Heliyon 10 (2024) e39545

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Dynamic changes in serum adenosine and the adenosine metabolism-based signature for prognosis in HER2-positive metastatic breast cancer patients

Lijun Wang, Yizhi Ge, Li Yin, Dan Zong, Yang Li, Jianfeng Wu, Xia He *

Department of Radiation Oncology, Jiangsu Cancer Hospital & Jiangsu Institute of Cancer Research & The Affiliated Cancer Hospital of Nanjing Medical University, Nanjing, Jiangsu, 210009, China

ARTICLE INFO

Keywords:
Adenosine metabolism
Metastatic breast cancer
HER2-Positive breast cancer
Trastuzumab
Prognostic biomarkers

ABSTRACT

Aims: Adenosine metabolism in the breast cancer microenvironment is critical for tumor immunity. However, the prognostic significance of adenosine in breast cancer remains unclear. We aimed to dynamically monitor serum adenosine levels in patients with HER2-positive metastatic breast cancer (MBC) patients and to explore its predictive significance in trastuzumab therapy.
Methods: The sequencing and clinical data were downloaded from TCGA and GSE176078. Adenosine-related differentially expressed genes was analyzed by "DESeq2" package. Multivariate Cox and lasso-penalized Cox regressions were used to construct prognostic risk signatures. The risk scores were calculated from the identified expression of the hub genes. Bioinformatic analyses were performed using R with related packages. We also enrolled the metastatic breast cancer patients with HER2-positive from in our center and classified them into different groups according to the clinical outcomes assessed by enhanced CT. The adenosine levels were dynamically detected, and the difference in immune microenvironment between the subgroups was assessed by the immune cells that were recorded in our center.
Results: A total of 109 breast cancer patients with HER2-positive MBC were enrolled, and the expressions of 22 adenosine-related genes were filtered and matched from the TCGA database. The survival model based on the 15 differentially expressed genes was established, and the risk scores of each patient were the prognostic risk factors. Single-cell transcriptome sequencing data identified transcriptomic differences in patients with HER2-positive breast cancer. We also confirmed the predictive value of serum adenosine in the clinical progression of HER2-positive MBC patients. The different immune microenvironment between the subgroups supported the reliability of the predictive ability of adenosine in HER2-positive MBC patients.
Conclusions: The dynamic change of adenosine is a predictive biomarker for monitoring disease progression. The adenosine metabolism-based signature has the potential application in the prognosis of HER2-positive MBC patients.

1. Introduction

According to the latest cancer statistics, there were more than two million cases of breast cancer worldwide in 2018, accounting for

* Corresponding author.
E-mail address: hexiabm@163.com (X. He).

https://doi.org/10.1016/j.heliyon.2024.e39545
Received 18 February 2024; Received in revised form 26 September 2024; Accepted 16 October 2024
Available online 24 October 2024

one-tenth of all cancers. It was also the second leading cause of cancer death [1]. There are four main molecular subtypes of breast cancer, among which the proportion of human epidermal growth factor receptor 2 (HER2) positive subtype accounts for about 25%–30 % of all breast cancers [2]. Generally, HER2-positive tumors are highly invasive, prone to recurrence and progression, and resistant to drug treatments, resulting in poor clinical prognosis [3]. Trastuzumab is a humanized monoclonal antibody targeting HER2, which can significantly reduce the risk of recurrence and death [4]. A large number of studies have confirmed that trastuzumab has significant clinical benefits for patients with metastatic breast cancer (MBC) as a single agent or in combination with chemotherapy [5]. In the treatment of MBC patients, the effective rate of trastuzumab reached 35 %, and the combined usage of trastuzumab with chemotherapy drugs could increase the response rate to 57.3 %. In addition, the median survival time was increased by more than five months [6].

Despite the abundance of treatment options available, the clinical benefit of HER2-targeted combination chemotherapy remains variable due to some patients not responding to the treatment [7]. Due to the biological heterogeneity of the tumors caused by gene mutations and tumor microenvironment, HER2 expression and amplification may also have intra-tumor heterogeneity, causing patients to have different sensitivity to HER2-targeted drugs [8]. A recent study showed that breast cancer patients with tumor heterogeneity were not sensitive to HER-2 targeted therapy and did not achieve clinical remission [9]. This has prompted research into the mechanisms of trastuzumab resistance and the identification of effective biomarkers to guide individualized therapy [10]. Although the mechanism of the immune system in HER2-positive breast cancer remains unclear, the conception that the immune mechanism is a key factor in regulating the action of HER2-targeted drugs is recognized [11]. Complex interactions between the immune system and tumor cells trigger the adaptive tumor-specific immune responses and immune escape. Tumor cells must exchange various substances and signal transduction with the microenvironment to recruit various immune cells to help in immune escape and surveillance [12], promoting tumor development and response to treatment. ATP metabolism plays a critical role in this process, as the breakdown of extracellular ATP leads to adenosine accumulation, which promotes an immunosuppressive environment. By modulating the activity of immune cells, adenosine fosters immune escape mechanisms that are essential for tumor growth and resistance to therapies.

To validate adenosine as a predictive biomarker, future studies should involve larger, multi-center trials that encompass a more diverse population of HER2-positive MBC patients. This will help assess the generalizability of the findings and refine the use of adenosine monitoring in clinical settings. In parallel with biomarker validation, there is an emerging interest in developing adenosine receptor inhibitors as part of cancer therapy. Investigating adenosine receptor antagonists in HER2-positive breast cancer, especially in combination with immunotherapy or HER2-targeted therapy, could offer new treatment strategies. Given the broad role of adenosine in tumor microenvironments, exploring its predictive value and therapeutic potential in other cancer types, such as lung and pancreatic cancers, could expand the clinical application of this research. Combining adenosine with other established or emerging biomarkers (e.g., PD-L1, tumor mutational burden) may enhance the precision of patient stratification and treatment decisions. Future research could explore multi-biomarker panels for better clinical outcomes.

Previous studies have shown that ATP metabolism is abnormal in tumor cells, and CD39 and CD73 break down ATP into the end product adenosine. Local accumulation of adenosine in the microenvironment inhibits dendritic cells and effective T cells and enhances the polarization of tumor-associated macrophages toward M2 phenotypes [13]. In addition, adenosine can also effectively regulate the progression of breast cancer and cell growth [14,15]. Therefore, adenosine metabolism in the breast cancer microenvironment is critical for tumor immunity. However, the prognostic significance of adenosine in breast cancer remains unclear, and data from large cohort studies are needed to fully understand its clinical relevance. In conclusion, adenosine has potential value in tumor targeting and clinical translation. This study aimed to dynamically monitor serum adenosine levels in HER2-positive MBC patients and to explore its predictive value in trastuzumab therapy. This study is of great significance for the early identification of the therapeutic benefit groups, further promotion of MBC targeting and immune synergistic therapy, and provides new ideas for MBC targeted therapy.

2. Material and methods

2.1. Research workflow

This study aimed to explore the prognostic value of adenosine metabolism in HER2-positive metastatic breast cancer (MBC) patients and its potential as a biomarker for predicting disease progression during trastuzumab therapy.

2.2. Data collection and patient enrollment

We collected gene expression data from public databases (TCGA and GEO) and prospectively enrolled 109 HER2-positive MBC patients from our center. Serum samples were taken at multiple time points to monitor adenosine levels during treatment.

2.3. Adenosine gene signature construction

Differential expression analysis identified key adenosine-related genes associated with HER2-positive breast cancer. Using lasso-penalized Cox regression, we developed a gene signature to calculate risk scores for each patient. These risk scores helped classify patients into high- and low-risk groups for survival prediction.

2.4. Survival and mutation analysis

We evaluated patient survival using Kaplan-Meier curves and Cox regression, revealing a strong association between higher adenosine-related risk scores and shorter survival. Mutation analysis from TCGA identified frequently mutated genes in HER2-positive MBC, highlighting potential interactions between these mutations and adenosine signaling.

2.5. Immune microenvironment and single-cell sequencing

We explored the tumor immune microenvironment using several computational tools, revealing distinct immune cell profiles between high- and low-risk groups. Single-cell RNA sequencing data provided insights into the cellular interactions and metabolic pathways influenced by adenosine in HER2-positive tumors.

