breast and colon carcinomas. *Oncotarget* **2016**, *7*, 16130–16145. [\[CrossRef\]](#) [\[PubMed\]](#)
102. Chen, Q.; He, L.; Li, X.; Xu, L.; Chen, T. Ruthenium complexes boost NK cell immunotherapy via sensitizing triple-negative breast cancer and shaping immuno-microenvironment. *Biomaterials* **2022**, *281*, 121371. [\[CrossRef\]](#) [\[PubMed\]](#)
103. Kaidun, P.; Holzmayer, S.J.; Greiner, S.M.; Seller, A.; Tegeler, C.M.; Hagelstein, I.; Mauermann, J.; Engler, T.; Koch, A.; Hartkopf, A.D.; et al. Targeting NKG2DL with Bispecific NKG2D-CD16 and NKG2D-CD3 Fusion Proteins on Triple-Negative Breast Cancer. *Int. J. Mol. Sci.* **2023**, *24*, 13156. [\[CrossRef\]](#) [\[PubMed\]](#)
104. Herault, A.; Mak, J.; de la Cruz-Chuh, J.; Dillon, M.A.; Ellerman, D.; Go, M.; Cosino, E.; Clark, R.; Carson, E.; Yeung, S.; et al. NKG2D-bispecific enhances NK and CD8+ T cell antitumor immunity. *Cancer Immunol. Immunother. CII* **2024**, *73*, 209. [\[CrossRef\]](#)

105. Wang, C.; Wu, L.; Li, X.; Mao, S.; Wang, Z.; Xie, H.; Xu, J.; Li, D.; Yang, H.; Wang, T.; et al. Frizzled-7-targeting antibody-derived bifunctional protein retargets NK cells against triple-negative breast cancer cells via MICA-NKG2D axis. *J. Immunother. Cancer* **2025**, *13*, e009621. [[CrossRef](#)]
106. Han, Y.; Xie, W.; Song, D.-G.; Powell, D.J. Control of triple-negative breast cancer using ex vivo self-enriched, costimulated NKG2D CAR T cells. *J. Hematol. Oncol.* **2018**, *11*, 92. [[CrossRef](#)]
107. Sommaggio, R.; Cappuzzello, E.; Dalla Pietà, A.; Tosi, A.; Palmerini, P.; Carpanese, D.; Nicolè, L.; Rosato, A. Adoptive cell therapy of triple negative breast cancer with redirected cytokine-induced killer cells. *Oncoimmunology* **2020**, *9*, 1777046. [[CrossRef](#)]
108. Alekseeva, N.A.; Boyko, A.A.; Shevchenko, M.A.; Grechikhina, M.V.; Streltsova, M.A.; Alekseeva, L.G.; Sapozhnikov, A.M.; Deyev, S.M.; Kovalenko, E.I. Three-Dimensional Model Analysis Revealed Differential Cytotoxic Effects of the NK-92 Cell Line and Primary NK Cells on Breast and Ovarian Carcinoma Cell Lines Mediated by Variations in Receptor-Ligand Interactions and Soluble Factor Profiles. *Biomedicines* **2024**, *12*, 2398. [[CrossRef](#)]
109. Hong, S.D.; Kang, M.S.; Katuwal, N.B.; Ghosh, M.; Park, N.; Cho, Y.B.; Pandey, K.; Kown, A.-Y.; Ahn, H.J.; Kim, S.-G.; et al. Intratumoral Injection of Allogeneic NK Cell With Chemotherapy in a Triple-negative Breast Cancer Preclinical Model. *Anticancer Res.* **2025**, *45*, 2669–2688. [[CrossRef](#)]
110. Saliu, M.A.; Wang, Q.; Salisu, M.D.; Ren, Y.; Zhang, P.; Suleiman, R.B.; Cao, B.; Xu, Y.; Liu, X.; Lluís, F.; et al. Mesothelin-targeted CAR-T cells secreting NKG2D-BiTEs exhibit potent efficacy against triple-negative breast cancer. *Exp. Hematol. Oncol.* **2025**, *14*, 27. [[CrossRef](#)]
111. Zamat, A.; Kim, C.; Sridhar, S.; Fabrega, S.; Sen, R.; Campbell, N.; Oliver, S.A.; Zha, Z.; Thiveaud, C.; Kulaksizoglu, E.; et al. Closed-loop sonothermogenetic control of CAR T cells for metronomic brain cancer therapy. *bioRxiv* **2025**. [[CrossRef](#)] [[PubMed](#)]
112. Routes, J.M.; Ryan, S.; Morris, K.; Takaki, R.; Cerwenka, A.; Lanier, L.L. Adenovirus serotype 5 E1A sensitizes tumor cells to NKG2D-dependent NK cell lysis and tumor rejection. *J. Exp. Med.* **2005**, *202*, 1477–1482. [[CrossRef](#)] [[PubMed](#)]
