Research, Society and Development, v. 12, n. 13, e40121344285, 2023
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v12i13.44285

Altered threonine tyrosine kinase (TTK) expression is associated with adverse clinical outcomes in breast tumors: An in silico approach

A expressão alterada de treonina tirosina quinase (TTK) está associada a resultados clínicos adversos em tumores de mama: Uma abordagem in silico

La expresión alterada de tirosina quinasa de treonina (TTK) se asocia con resultados clínicos adversos en tumores de mama: Un enfoque in silico

Received: 11/21/2023 | Revised: 11/25/2023 | Accepted: 11/25/2023 | Published: 11/27/2023

Gustavo Barboza Roberto da Silva
ORCID: https://orcid.org/0009-0000-7794-9263
Beneficent Society São Camilo, Brazil
E-mail: gu.barboza@hotmail.com
Beatriz Silva Cruz
ORCID: https://orcid.org/0009-0007-6532-7601
Anhembi Morumbi University, Brazil
E-mail: beatrizsvcruz@gmail.com
Julia Gonçalves Vieira
ORCID: https://orcid.org/0009-0005-1791-4866
Anhembi Morumbi University, Brazil
E-mail: jugoncalves0807@hotmail.com
Julia Montorso Uzum
ORCID: https://orcid.org/0009-0001-4277-9185
União Química Farmacêutica Nacional s/a, Brazil
E-mail: jmontorso@gmail.com
Renan Gomes do Nascimento
ORCID: https://orcid.org/0000-0002-7579-0671
Instituto Brasileiro de Controle do Câncer, Brazil
E-mail: renangomesfarmaceutico@gmail.com
Gustavo José Vasco Pereira
ORCID: https://orcid.org/0000-0001-9811-7220
Anhembi Morumbi University, Brazil
E-mail: gustavo.vasco@animaeducação.com.br

Abstract
The most common and lethal malignancy in women on a global scale remains breast cancer. Threonine tyrosine kinase (TTK) has been shown to be a critical mitotic spindle assembly checkpoint (SAC) molecule, resulting in correct chromosome segregation and maintenance of genomic stability. Therefore, the present study was carried out to evaluate the expression pattern of threonine tyrosine kinase (TTK) in breast cancer and its potential prognostic and predictive value for therapeutic response using bioinformatics tools. Web platforms containing clinical information and cDNA microarray data were selected to perform in silico analyzes of the potential threonine tyrosine kinase (TTK) marker. The threonine tyrosine kinase (TTK) gene was found to be differentially expressed in tumor samples when compared to healthy breast tissue samples ($p<0.0001$) and the TNBC subtype exhibited the highest expression of threonine tyrosine kinase (TTK) relative to the other subtypes ($p<0.0001$). Furthermore, Kaplan-Meier curves revealed that high threonine tyrosine kinase (TTK) levels corresponded to an unfavorable outcome for overall survival ($p<0.0001$), as well as for recurrence-free survival ($p<0.0001$) and distant metastasis-free survival ($p<0.0001$). Finally, differential expression of threonine tyrosine kinase (TTK) was related to the response of breast cancer patients to different therapies. Our cumulative results demonstrate that threonine tyrosine kinase (TTK) may be a promising biomarker for predicting prognosis and therapeutic response in breast cancer patients.
Keywords: Biomarkers; Breast neoplasms; Computational biology.

Resumo
A neoplasia maligna mais incidente e letal em mulheres em escala global permanece sendo a de mama. Foi demonstrado que treonina tirosina quinase (TTK) é uma molécula crítica do ponto de verificação da montagem do fuso mitótico (SAC), resultando em segregação cromossômica correta e manutenção da estabilidade genômica. Portanto, o presente estudo foi realizado para avaliar o padrão de expressão de treonina tirosina quinase (TTK) no câncer de mama e seu potencial valor prognóstico e preditivo para resposta terapêutica utilizando ferramentas de bioinformática. Plataformas de web contendo informações clínicas e dados de cDNAmicroarray foram selecionadas