2.6. Clinical validation

Serum adenosine levels were monitored throughout trastuzumab therapy. Patients with progressive disease (PD) had higher adenosine levels compared to those with stable disease (SD), confirming the potential of adenosine as a biomarker for tracking treatment response and disease progression.

2.7. Patients' source and adenosine-related classification

Breast cancer patients with HER positive status and survival of at least 30 days were downloaded from The Cancer Genome Atlas Program (TCGA). The adenosine-related genes were archived from a previous study [16]. The analysis of differentially expressed genes was performed using the "DESeq2" package. Multivariate Cox and lasso-penalized Cox regressions were used to construct the optimal prognostic risk signatures. The risk scores were calculated from the identified expression of the hub genes.

Female patients with Her2-positive breast cancer from our hospital were enrolled in this study. All patients received simultaneous treatment with trastuzumab and docetaxel. Trastuzumab was administered at an initial dose of 8 mg/kg, followed by maintenance therapy every three weeks at a dose of 6 mg/kg until disease progression or intolerable side effects occur. Docetaxel was administered at a dose of 75 mg/m^2 every three weeks for eight cycles (at least six cycles were completed). The treatment period was one year.

2.8. Somatic mutation analysis

Mutant data were obtained and visualized using the "maftools" package. The "somaticInteractions" function was used to analyze co-occurring or exclusiveness between mutated genes using paired Fisher's precise tests.

2.9. Construction of prognostic model and survival analyses

Patients were classified into high- and low-risk groups using the median of risk score for each cohort. Kaplan-Meier survival and the Receiver Operating Characteristic (ROC) analyses were used to analyze the association between adenosine-related risk scores and overall survival (OS), and the accuracy of the model. The interaction of a BRCA1/BRCA2 mutation and Homologous Recombination Deficiency (HRD) with the adenosine-related signature was assessed by a hierarchical analysis and interaction *P* value using a Wald test. The Number of Telomeric Allelic Imbalances (NtAI) count, Large-scale State Transitions (LST) count, Homologous Recombination Deficiency (HRD) scores, and Loss of heterozygosity (HRD-LOH) for the TCGA samples were obtained from a previous pan-cancer report [17].

2.10. ssGESA enrichment and biological immune features analyses

The enrichment score within the patients with discrepant adenosine signature to explore the molecular mechanism was calculated using the ssGSVA method implemented in the "GSVA" package. In addition, the immune infiltration signature, the association between the adenosine risk group and immune microenvironment, was calculated using CIBERSORT, ESTIMATE, MCPcounter, TIMER, and TIDE algorithms and was compared using a Wilcoxon rank-sum test. The expression of immune checkpoint genes was also analyzed.

2.11. Single cell sequencing data of breast cancer patients with HER2-positive download and processing

The GSE176078 single-cell breast cancer was downloaded from the GEO public database (https://www.ncbi.nlm.nih.gov). Five HER2-positive breast cancer patients were included in the GSE176078. The Seurat object was constructed under the conditions of min. cells = 3 and min. features = 300, and the correlation between nFearure, count, and sequencing depth was calculated. The percentage of mitochondrial genes, ribosomal genes, and erythrocyte genes was calculated. Cells were filtered using nFeature_RNA >4000, mitochondrial gene percentage ≥15 %, count number ≥10,000, and erythrocyte gene percentage≥1 %. The following settings were used: Normality. method = "LogNormalize", scale. factor = 10,000 for the "NormalizeData" function. "FindVariableFeatures" calculated the first 3000 highly variable genes. The dimensionality of the data was reduced using the UMAP method, and the cells were clustered using the "KNN" (k-nearest neighbor) method with a resolution of 0.2. Subsequently, annotations were made using known

cell-specific markers [18].

2.12. Serum samples collection and adenosine content detection

During the treatment, the serum samples were collected every two treatment cycles. For 24 weeks after the end of treatment, serum samples were collected every 8 weeks. For 24–48 weeks after the end of treatment, serum samples are collected every 12 weeks or until disease progression occurs or the end of the study. A total of 16 patients met inclusion criteria from October 2021 to September 2022 in our hospital were enrolled. Lesions with at least one diameter line that can be accurately measured with CT evaluation according to RECIST 1.1. Specifically, the maximum diameter of the lesion was at least 10 mm (2 layers thick, 5–8 mm thick). The minimum sum of the target lesion diameters measured throughout the experimental study was used as a reference. The patients with total diameter of all measurable target lesions was $\geq$30 % below baseline were grouped into partial remission (PR). The diameters increase of at least 20 % and the presence of one or more new lesions were considered disease progression (PD). The degree of decrease or increase of target lesions was between PR and PD was regarded as stable disease (SD). The human Adenosine assay kit (**Abcam**) was used to detect serum adenosine level in each patient. Briefly, serum samples were collected as approximately 500 μL specimens preserved after hematological examination during the treatment or post-treatment follow-up. Add plasma to prepared reaction and background mix, and incubate them for 15 min at RT. Measure fluorescence immediately on a microplate reader at Ex/Em = 535/587 nm. Concentration of Adenosine in the test samples is calculated as manual. This research was approved by the Research Ethics Committee of Jiangsu Cancer Hospital. All patients have written informed consent for participation in this study. The thioredoxin reductase (TR) activity and circulating free DNA in the plasma (cfDNA) levels were detected by the TR test kit (No. 20110901) and QuantiDNA™ DNA Measurement Assay (No. DC-08-0092R), respectively. All samples to be tested were retrieved from the residual serum samples for clinical testing. The immune cell counts were analyzed by flow cytometry. Briefly, single-cell suspension was prepared, and the antibodies were added in an appropriate volume of flow cytometry staining solution and the cells. Gently pulse vortex mixing and incubate on ice for more than 30 min. The cells were washed with flow cytometry staining solution and centrifuged at room temperature 500 g for 5 min. Resuspend the cells in the staining solution and analyze the samples flow cytometry. Natural killer (NK) cells were marked by $CD3^{-}CD16^{+}CD56^{+}$, natural killer T (NKT) cells were marked by $CD3^{+}CD16^{+}CD56^{+}$, B cells were marked by $CD19^{+}$, active T cells were marked by $CD3^{+}$HLA-DR^{+}, and Regulatory T (Treg) cells were marked by $CD4^{+}CD25^{+}CD127^{low}$.

2.13. Statistical analysis

All statistical analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, USA) and GraphPad Prism version 9.0 (GraphPad Software, La Jolla, USA). Data are presented as mean ± standard error of the mean (SEM). A two-tailed P-value of <0.05 was considered statistically significant. Differentially expressed genes (DEGs) between groups were analyzed using the "DESeq2" package in R. The thresholds for significance were set at an absolute log2 (fold change) > 1 and a false discovery rate (FDR) < 0.05. DEGs were visualized through volcano plots and heatmaps generated using the "ggplot2" package. To assess the association between adenosine-related risk scores and overall survival (OS), we constructed a multivariate Cox proportional hazards regression model. Additionally, we used the lasso-penalized Cox regression method for variable selection and to prevent overfitting. The optimal tuning parameter (λ) for the lasso model was selected through 10-fold cross-validation, using the minimum partial likelihood deviance as the criterion. Hazard ratios (HR) with 95 % confidence intervals (CI) were calculated to estimate the strength of the association between risk scores and survival. Kaplan-Meier survival curves were plotted to compare survival between high- and low-risk groups, with differences assessed using the log-rank test. The prognostic performance of the risk model was evaluated using time-dependent Receiver Operating Characteristic (ROC) curve analysis, with the area under the curve (AUC) used to measure the model's accuracy. To investigate the association between adenosine-related gene expression and immune microenvironment features, we applied several immune infiltration algorithms, including CIBERSORT, ESTIMATE, MCPcounter, TIMER, and TIDE. The Wilcoxon rank-sum test was used to compare immune cell populations between high- and low-risk groups. We also performed gene set enrichment analysis (GSEA) to identify the top enriched pathways in different adenosine risk groups, using the "clusterProfiler" package in R. For mutation analysis, we used the "maftools" package to explore the mutation landscape in breast cancer patients. The "somaticInteractions" function was employed to evaluate co-occurring and mutually exclusive mutations between genes, with Fisher's exact test applied for statistical assessment. We also analyzed the correlation between risk scores and genomic instability markers, including the number of telomeric allelic imbalances (NtAI), large-scale state transitions (LST), and homologous recombination deficiency (HRD) scores, using hierarchical clustering and Wilcoxon rank-sum tests. The robustness of the prognostic model was validated using internal cross-validation and additional stratified analyses. Patients were stratified based on clinical parameters such as homologous recombination deficiency (HRD) scores. Interaction tests were performed using a Wald test to assess the combined effect of the adenosine signature and HRD status on survival outcomes.