113. Mahmoodi, S.; Nezafat, N.; Barzegar, A.; Negahdaripour, M.; Nikanfar, A.R.; Zarghami, N.; Ghasemi, Y. Harnessing Bioinformatics for Designing a Novel Multiepitope Peptide Vaccine Against Breast Cancer. *Curr. Pharm. Biotechnol.* **2016**, *17*, 1100–1114. [[CrossRef](#)] [[PubMed](#)]
114. Pan, J.; Shen, J.; Si, W.; Du, C.; Chen, D.; Xu, L.; Yao, M.; Fu, P.; Fan, W. Resveratrol promotes MICA/B expression and natural killer cell lysis of breast cancer cells by suppressing c-Myc/miR-17 pathway. *Oncotarget* **2017**, *8*, 65743–65758. [[CrossRef](#)]
115. Ding, B.; Li, J.; Yan, J.-L.; Jiang, C.-Y.; Qian, L.-B.; Pan, J. Resveratrol contributes to NK cell-mediated breast cancer cytotoxicity by upregulating ULBP2 through miR-17-5p downmodulation and activation of MINK1/JNK/c-Jun signaling. *Front. Immunol.* **2025**, *16*, 1515605. [[CrossRef](#)]
116. Abdelhamid, A.M.; Zeinelabdeen, Y.; Manie, T.; Khallaf, E.; Assal, R.A.; Youness, R.A. miR-17-5p/STAT3/H19: A novel regulatory axis tuning ULBP2 expression in young breast cancer patients. *Pathol. Res. Pract.* **2024**, *263*, 155638. [[CrossRef](#)]
117. El-Deeb, N.M.; El-Adawi, H.I.; El-Wahab, A.E.A.; Haddad, A.M.; El Enshasy, H.A.; He, Y.-W.; Davis, K.R. Modulation of NKG2D, KIR2DL and Cytokine Production by Pleurotus ostreatus Glucan Enhances Natural Killer Cell Cytotoxicity Toward Cancer Cells. *Front. Cell Dev. Biol.* **2019**, *7*, 165. [[CrossRef](#)]
118. Awad, A.R.; Youness, R.A.; Ibrahim, M.; Motaal, A.A.; El-Askary, H.I.; Assal, R.A.; Gad, M.Z. An acetylated derivative of vitexin halts MDA-MB-231 cellular progression and improves its immunogenic profile through tuning miR-20a-MICA/B axis. *Nat. Prod. Res.* **2021**, *35*, 3126–3130. [[CrossRef](#)]
119. Yang, C.; Qian, C.; Zhang, T.; Zheng, W.; Zhang, S.; Wang, S.; Wang, A.; Zhao, Y.; Lu, Y. The killing effect of Tanshinol on breast cancer cells: Insight into the reversion of TGF- β 1-mediated suppression of NK cell functions. *Front. Biosci.* **2021**, *26*, 1106–1118. [[CrossRef](#)]
120. Askar, M.A.; El-Sayyad, G.S.; Guida, M.S.; Khalifa, E.; Shabana, E.S.; Abdelrahman, I.Y. Amygdalin-folic acid-nanoparticles inhibit the proliferation of breast cancer and enhance the effect of radiotherapy through the modulation of tumor-promoting factors/immunosuppressive modulators in vitro. *BMC Complement. Med. Ther.* **2023**, *23*, 162. [[CrossRef](#)]
121. Yang, C.; Qian, C.; Zheng, W.; Dong, G.; Zhang, S.; Wang, F.; Wei, Z.; Xu, Y.; Wang, A.; Zhao, Y.; et al. Ginsenoside Rh2 enhances immune surveillance of natural killer (NK) cells via inhibition of ERp5 in breast cancer. *Phytomed. Int. J. Phytother. Phytopharm.* **2024**, *123*, 155180. [[CrossRef](#)] [[PubMed](#)]
122. Thepmalee, C.; Jenkham, P.; Ramwarungkura, B.; Suwannasom, N.; Khoothiam, K.; Thephinlap, C.; Sawasdee, N.; Panya, A.; Yenchitsomanus, P.-T. Enhancing cancer immunotherapy using cordycepin and Cordyceps militaris extract to sensitize cancer cells and modulate immune responses. *Sci. Rep.* **2024**, *14*, 21907. [[CrossRef](#)] [[PubMed](#)]
123. Belkahla, S. Dichloroacetate enhances Chemo-sensitivity in wild-type P53 breast cancer cells by modulating ABCG2 and NKG2DL. *Sci. Rep.* **2025**, *15*, 29447. [[CrossRef](#)] [[PubMed](#)]
124. Dhar, S.; Chiplunkar, S.V. Lysis of aminobisphosphonate-sensitized MCF-7 breast tumor cells by V γ 9V δ 2 T cells. *Cancer Immun.* **2010**, *10*, 10.