para realizar análises in silico do potencial marcador treonina tirosina quinase (TTK). Foi descoberto que o gene treonina tirosina quinase (TTK) é diferencialmente expresso em amostras de tumores quando comparados com amostras de tecido mamário saudável ($p<0.0001$) e o subtipo TNBC exibiu a expressão mais elevada de treonina tirosina quinase (TTK) em relação aos outros subtipos ($p<0,0001$). Além disso, as curvas de Kaplan-Meier revelaram que níveis elevados de treonina tirosina quinase (TTK) correspondiam a um desfecho desfavorável para a sobrevida global ($p<0.0001$), bem como para a sobrevida livre de recidiva ($p<0.0001$) e sobrevida livre de metástase distante ($p<0.0001$). Por fim, a expressão diferencial de treonina tirosina quinase (TTK) foi relacionado com a resposta de pacientes com câncer de mama a diferentes terapias. Nossos resultados cumulativos demonstram que treonina tirosina quinase (TTK) pode ser um biomarcador promissor para prever o prognóstico e a resposta terapêutica em pacientes com câncer de mama.
Palavras-chave: Biomarcadores; Neoplasias da mama; Biologia computacional.

Resumen
La neoplasia maligna más incidente y letal en mujeres a nivel global sigue siendo la de mama. Se ha demostrado que tirosina quinasa de treonina (TTK) es una molécula crítica en el punto de control de ensamblaje del huso mitótico (SAC), lo que resulta en una correcta segregación cromosómica y mantenimiento de la estabilidad genómica. Por lo tanto, el presente estudio se realizó para evaluar el patrón de expresión del tirosina quinasa de treonina (TTK) en el cáncer de mama y su potencial valor pronóstico y predictivo para la respuesta terapéutica utilizando herramientas de bioinformática. Se seleccionaron plataformas web que contienen información clínica y datos de microarreglos de cDNA para realizar análisis in silico del potencial marcador tirosina quinasa de treonina (TTK). Se descubrió que el gen tirosina quinasa de treonina (TTK) se expresa diferencialmente en muestras tumorales en comparación con muestras de tejido mamario sano ($p<0.0001$), y el subtipo TNBC exhibió la expresión más alta de tirosina quinasa de treonina (TTK) en comparación con otros subtipos ($p<0.0001$). Además, las curvas de Kaplan-Meier revelaron que niveles altos de tirosina quinasa de treonina (TTK) correspondieron a un resultado desfavorable para la supervivencia global ($p<0.0001$), así como para la supervivencia libre de recurrencia ($p<0.0001$) y la supervivencia libre de metástasis distante ($p<0.0001$). Por último, la expresión diferencial de tirosina quinasa de treonina (TTK) se relacionó con la respuesta de pacientes con cáncer de mama a diferentes terapias. Nuestros resultados acumulativos demuestran que tirosina quinasa de treonina (TTK) puede ser un biomarcador prometedor para predecir el pronóstico y la respuesta terapéutica en pacientes con cáncer de mama.
Palabras clave: Biomarcadores; Neoplasias de la mama; Biología computacional.

1. Introduction

Breast cancer, no different from other malignant neoplasms, encompasses a set of genetically and phenotypically specific diseases, culminating in different histopathological and molecular profiles, variations in patients' clinical results and varying response rates to commonly used treatments (Bhushan et al., 2021). The most common and lethal solid tumor in women on a global scale remains the breast type, representing more than 2.2 million new cases and approximately 680,000 deaths annually (Sung et al., 2021).

Monopolar spindle 1 (Mps1), also known as threonine tyrosine kinase (TTK) is a critical mitotic spindle assembly checkpoint (SAC) molecule, resulting in correct chromosome segregation and maintenance of genomic stability (Xie et al., 2017). Aberrant expression of threonine tyrosine kinase (TTK) has been reported in different malignancies due to promotion of aneuploidy, genomic instability, proliferation and survival of cancer cells (Xie et al., 2017). In breast cancer, a recent study by Gao and colleagues revealed that elevated threonine tyrosine kinase (TTK) expression was associated with tumor progression and low disease-free and overall survival rates of patients with triple-positive (ER+, PR+, and HER2+) breast tumors (Gao et al., 2022).

The objective of this study is to use bioinformatics tools to comprehensively analyze the clinical and therapeutic implications associated with threonine tyrosine kinase (TTK) expression, contributing to the advancement of knowledge on biomarkers and personalized treatment strategies in the context of breast cancer.