3. Results

3.1. Patients screening and adenosine-related classification

Since adenosine signaling had immunosuppressive effects in the microenvironment [19], we first screened the significant differentially expressed genes (DEGs) that and intersected with adenosine genes. Fifteen DEGs related to adenosine signaling were identified (Table 1). A total of 109 HER2-positive breast cancer patients and the expression of 22 adenosine-related genes were filtered and

Table 1
The differentially expressed genes related to ABO between normal and tumor samples.

Gene	baseMean	log2FoldChange	lfcSE	Stat	pvalue	padj
ENTPD2	952.670130574462	0.414725399064061	0.124977016020519	3.31841335526821	0.000905304042015714	0.00177058153457449
AK1	288.289563557561	0.394314201489413	0.0777558122237255	5.07118619447842	3.95E-07	1.10E-06
ENTPD8	226.219280309336	2.67770947882277	0.186581674930798	14.3514065881117	1.04E-46	1.93E-45
NT5E	1307.52029930707	−1.24592216632621	0.104619338597197	−11.9091000099248	1.06E-32	1.17E-31
ALPL	1515.08729338468	−1.193784169436	0.147620372624849	−8.08685243241995	6.12E-16	3.10E-15
ENPP1	5667.69412874279	0.67650604620572	0.117994118747843	5.73338784496061	9.84E-09	3.14E-08
ADORA2B	197.190448147734	−0.392377127539948	0.127653756844643	−3.07376090793377	0.00211378763836155	0.00393094954382241
NME2P2	0.322454147418054	0.181401686592126	0.409300185878518	0.443199619376588	0.657621354179224	0.720433583955831
PNP	1617.8884809155	0.951771030797893	0.0804939437417282	11.8241321838042	2.93E-32	3.17E-31
NME1	1474.30684379113	1.94890981536656	0.104748486239671	18.6056131723693	2.89E-77	1.39E-75
AK2	7690.17309007792	0.214069319176001	0.0469360336935911	4.56087364717464	5.09E-06	1.28E-05
ADA	470.439502283794	0.415081435217094	0.0890814030131249	4.65957451473836	3.17E-06	8.14E-06
BST1	302.50314810715	−1.18727408022652	0.0809055572520613	−14.6748149391959	9.35E-49	1.87E-47
ADORA3	311.353093686292	0.537072504434687	0.0963621542513669	5.57347963634872	2.50E-08	7.70E-08
ADORA2A	12.1484333849252	1.09004401106691	0.163239150085794	6.67758935582558	2.43E-11	9.31E-11

matched from the TCGA database. Considering the close relationship between adenosine signaling and the immune microenvironment, we established a survival model based on the adenosine signaling DEGs using a lasso regression model (Fig. 1A–B). The formula used to calculate the adenosine-related risk signature in HER2-positive breast cancer patients was:

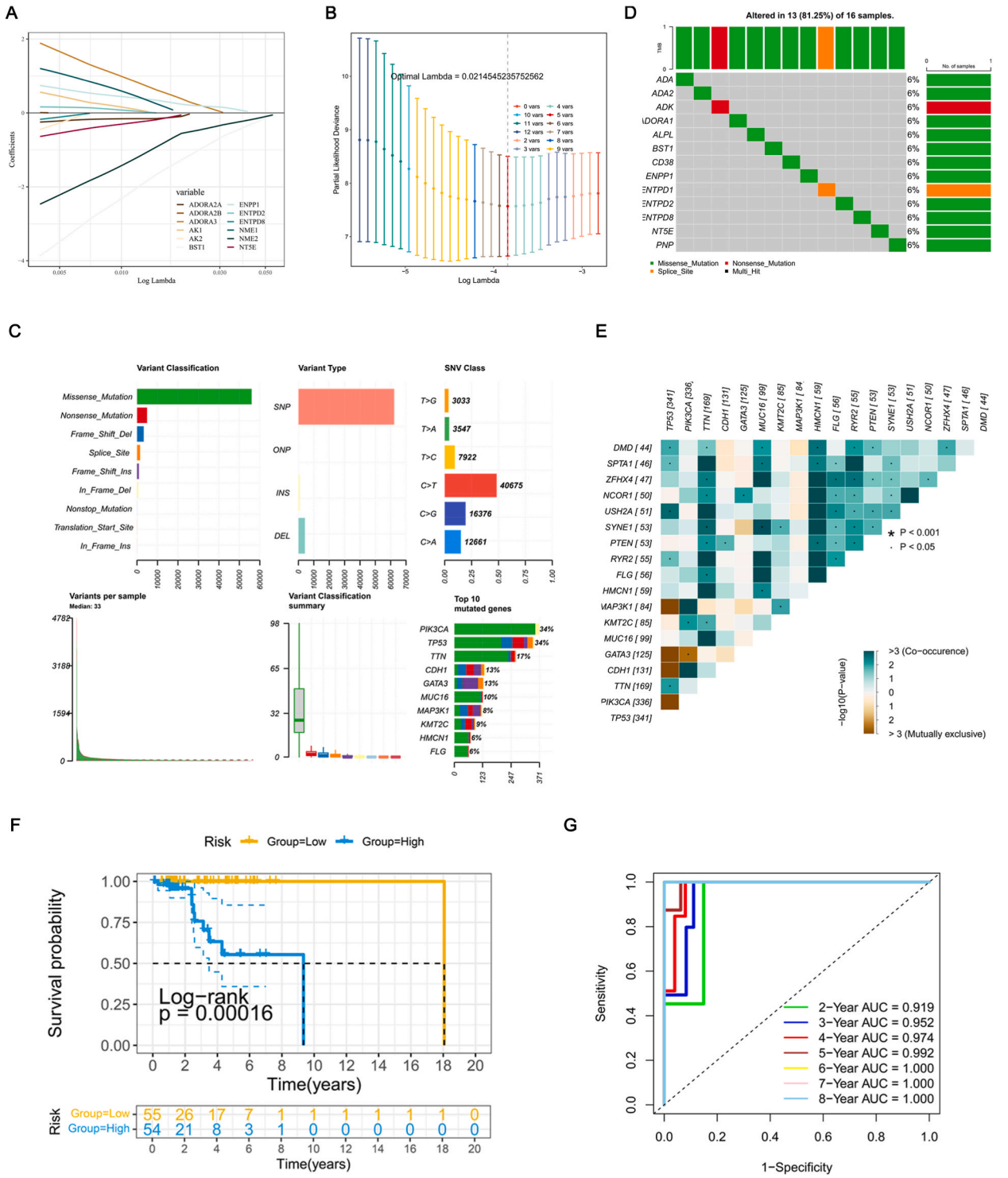


Fig. 1. A–B. Lasso regression model based on the DEGs of adenosine signaling. C. Overview of breast cancer cohort mutations. D. Waterfall of the top 30 mutated genes in the TCGA breast cancer cohort. E. Somatic Interactions analysis. F. Survival curve between high- and low-risk groups. G. ROC curve of the adenosine-based risk signature.

ENPP1*0.039+NME2*0.033+BST1*0.058-ADORA3*0.0744+ADORA2A*0.044. The risk scores of each patient were calculated from the expression of hub adenosine genes in HER2-positive breast cancer patients. All patients were assigned to the high- or low-risk groups based on the median score value.