125. Rahimian, L.; Kalantari Khandani, B.; Nemati, M.; Hoseini-Shahrestanak, S.; Aminizadeh, N.; Jafarzadeh, A. Reduced Expression of Natural Killer Cell-Related Activating Receptors by Peripheral Blood Mononuclear Cells from Patients with Breast Cancer and Their Improvement by Zoledronic Acid. *Asian Pac. J. Cancer Prev. APJCP* **2022**, *23*, 1661–1669. [CrossRef]
126. Okita, R.; Mougiakakos, D.; Ando, T.; Mao, Y.; Sarhan, D.; Wennerberg, E.; Seliger, B.; Lundqvist, A.; Mimura, K.; Kiessling, R. HER2/HER3 signaling regulates NK cell-mediated cytotoxicity via MHC class I chain-related molecule A and B expression in human breast cancer cell lines. *J. Immunol.* **2012**, *188*, 2136–2145. [CrossRef]
127. Stephan, S.B.; Taber, A.M.; Jileeva, I.; Pegues, E.P.; Sentman, C.L.; Stephan, M.T. Biopolymer implants enhance the efficacy of adoptive T-cell therapy. *Nat. Biotechnol.* **2015**, *33*, 97–101. [CrossRef]
128. Liu, C.; Lai, H.; Chen, T. Boosting Natural Killer Cell-Based Cancer Immunotherapy with Selenocystine/Transforming Growth Factor-Beta Inhibitor-Encapsulated Nanoemulsion. *ACS Nano* **2020**, *14*, 11067–11082. [CrossRef]
129. Yang, Y.; Jiang, Y.; Xie, B.; Shi, S.; Pi, F.; Chen, M.; Sang, C.; Xu, L.; Chen, T. Selenadiazole derivative-loaded metal azolate frameworks facilitate NK cell immunotherapy by sensitizing tumor cells and shaping immuno-suppressive microenvironments. *Biomater. Sci.* **2023**, *11*, 1517–1529. [CrossRef]
130. Mathieu, C.; Ghosh, S.; Draussin, J.; Gasser, A.; Jacquot, G.; Banerjee, M.; Gupta, T.; Schmutz, M.; Mirjolet, C.; Tillement, O.; et al. Supramolecular Heterodimer Peptides Assembly for Nanoparticles Functionalization. *Adv. Healthc. Mater.* **2024**, *13*, e2304250. [CrossRef]
131. Pal, A.; Schneider, J.; Schlüter, K.; Steindorf, K.; Wiskemann, J.; Rosenberger, F.; Zimmer, P. Different endurance exercises modulate NK cell cytotoxic and inhibiting receptors. *Eur. J. Appl. Physiol.* **2021**, *121*, 3379–3387. [CrossRef] [PubMed]
132. Khan, A.U.H.; Blimkie, M.; Yang, D.S.; Serran, M.; Pack, T.; Wu, J.; Kang, J.-Y.; Laakso, H.; Lee, S.-H.; Le, Y. Effects of Chronic Low-Dose Internal Radiation on Immune-Stimulatory Responses in Mice. *Int. J. Mol. Sci.* **2021**, *22*, 7303. [CrossRef] [PubMed]
133. Eagle, R.A.; Jafferji, I.; Barrow, A.D. Beyond Stressed Self: Evidence for NKG2D Ligand Expression on Healthy Cells. *Curr. Immunol. Rev.* **2009**, *5*, 22–34. [CrossRef] [PubMed]
134. McGilvray, R.W.; Eagle, R.A.; Watson, N.F.S.; Al-Attar, A.; Ball, G.; Jafferji, I.; Trowsdale, J.; Durrant, L.G. NKG2D ligand expression in human colorectal cancer reveals associations with prognosis and evidence for immunoediting. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* **2009**, *15*, 6993–7002. [CrossRef]
135. McGilvray, R.W.; Eagle, R.A.; Rolland, P.; Jafferji, I.; Trowsdale, J.; Durrant, L.G. ULBP2 and RAET1E NKG2D ligands are independent predictors of poor prognosis in ovarian cancer patients. *Int. J. Cancer* **2010**, *127*, 1412–1420. [CrossRef]