2. Methodology

In our research, we used specific cancer-related gene expression programs that provide a new basis for classification. With the advent of microarray technology, we globally analyzed these profiles instead of gene by gene, allowing us to

understand the biology associated with certain types or subtypes of cancer, including their genetic mutations and aberrant biological pathways. In the analyses led by Beatriz Cruz, Julia Montorso, Gustavo Barboza, and Julia Gonçalves, we were able to analyze breast cancer based on specific variables such as organ type or subtype, patient prognosis, treatment response prediction, or site of metastasis. The platforms on which we conducted the analyses and their utilities are described below:

UALCAN is a free online platform for accessing cancer data through the science of "OMICS" (Chandrashekar et al., 2022). In this way, we access the expression profile of the threonine tyrosine kinase (TTK) biomarker, in association with survival and gene regulation data, generating a robust analysis in preclinical trials.

Bc-GenExMiner v.5 is a microarray and RNA-seq data mining tool containing data from breast cancer patients only (Jézéquel et al., 2021). For this study, we only considered microarray data to analyze threonine tyrosine kinase (TTK) expression associated with clinicopathological parameters, with regard to classical breast cancer biomarkers and different molecular subtypes. The median expression was used as the cutoff point.

Kaplan-Meier (KM) Plotter is a publicly available platform with data from 21 different types of neoplasms (Győrffy, 2023). For this study, we chose to enable the option to select the best probe corresponding to the threonine tyrosine kinase (TTK) gene (204822_at). Overall survival (OS) and recurrence-free survival (RFS) were adjusted for the total follow-up time of 120 months. Patients were also stratified by high and low expression of the target gene, since the best cutoff point between the lower and upper quartile was selected. Log-rank p values and hazard ratio (HR) with 95% confidence interval (CI) were automatically determined.

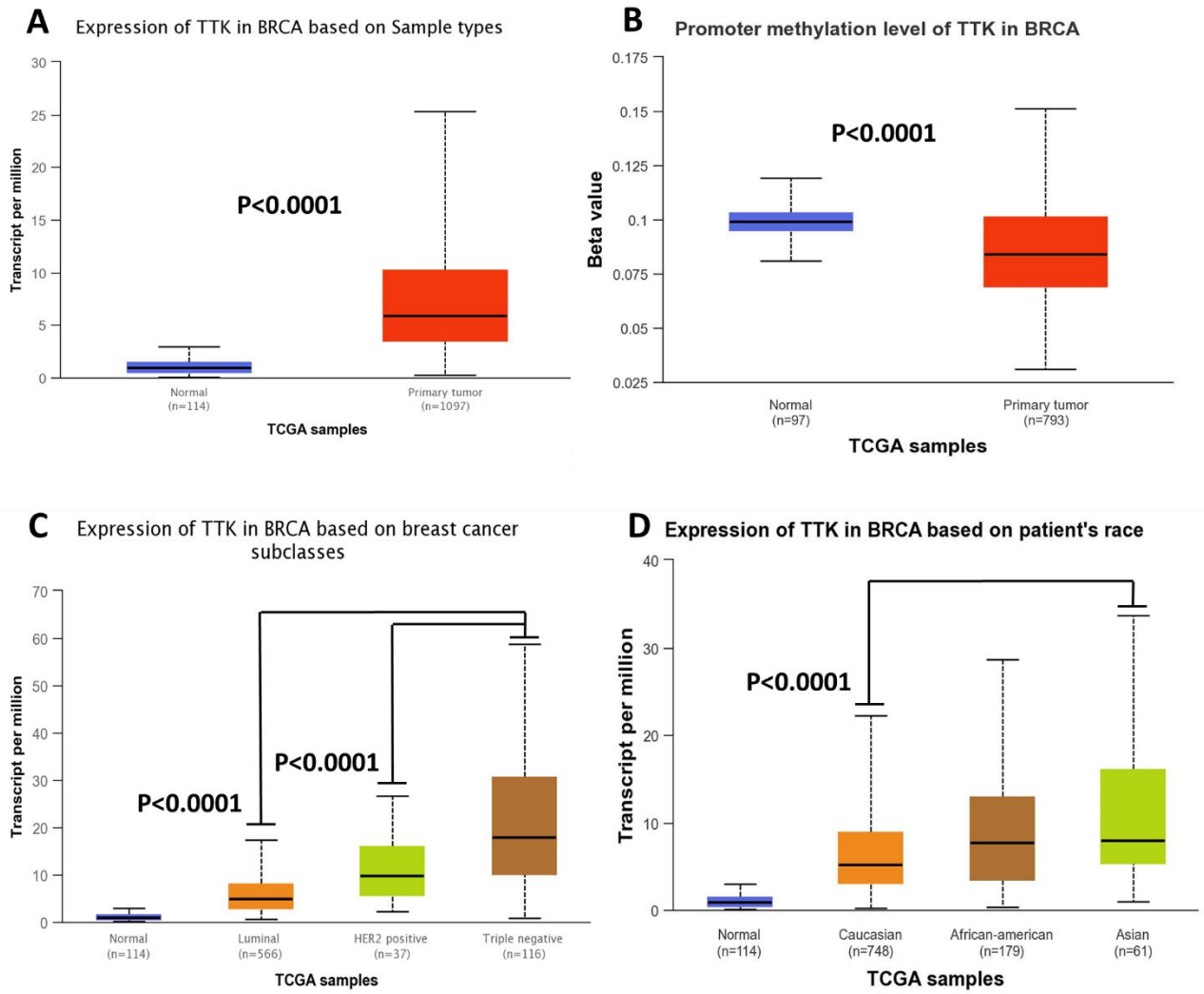
The ROC Plotter is an interactive and easy-to-use online tool containing transcriptomic data from more than 3,000 breast cancer patients treated and not treated with endocrine therapy, anti-HER2 therapy or chemotherapy (Fekete & Győrffy, 2019). Here we quickly evaluated the pattern of threonine tyrosine kinase (TTK) expression based on the treatment received by the patients and the 5-year recurrence-free survival (RFS).