3.2. Mutation landscape and adenosine-related survival analyses

The mutant database of breast cancer patients was obtained from the TCGA. For all the patients, *Missense_Mutation*, *SNP*, and $C > T$

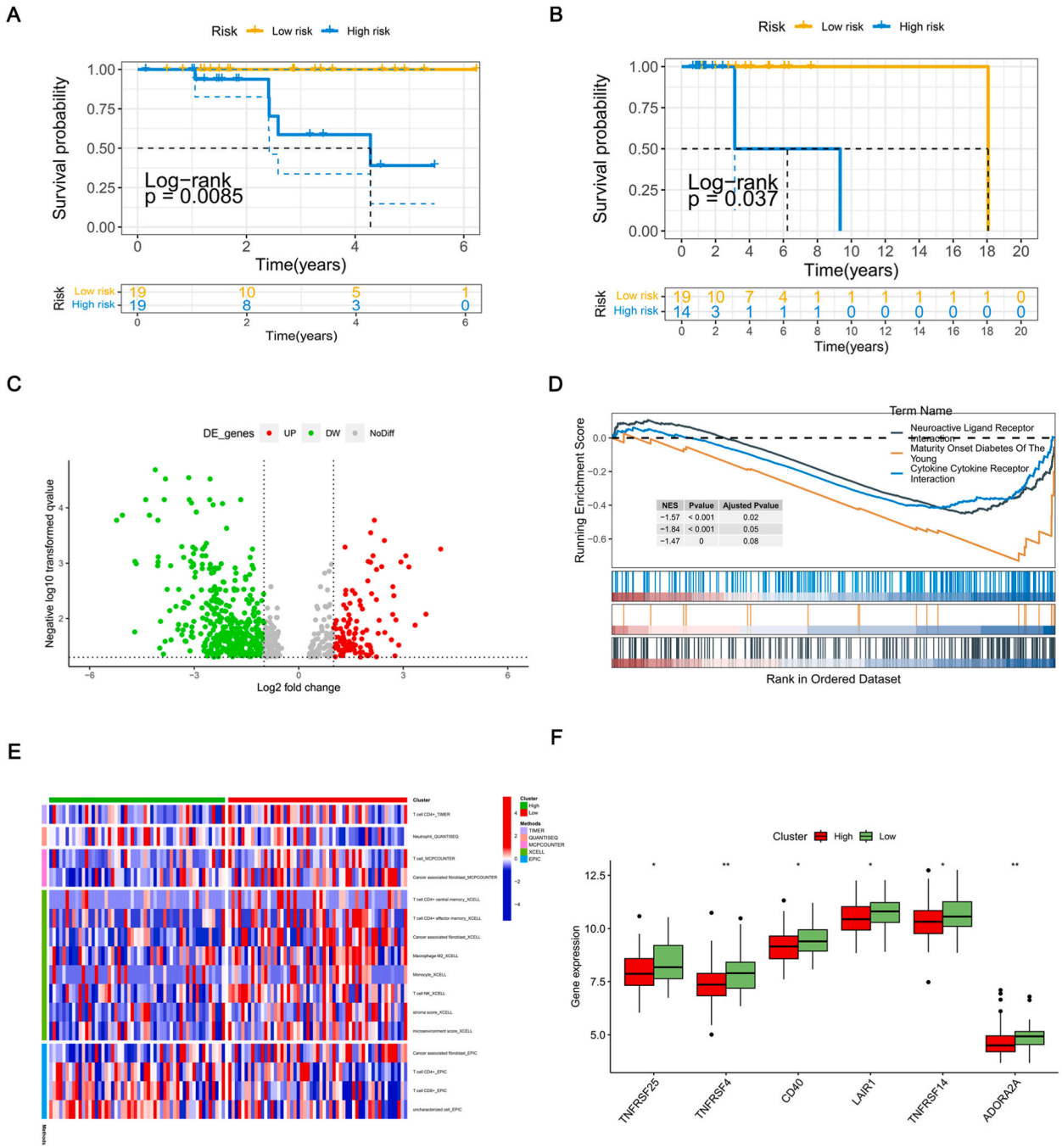


Fig. 2. A–B. OS survival curve between the adenosine groups in subgroups of patients with high or low HRD scores. C. Volcano plot of DEGs between adenosine groups. D. Enriched top terms in the adenosine group by ssGSEA. E. Heatmap of the infiltrated immune signatures score based on five algorithms. F. Box plot of the immune checkpoint genes expressions between the two adenosine-related risk groups.

mutation were the most common terms (Fig. 1C). *TP53*, *PIK3CA*, *TTN*, *CDH1*, *GATA3*, and *MUC16* had higher somatic mutations status in the waterfall diagram using the MutSigCV algorithm (Fig. 1D). Many disease-causing genes had the features of co-occurring or exclusiveness in their mutation patterns. The correlation analysis of mutated genes indicated there was strong exclusiveness between *TP53* and *PIK3CA*, which is consistent with previous reports (Fig. 1E).

Patients with high adenosine-based risk scores had shorter OS than those with low-risk scores (HR, 17; 95 % CI, 5–56, $P < 0.001$, Fig. 1F). The AUC value further indicated the excellent advantages of the signature both in short- and long-term survival forecasts (Fig. 1G). Limited to the number of patients, we further validated the reliability of the adenosine-related risk scores.

3.3. HRD score and biological immune features of the adenosine groups

Since HRD can be considered as a type of genomic instability score, we further validated the prognostic value of the adenosine signature based on the HRD score subgroup. There was no significant difference in the NtAI and LST counts and the HRD-LOH and HRD scores between the high-and low-risk groups (Supplementary Fig. 1A). Patients were divided into two subgroups based on the median HRD score. The patients with a high HRD score had the significant prognostic differences between high- or low-risk adenosine signature scores, as did those with a low HRD score (Fig. 2A–B). This indicated the strong survival benefits of the adenosine signature in HRD patients.

To further explore the variant genomic patterns between the adenosine-related risk groups, we performed a DEGs analysis and revealed the molecular mechanisms via gene set enrichment analysis enrichment analysis (GSEA). The top enriched terms were *Neuroactive Ligand Receptor Interaction*, *Maturity Onset Diabetes of The Young*, and *Cytokine Receptor Interaction* (Fig. 2C–D). In addition, five immune signature algorithms were applied to explore the correlation between the adenosine score and the immune microenvironment. The result showed that the immune features of the low adenosine group were enriched in carcinoma-associated fibroblasts, NK cells, and M2 macrophages (Fig. 2E). In contrast, the high-risk group had decreased expression of the checkpoint genes *TNFRSF2*, *TNFRSF4*, *TNFRSF14*, *CD40*, *LAIR1*, and *ADORA2A* compared with the low-risk group (Fig. 2F).

3.4. Single-cell transcriptomic characteristics of HER2-positive breast cancer patients

Single-cell transcriptomic data of five HER2-positive breast cancer patients were re-analyzed and clustered, and nine clusters were identified (Fig. 3A). The cell type ratios between the samples were determined, and found that T cells made up the majority percentage of the cells (Fig. 3B). The other clusters were *B cells, Cancer Epithelial*, *Endothelial*, *perivascular−like cells*, *Myeloid*, *Plasmablasts*, *and cancer associated fibroblasts (CAFs)*. Considering that metabolic reprogramming is an important hallmark of cancer cells and TME cells, we further presented the activated metabolic pathways in HER2-positive breast patients (Fig. 3C). The most well-known classic pathways were activated, such as oxidative-phosphorylation, glycolysis and gluconeogenesis, and glutathione metabolism (Fig. 3D and Supplementary Figs. 1B–C), which indicated significant mitochondrial activity. Benefiting from the advantages of scRNA-seq technology, we revealed the high-resolution interactions among the various subgroups (Fig. 3E). ANGPTL was validated as the dependent mediator of CAF pro-tumorigenic functions, and it showed the highest communication strength with the SDC and CDH families (Fig. 3F–G). Additionally, CAFs derived from the HER2-positive subtype were unique compared with other types of breast cancers. We further explored the cellular developmental trajectory using a pseudotime trajectory analysis. The CAFs cells were re-clustered and marked by MEST, CADM1, GRP, ARC, CFD, IDO1, and HIGD1B (Fig. 3H–I and Supplementary Figs. 1D–E). We also showed the evolutionary trajectory of the model genes we presented and found that NME2 had the most obvious magnitude of change (Fig. 3J–K).