136. Home | ClinicalTrials.gov. Available online: <https://clinicaltrials.gov/> (accessed on 30 December 2025).
137. BioArt. Available online: <https://bioart.niaid.nih.gov/> (accessed on 5 February 2026).
138. Wei, H.; Xiang, L.; Zhou, Y.; Huang, Y.; Liu, L.; Cao, D. Targeting NKG2D/NKG2DL axis in cancer immunotherapy: Mechanisms and therapeutic applications. *J. Transl. Med.* **2026**, *24*, 126. [CrossRef]
139. Chmielewski, M.; Abken, H. TRUCKs: The fourth generation of CARs. *Expert Opin. Biol. Ther.* **2015**, *15*, 1145–1154. [CrossRef]
140. Martínez, A.P. A Phase I Trial of Memory T Cells Expressing an NKG2D Chimeric Antigen Receptor in Children, Adolescents and Young Adults with Advanced Sarcoma. Report No.: NCT06087341. 2024. Available online: <https://clinicaltrials.gov/study/NCT06087341> (accessed on 5 February 2026).
141. Mahadeo, K.M.; Khazal, S.J.; Abdel-Azim, H.; Fitzgerald, J.C.; Taraseviciute, A.; Bollard, C.M.; Tewari, P.; Duncan, C.; Traube, C.; McCall, D.; et al. Management guidelines for paediatric patients receiving chimeric antigen receptor T cell therapy. *Nat. Rev. Clin. Oncol.* **2019**, *16*, 45–63. [CrossRef]
142. Leung, W.-H.; Vong, Q.P.; Lin, W.; Janke, L.; Chen, T.; Leung, W. Modulation of NKG2D ligand expression and metastasis in tumors by spironolactone via RXR γ activation. *J. Exp. Med.* **2013**, *210*, 2675–2692. [CrossRef]
143. Expression of MICA in Cancer—Summary—The Human Protein Atlas. Available online: <https://www.proteinatlas.org/ENSG00000204520-MICA/cancer> (accessed on 14 January 2026).
144. Expression of MICB in Cancer—Summary—The Human Protein Atlas. Available online: <https://www.proteinatlas.org/ENSG00000204516-MICB/cancer> (accessed on 20 January 2026).
145. Expression of ULBP1 in Cancer—Summary—The Human Protein Atlas. Available online: <https://www.proteinatlas.org/ENSG00000111981-ULBP1/cancer> (accessed on 20 January 2026).
146. Expression of ULBP2 in Cancer—Summary—The Human Protein Atlas. Available online: <https://www.proteinatlas.org/ENSG00000131015-ULBP2/cancer> (accessed on 20 January 2026).
147. Expression of ULBP3 in Cancer—Summary—The Human Protein Atlas. Available online: <https://www.proteinatlas.org/ENSG00000131019-ULBP3/cancer> (accessed on 20 January 2026).
148. Expression of RAET1E in Cancer—Summary—The Human Protein Atlas. Available online: <https://www.proteinatlas.org/ENSG00000164520-RAET1E/cancer> (accessed on 20 January 2026).

149. Expression of RAET1G in Cancer—Summary—The Human Protein Atlas. Available online: <https://www.proteinatlas.org/ENSG00000203722-RAET1G/cancer> (accessed on 20 January 2026).
150. Expression of RAET1L in Cancer—Summary—The Human Protein Atlas. Available online: <https://www.proteinatlas.org/ENSG00000155918-RAET1L/cancer> (accessed on 20 January 2026).

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