3. Results

3.1 Threonine tyrosine kinase (TTK) expression in samples from breast cancer patients

Using TCGA data analyzed by the UALCAN platform, we found that threonine tyrosine kinase (TTK) showed elevated expression in breast cancer tumor tissues when compared to adjacent normal tissue (Figure 1A; $p<0.0001$). Furthermore, hypomethylation of the threonine tyrosine kinase (TTK) promoter region was observed in breast cancer tissues compared to healthy tissues (Figure 1B; $p<0.0001$). Finally, we investigated threonine tyrosine kinase (TTK) expression patterns in relation to population ethnic classification and molecular classification of breast neoplasia. Triple negative breast cancer (TNBC) exhibited increased transcriptional distribution compared to Luminal and HER2+ subtypes (Figure 1C; $p<0.0001$, respectively). Regarding ethnicity, Asian patients had breast tumors with higher rates of gene expression when compared to Caucasians, but not to African-Americans (Figure 1D; $p<0.0001$ and $p=0.17$, respectively).

Figure 1 - Threonine tyrosine kinase (TTK) expression in breast cancer patients.



Source: Own authors.

In Figure 1A, we can see the expression of threonine tyrosine kinase (TTK) in tumor and healthy breast samples, whose comparison resulted in a p-value of <0.0001, meaning it is extremely significant. We can notice a similar expression in Figure 1B, which shows the methylation profile of the threonine tyrosine kinase (TTK) promoter region in tumor and healthy breast samples. In Figure 1C, the expression of threonine tyrosine kinase (TTK) can be analyzed in different molecular subtypes of breast cancer. The result reveals that the marker is significantly expressed in all subtypes, but it is noteworthy that in the TNBC and Luminal subclasses, the expression level is higher than in the HER2 positive subtype. Finally, it can be observed in Figure 1D that the expression of TTK varies based on different ethnicities, resulting in higher expression in Asian patients.

3.2 Association of threonine tyrosine kinase (TTK) Expression with Clinicopathological Features

The open source database, bc-GenExMiner, was used for this analysis. The sample evidence allowed us to demonstrate significant associations between the differential expression of threonine tyrosine kinase (TTK) and age (p<0.0001), nodal status (p<0.0001), Scarff-Bloom-Richardson (SBR) classification (p<0.0001), molecular classification (p<0.0001) and the expression status of the markers ER (p<0.0001), PR (p<0.0001), HER2 (p<0.0001) and TP53 (p<0.0001) (Table 1).