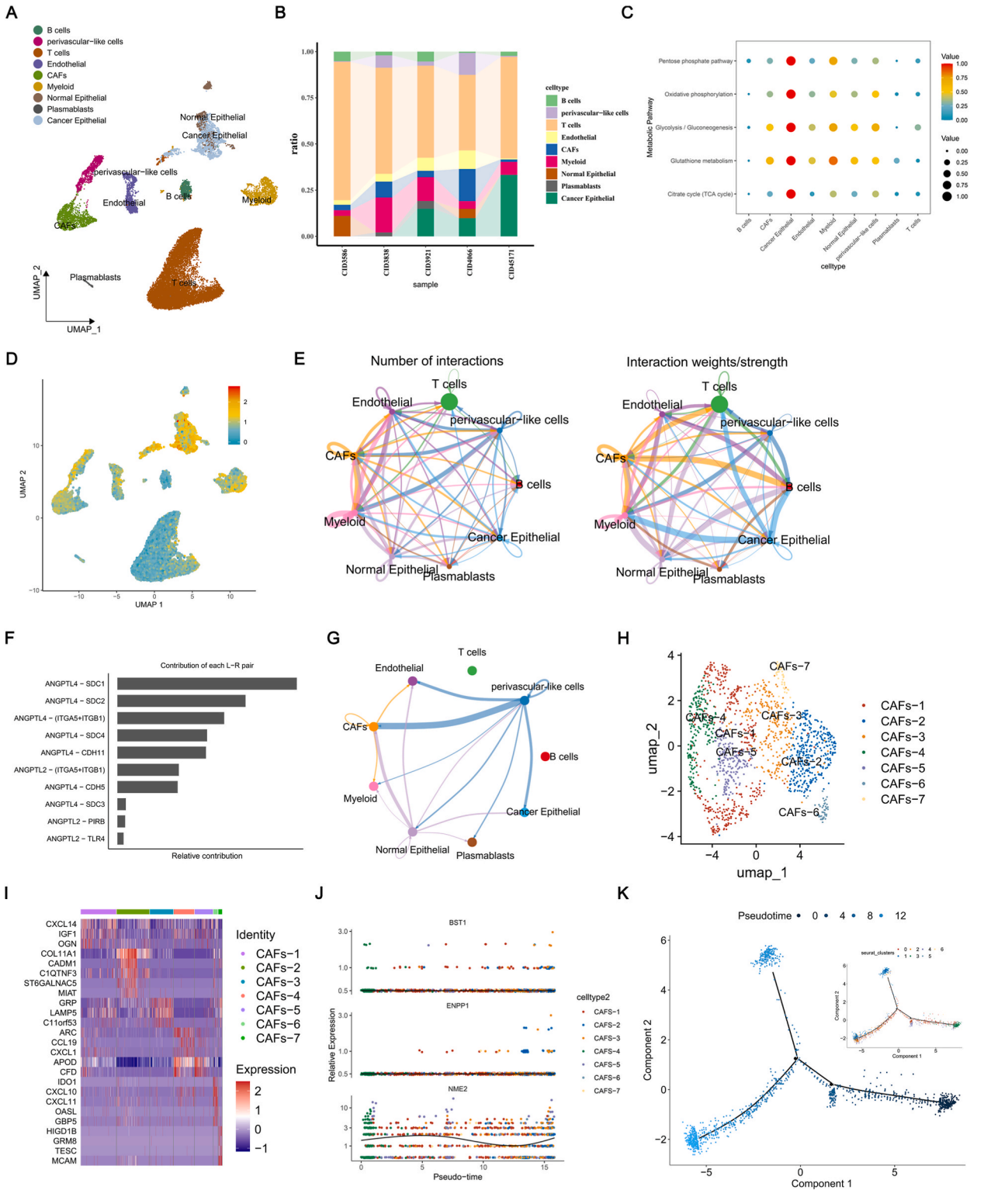
3.5. Predictive value of serum adenosine in clinical progression of HER2-positive MBC patients

Since proteins are the most direct response to the biological processes and are convenient to detect in clinical practice, we detected the adenosine levels, a regulated end product of the aforementioned signature genes. A total of fifteen HER2-positive breast cancer patients from our hospital were enrolled in this study. The left ventricular ejection fraction was above 50 % of all patients aged between 18 and 70. The medium follow-up was 22 (5–23) months, the medium age was 55 (34–75), and 73 % (11/15) were diagnosed with varying degrees of distant metastases at the initial diagnosis. Among the patients, the primary lesion site was the left breast in six cases, and right breast in eight cases, and one patient was diagnosed with bilateral breast cancers. At the latest examination, ten patients had a stable disease (SD) after reexamination by imaging, and five had a progressive disease (PD).

The serum adenosine levels were statistically different and were higher in the disease progression group (Fig. 4A). Moreover, the serum adenosine levels fluctuated with the clinical outcomes (Fig. 4B–D) and were stable in the patients who had an SD (Fig. 4E). In addition, TR and cfDNA were also included in the follow-up analyses. The significantly increased TR activity significantly suggested the hyperactive histiocytosis in vivo. CfDNA levels can be used to evaluate the curative effect, risk analysis of the tumor metastasis, and tumor recurrence monitoring. A decreased trend in the cfDNA level is generally indicative of a better response to treatments. Herein, we observed that the TR and cfDNA levels were higher in the PD group (Fig. 4F–G). These results indicated that the reduced adenosine level predicted an SD condition according to the CT examination and tumor markers.

3.6. Difference in the immune microenvironment and clinical outcomes between the PD and SD groups of HER2-positive MBC patients

To further explore the immune microenvironment between the PD and SD groups of HER2-positive MBC patient, we performed the immune cell counts at our hospital. We found that the $CD4^+$, NKT^+, and $CD4^+CD25^+$ cells were significantly increased (Fig. 5A–C),



(caption on next page)

Fig. 3. Single-cell transcriptomic characteristics resolved atlas of HER2-positive breast cancers A. UMAP visualization of the sequencing data of HER2-positive at the single cell level from five breast cancer patients. B. Percentage of each cell among the samples. C. Activated metabolic pathways in HER2-positive breast patients. D. UMAP visualization of the oxidative-phosphorylation pathway. E. Visualization of the interaction weights of HER2-positive breast cancer subpopulations. F-G. Ligand-receptor interactions analysis and circle aggregate visualization of ANGPTL. H. Heatmap visualization of the re-clustered CAFs. I. Heatmap plots of the marker genes for CAFs clusters in HER2-positive breast cancer patients. J-K. Cell states and the expression of genes that changed as a function of pseudotime for CAFs.

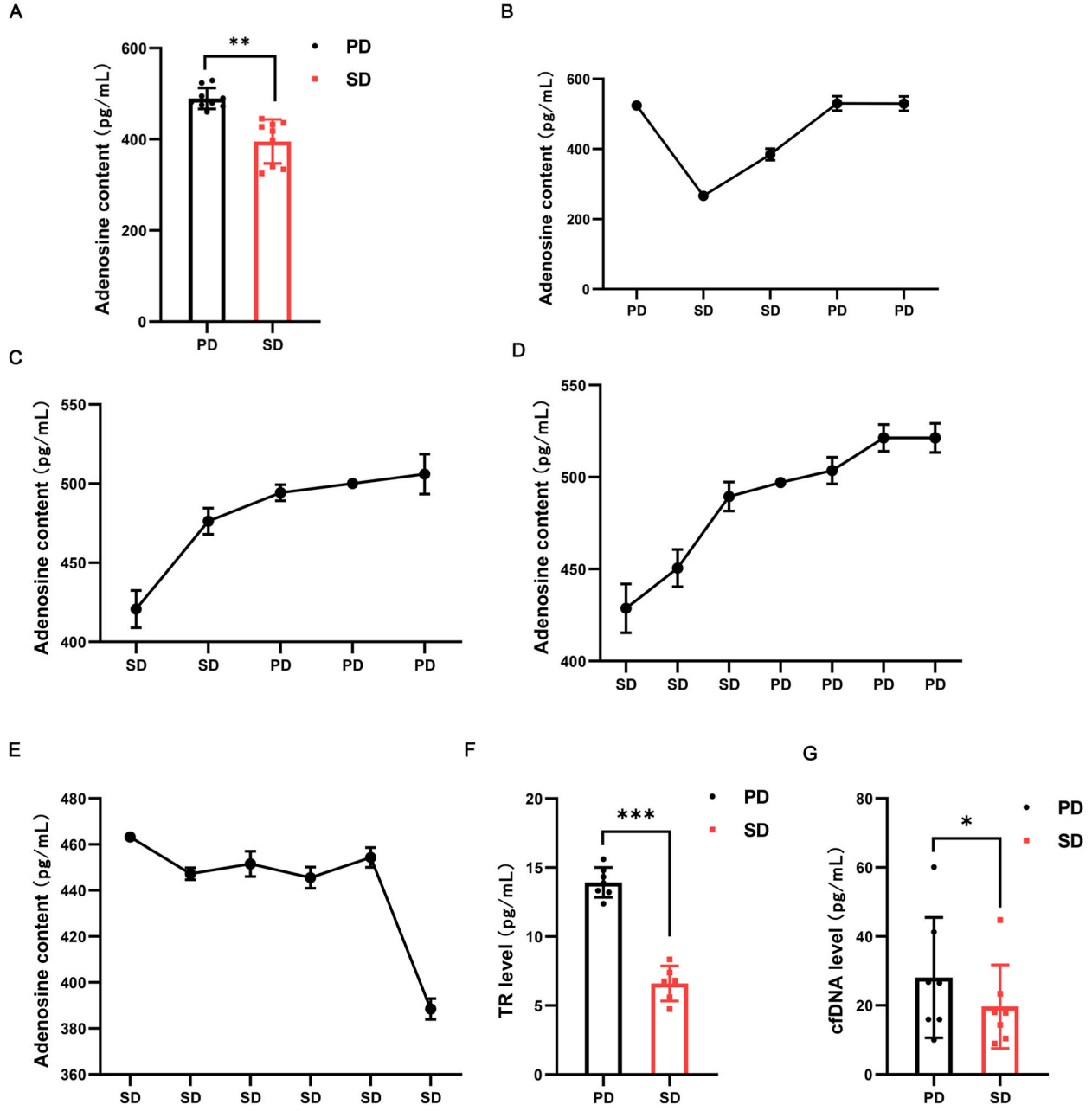


Fig. 4. Predictive value of serum adenosine in clinical progression of MBC patients with HER2-positive status A. The serum adenosine levels of 15 HER2-positive MBC patients. B. One patient was evaluated as stable and progressed after the initial PD status. C-D. The patients were evaluated as PD after the initial SD status. E. A representative HER2-positive MBC patients was evaluated as SD during the follow-up. The serum TR (F) and cfDNA (G) levels of 15 HER2-positive MBC patients. TR, thioredoxin reductase; cfDNA, circulating free DNA. MBC, metastatic breast cancer.

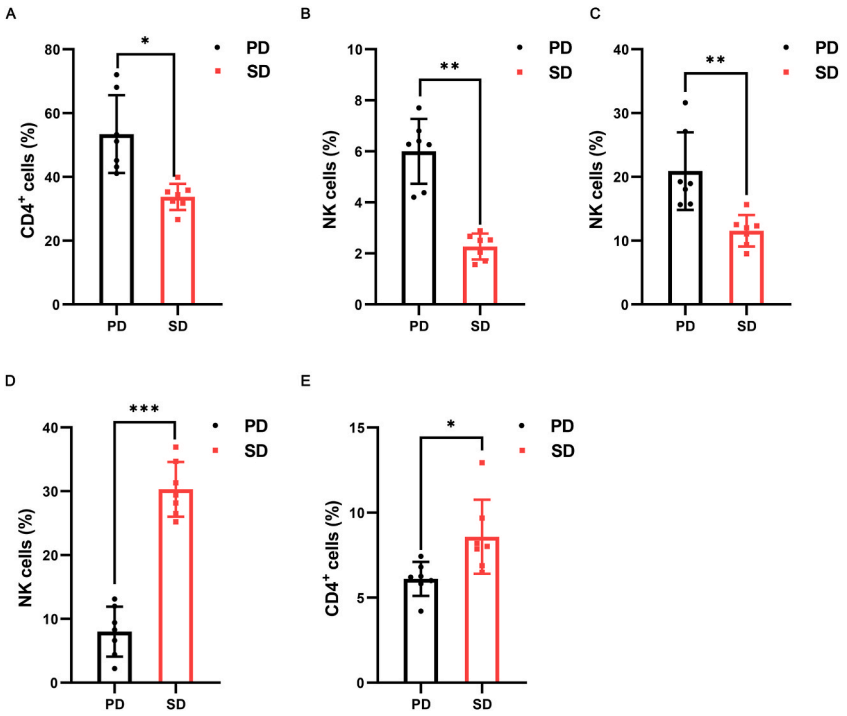


Fig. 5. Difference in immune microenvironment between PD and SD groups of HER2-positive MBC patients (A). $CD4^+$ cell, (B). NKT, (C). $CD4^+CD25^+$ cells, (D). NK, and (E). Treg cells proportions between the PD and SD groups of HER2-positive MBC patients.

while the NK^+ and Treg cells were decreased in the PD group (Fig. 5D–E). The $CD8^+$, B cell, and active T cells had no difference (Figs. S2A–C).

The imagological examinations were applied to assess the clinical outcomes and confirmed the predictive role of serum adenosine in HER2-positive MBC patients. We found that the patients with the elevated adenosine levels had new lesions and were assessed as having progressive disease (Fig. 6A–D). The other ten patients had stable adenosine levels, and their clinical outcomes were regarded as stable after evaluation (Fig. 6E).

4. Discussion

Trastuzumab is a monoclonal antibody that targets HER2-positive tumor cells through immune mechanisms, such as antibody-dependent cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) [20]. Clinical studies have found that the characteristics of immune infiltration are associated with the clinical benefit of trastuzumab [21,22]. Intensive research has demonstrated that the more tumor-infiltrating T lymphocytes (TILs), the greater the clinical benefit from HER2-targeted therapy and the higher the complete response rate. In addition, the type of lymphocytes and circulating tumor cells (CTCS) are also valuable for the prediction of efficacy and prognosis [23]. Some studies have screened the benefit indicators of targeted HER2 therapy through gene sequencing and other aspects. Therefore, the search for a screen simple, stable, and effective biomarkers has become an urgent issue in clinical personalized medicine. This study dynamically monitored the serum adenosine levels in HER2-positive MBC patients during trastuzumab treatment. We confirmed that the adenosine level may be a potential early indicator for predicting the therapeutic effect of trastuzumab and disease progression.

As with most solid tumors, breast cancers have higher energy metabolism and extracellular ATP release, and consequently, ATP, ADP, and AMP hydrolysis and extracellular adenosine accumulation [24]. In the cancer microenvironment, adenosine binds to its ligands and then triggers signal transductions, such as cAMP and NF-κB, thereby inhibiting the anti-tumor functions of immune cells [25]. Atezolizumab is the only FDA-approved immunotherapy agent for breast cancer, which is unfortunately limited to MBC [26]. Non-responsiveness to current immune checkpoint inhibitors can be attributed to post-treatment upregulation of other compensatory immune checkpoints [27]. Furthermore, HIF-1α, a biomarker of hypoxic tumor microenvironment in cancers, could serve as a potent enhancer of CD39 and CD73 ectonucleotides expressions, which in turn mediate extracellular adenosine accumulation and consequently the establishment of immunosuppression [28–30]. These conclusions sparked our interest in exploring the clinical predictive value of adenosine in HER2-positive MBC patients.

Considering the higher economic cost of multigene testing and the limited scope of application in clinical institutions or hospitals, it is reasonable to find alternative tests that are easier. In contrast, metabolic products could assess the metabolic status and associated disease risk by directly analyzing the concentration and changes of metabolites in an individual. It is relatively simpler and could

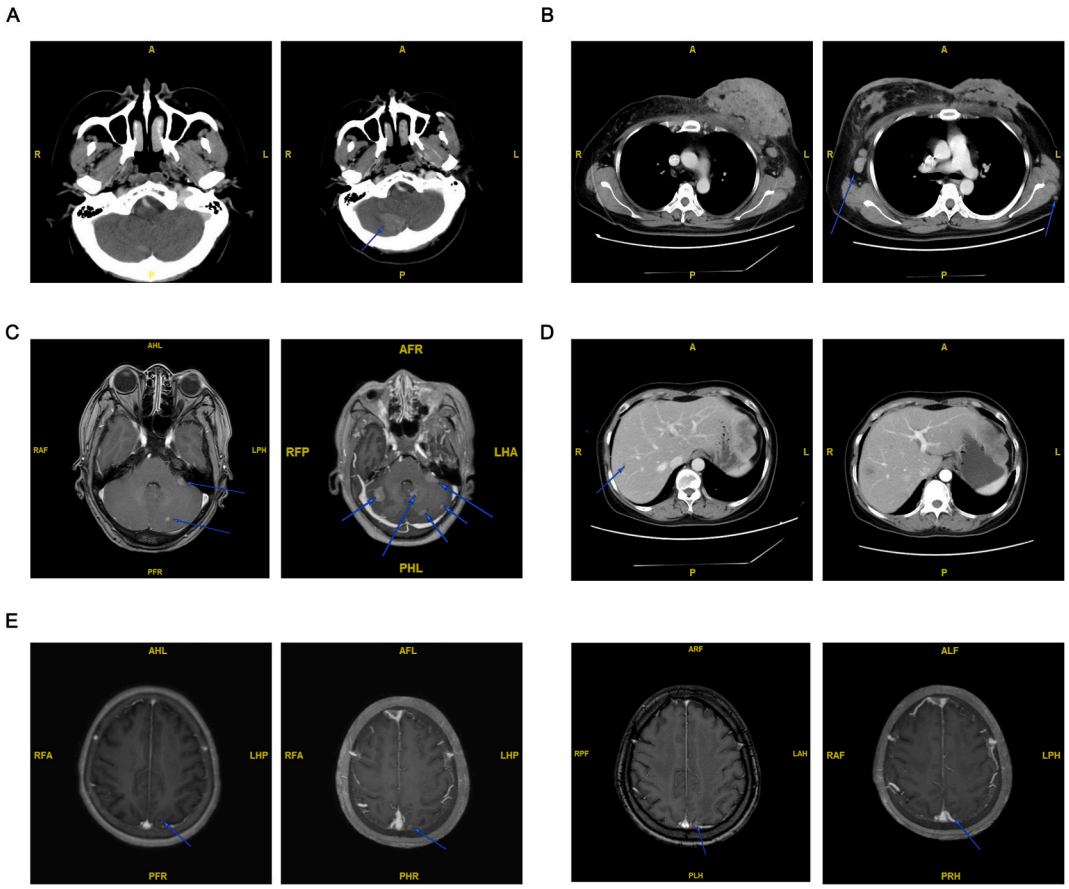


Fig. 6. Representative CT images of HER2-positive MBC patients grouped by PD and SD subgroups (A–D). The patients with elevated adenosine levels had new lesions in both the brain (A and C), lymph nodes (B), and liver (D). They were assessed as PD. (E). The representative brain images of an MBC patient with stable adenosine levels. They were assessed as SD. MBC, metastatic breast cancer patients; PD, progressive disease; SD, stable disease.

directly reflect the current metabolic status, which has certain practical value. Adenosine is an endogenous purine nucleoside that mediates intracellular cAMP by acting through four G-protein-coupled receptors [31]. Most reports have explored the association between adenosine deaminase 2 (ADA2), which is a polymorphic enzyme that regulates adenosine concentrations, and various clinical outcomes [32]. Few reports have implied the role of peripheral adenosine levels in physiological and pathological processes [33–35]. However, the predictive role of adenosine in malignant tumors has not yet been reported. Several reports have indicated that the type and density of immune cells within the tumor microenvironment play a central role in disease progression [36–38]. Immunological analyses of the tumor microenvironment have shown great promise in predicting prognosis and predicting therapeutic response. $CD4^+$ T cells play diverse roles in restraining inflammation and constraining adaptive immune responses [39]. NK cells are innate immune cells with the ability to kill malignant cells directly [40]. Treg cell-mediated suppression serves as a vital mechanism of negative regulation of immune-mediated inflammation [41]. CD4CD25 regulatory T lymphocytes are a class of immunomodulatory cells that have been recently recognized. They have two major characteristics: immunosuppression and immune anergy. They also have potential application value in the treatment of autoimmune diseases, immunotherapy of tumors, and induction of transplantation tolerance [42]. It has been demonstrated that adenosine mediates pro-tumor activities, tumor tissue angiogenesis, and chemoresistance [38]. Adenosine regulates anti-tumor immune responses and facilitates tumor immune escape [43]. In HER2-positive metastatic breast cancer, elevated adenosine levels have been shown to suppress the activity of critical immune cells, including natural killer (NK) cells and cytotoxic T lymphocytes, which are essential for the anti-tumor immune response. Furthermore, adenosine promotes the expansion of regulatory T cells (Tregs), which contribute to immunosuppression in the tumor microenvironment. This immunomodulatory role of adenosine correlates with poor clinical outcomes, as higher serum adenosine levels are associated with more aggressive disease progression. Therefore, adenosine is favorable and indispensable in down-regulating anti-tumor immune responses and tumor progression. This study revealed that serum adenosine robustly associates with the progression of MBC and would be a promising biomarker for therapeutic response. This study revealed that serum adenosine robustly associates with the progression of MBC and would be a promising biomarker for therapeutic response. In healthy individuals, adenosine plays a role in physiological processes such as regulating inflammation and maintaining tissue homeostasis. However, in metastatic breast cancer, the

accumulation of adenosine is significantly heightened due to the hypoxic conditions of the tumor microenvironment. This selective elevation in adenosine levels in cancer patients, compared to healthy individuals, makes adenosine a promising target for therapeutic intervention, as it specifically contributes to the immune evasion mechanisms in the tumor.

We also constructed an adenosine-based prognostic model and validated its prognostic ability and predictive power. The single-cell sourced data from available public data revealed the transcriptomic characteristics atlas of HER2-positive breast cancers. Therefore, it is reliable and reasonable to further confirm and establish the role of adenosine in predicting clinical outcomes and the efficacy of targeted therapy.

There are several limitations of this study. First, the limited number of patients attenuates the reliability of these results. Larger numbers and external validation cohorts are needed in the future work. Moreover, Systematic Reliability Optimization (ASRO) and Novel Techniques might be helpful for the complex algorithm and patients grouping [44,45]. Second, some clinical parameters were incomplete, which restricted the comprehensive analyses. However, the dynamic monitoring of serum adenosine adds to the credibility of these results to some degree.

5. Conclusion

This study firstly revealed the predictive ability of serum adenosine on disease progression and targeted therapeutic response in metastatic breast cancer patients with HER2-positive.

CRediT authorship contribution statement

Lijun Wang: Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yizhi Ge:** Writing – original draft, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Li Yin:** Software, Project administration, Methodology, Data curation. **Dan Zong:** Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yang Li:** Software, Resources, Investigation, Formal analysis, Data curation. **Jianfeng Wu:** Supervision, Resources, Formal analysis, Conceptualization. **Xia He:** Writing – review & editing, Visualization, Validation, Supervision, Investigation, Funding acquisition, Conceptualization.

Data availability statements

The information of TCGA and GSE176078 can be found at the following website: https://portal.gdc.cancer.gov/, https://www.ncbi.nlm.nih.gov/. The data that support the findings of this study are available in this article and its supplementary files. Further inquiries can be directed to the corresponding authors.

Ethics approval

The work described in this study has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). This research was approved by the Research Ethics Committee of Jiangsu Cancer Hospital (No., 2021-012). All patients have written informed consent for participation in this study.

Funding

This work was supported by the Wu Jieping Medical Foundation [320.6750.2021-10-52], and the National Natural Science Foundation of China [No. 8217101863].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank the TCGA and GSE176078 databases for kindly providing the data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e39545.

References

[1] A.G. Waks, E.P. Winer, Breast cancer treatment: a review, JAMA 321 (2019) 288–300.
[2] M.A. Cobleigh, C.L. Vogel, D. Tripathy, et al., Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease, J. Clin. Oncol.: Offic. J. Am. Soc. Clin. Oncol. 17 (1999) 2639–2648.
[3] M. Tapia, C. Hernando, M.T. Martínez, et al., Clinical impact of new treatment strategies for HER2-positive metastatic breast cancer patients with resistance to classical anti-HER therapies, Cancers 15 (2023).
[4] E.A. Perez, E.H. Romond, V.J. Suman, et al., Four-year follow-up of trastuzumab plus adjuvant chemotherapy for operable human epidermal growth factor receptor 2-positive breast cancer: joint analysis of data from NCCTG N9831 and NSABP B-31, J. Clin. Oncol. : Offic. J. Am. Soc. Clin. Oncol. 29 (2011) 3366–3373.
[5] H.J. Stemmler, S. Kahlert, W. Siekiera, et al., Prolonged survival of patients receiving trastuzumab beyond disease progression for HER2 overexpressing metastatic breast cancer (MBC), Onkologie 28 (2005) 582–586.
[6] M.V. Dieci, F. Miglietta, G. Griguolo, et al., Biomarkers for HER2-positive metastatic breast cancer: beyond hormone receptors, Cancer Treat Rev. 88 (2020) 102064.
[7] G. Bianchini, L. Gianni, The immune system and response to HER2-targeted treatment in breast cancer, Lancet Oncol. 15 (2014) e58–e68.
[8] M.V. Dieci, F. Miglietta, G. Griguolo, et al., Biomarkers for HER2-Positive Metastatic Breast Cancer: beyond Hormone Receptors, 2024.
[9] O. MF, G. V, L. T, et al., HER2 heterogeneity as a predictor of response to neoadjuvant T-DM1 plus pertuzumab: results from a prospective clinical trial, in: Abstract 502 Presented at 2019 ASCO Annual Meeting, Chicago, US, June 3, 2019.166, 2019.
[10] Y. Hou, H. Nitta, L. Wei, et al., HER2 intratumoral heterogeneity is independently associated with incomplete response to anti-HER2 neoadjuvant chemotherapy in HER2-positive breast carcinoma, Breast Cancer Res. Treat. 166 (2017) 447–457.
[11] F. Bellati, C. Napoletano, I. Ruscito, et al., Cellular adaptive immune system plays a crucial role in trastuzumab clinical efficacy, J. Clin. Oncol. : Offic. J. Am. Soc. Clin. Oncol. 28 (2010) e369–e370.
[12] M. De Palma, D. Biziato, T.V. Petrova, Microenvironmental regulation of tumour angiogenesis, Nat. Rev. Cancer 17 (2017) 457–474.
[13] C. Cekic, Y.J. Day, D. Sag, et al., Myeloid expression of adenosine A2A receptor suppresses T and NK cell responses in the solid tumor microenvironment, Cancer Res. 74 (2014) 7250–7259.
[14] A. Bahreyni, S.S. Samani, F. Rahmani, et al., Role of adenosine signaling in the pathogenesis of breast cancer, J. Cell. Physiol. 233 (2018) 1836–1843.
[15] B. Kutryb-Zajac, P. Koszalka, P. Mierzejewska, et al., Adenosine deaminase inhibition suppresses progression of 4T1 murine breast cancer by adenosine receptor-dependent mechanisms, J. Cell Mol. Med. 22 (2018) 5939–5954.
[16] D. Boison, G.G. Yegutkin, Adenosine metabolism: emerging concepts for cancer therapy, Cancer Cell 36 (2019) 582–596.
[17] A.M. Marquard, A.C. Eklund, T. Joshi, et al., Pan-cancer analysis of genomic scar signatures associated with homologous recombination deficiency suggests novel indications for existing cancer drugs, Biomark. Res. 3 (2015) 9.
[18] X. Zhang, Y. Lan, J. Xu, et al., CellMarker: a manually curated resource of cell markers in human and mouse, Nucleic Acids Res. 47 (2019) D721–d728.
[19] T. Maj, W. Wang, J. Crespo, et al., Oxidative stress controls regulatory T cell apoptosis and suppressor activity and PD-L1-blockade resistance in tumor, Nat. Immunol. 18 (2017) 1332–1341.
[20] S. Liu, B. Chen, S. Burugu, et al., Role of cytotoxic tumor-infiltrating lymphocytes in predicting outcomes in metastatic HER2-positive breast cancer: a secondary analysis of a randomized clinical trial, JAMA Oncol. 3 (2017) e172085.
[21] A. Glajcar, J. Szpor, D. Hodorowicz-Zaniewska, et al., The composition of T cell infiltrates varies in primary invasive breast cancer of different molecular subtypes as well as according to tumor size and nodal status, Virchows Arch. : Int. J. Pathol. 475 (2019) 13–23.
[22] G. Fu, L. Miao, M. Wang, et al., The Postoperative Immunosuppressive Phenotypes of Peripheral T Helper Cells Are Associated with Poor Prognosis of Breast Cancer Patients, 2024.
[23] Y. Liu, Q. Liu, T. Wang, et al., Circulating tumor cells in HER2-positive metastatic breast cancer patients: a valuable prognostic and predictive biomarker, BMC Cancer 13 (2013) 202.
[24] B. Allard, D. Allard, L. Buisseret, et al., The adenosine pathway in immuno-oncology, Nat. Rev. Clin. Oncol. 17 (2020) 611–629.
[25] C. Sun, B. Wang, S. Hao, Adenosine-A2A receptor pathway in cancer immunotherapy, Front. Immunol. 13 (2022) 837230.
[26] P. Narayan, S. Wahby, J.J. Gao, et al., FDA approval summary: atezolizumab plus paclitaxel protein-bound for the treatment of patients with advanced or metastatic TNBC whose tumors express PD-L1, Clin. Cancer Res. 26 (2020) 2284–2289.
[27] L. Fong, A. Hotson, J.D. Powderly, et al., Adenosine 2A receptor blockade as an immunotherapy for treatment-refractory renal cell cancer, Cancer Discov. 10 (2020) 40–53.
[28] L.B. Jarvis, D.B. Rainbow, V. Coppard, et al., Therapeutically expanded human regulatory T-cells are super-suppressive due to HIF1A induced expression of CD73, Commun. Biol. 4 (2021) 1186.
[29] H.K. Eltzschig, D. Köhler, T. Eckle, et al., Central role of Sp1-regulated CD39 in hypoxia/ischemia protection, Blood 113 (2009) 224–232.
[30] D. Vijayan, A. Young, M.W.L. Teng, et al., Targeting immunosuppressive adenosine in cancer, Nat. Rev. Cancer 17 (2017) 709–724.
[31] B.B. Fredholm, M.P. Abbracchio, G. Burnstock, et al., Nomenclature and classification of purinoceptors, Pharmacol. Rev. 46 (1994) 143–156.
[32] A. Niraula, S. Thapa, S. Kunwar, et al., Adenosine deaminase activity in type 2 diabetes mellitus: does it have any role? BMC Endocr. Disord. 18 (2018) 58.
[33] T. Murata, S. Suzuki, T. Takeuchi, et al., Relation between plasma adenosine and serum TSH levels in women with hyperemesis gravidarum, Arch. Gynecol. Obstet. 273 (2006) 331–336.
[34] C. Gubert, C.E. Jacintho Moritz, M.P. Vasconcelos-Moreno, et al., Peripheral adenosine levels in euthymic patients with bipolar disorder, Psychiatr. Res. 246 (2016) 421–426.
[35] Z. Lazurova, P. Mitro, M. Popovnakova, The role of adenosine and its degradation enzymes - adenosinedeaminase and adenosinekinase in pathogenesis of vasovagal syncope, Eur. J. Intern. Med. 105 (2022) 77–81.
[36] T.J. Welsh, R.H. Green, D. Richardson, et al., Macrophage and mast-cell invasion of tumor cell islets confers a marked survival advantage in non–small-cell lung cancer, J. Clin. Oncol. 23 (2005) 8959–8967.
[37] X. Li, D. Wen, X. Li, et al., Identification of an immune signature predicting prognosis risk and lymphocyte infiltration in colon cancer, Front. Immunol. 11 (2020) 1678.
[38] D. Liu, L.R. Heij, Z. Czigany, et al., The role of tumor-infiltrating lymphocytes in cholangiocarcinoma, J. Exp. Clin. Cancer Res. : CR 41 (2022) 127.
[39] S.Z. Josefowicz, L.F. Lu, A.Y. Rudensky, Regulatory T cells: mechanisms of differentiation and function, Annu. Rev. Immunol. 30 (2012) 531–564.
[40] F. Fang, S. Xie, M. Chen, et al., Advances in NK cell production, Cell. Mol. Immunol. 19 (2022) 460–481.
[41] P.A. Savage, D.E.J. Klawon, C.H. Miller, Regulatory T cell development, Annu. Rev. Immunol. 38 (2020) 421–453.
[42] H.Z. Shi, X.J. Qin, CD4CD25 regulatory T lymphocytes in allergy and asthma, Allergy 60 (2005) 986–995.
[43] J. Xing, J. Zhang, J. Wang, The immune regulatory role of adenosine in the tumor microenvironment, Int. J. Mol. Sci. 24 (2023).
[44] H.S. Kim, C. Park, V. Govindan, et al., Systematic reliability optimization (ASRO), Babylonian J. Math. 2023 (2023) 50–53.
[45] I. Ahmad, R. Jan, M. Nur Alam, et al., Novel Techniques for classifying exotic spheres in high dimensions, Babylonian J. Math. 2023 (2023) 36–39.