Table 1 - Relationship between threonine tyrosine kinase (TTK) expression and clinical parameters of breast cancer patients using the bc-GenExMiner database.

Variables	Patient Number	TTK microarray	*P* value
Age			*P*<0.0001
≤51	2870	Increased	
>51	4707	-	
Nodal Status			*P*<0.0001
Negative	4383	-	
Positive	3450	Increased	
SBR			*P*<0.0001
1	1022	Decreased	
2	3276	-	
3	3429	-	
Status TP53			*P*<0.0001
Wild-type	643	-	
Mutated	299	Increased	
Estrogen Receptor			*P*<0.0001
Negative	2602	Increased	
Positive	7066	-	
Progesterone Receptor			*P*<0.0001
Negative	2649	Increased	
Positive	3405	-	
HER2			*P*<0.0001
Negative	4644	-	
Positive	793	Increased	
Molecular Subtypes			*P*<0.0001
Luminal A	3348	Decreased	
Luminal B	3030	-	
HER2	1405	-	
Triple Negative	2024	Increased	

Source: Own authors.

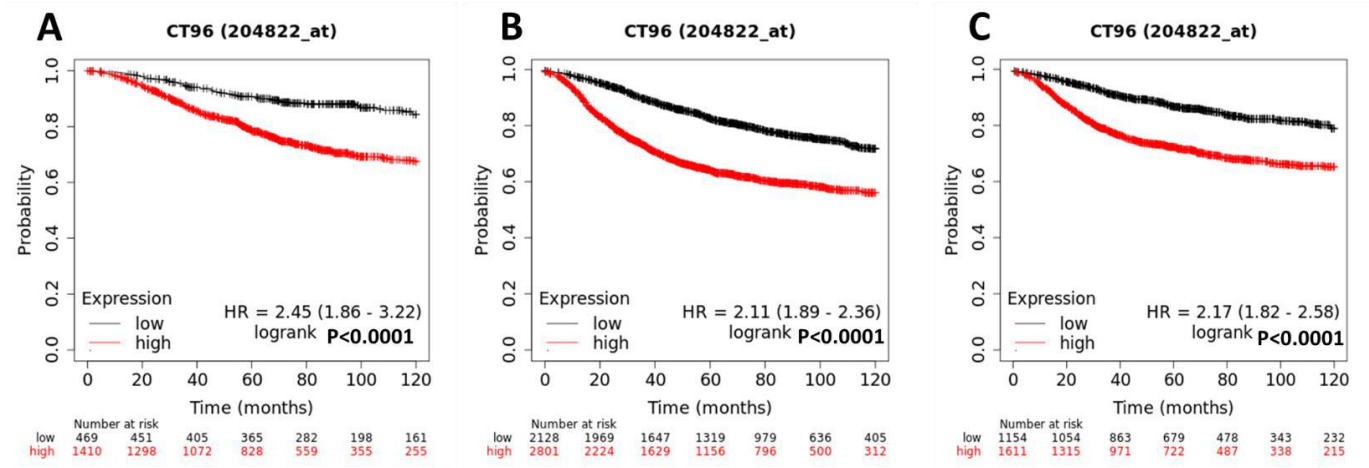
Significant p-values are show in bold. TTK: Threonine tyrosine kinase; SBR: Scarff-Bloom-Richardson; NPI: Nottingham prognostic index; HER2: Human epidermal growth factor receptor-2.

3.3 Threonine tyrosine kinase (TTK) expression and prognostic value in breast cancer patients

Next, we investigated the prognostic value of threonine tyrosine kinase (TTK) using the KM Plotter platform. Notably, high threonine tyrosine kinase (TTK) mRNA expression levels were significantly correlated with a poor prognosis in the investigations of overall survival (OS), recurrence-free survival (RFS) and distant metastasis-free survival (DMFS) (Figures 2A, 2B and 2C; p<0.0001; respectively).

Figure 2 - Survival curves derived from the Kaplan-Meier Plotter evaluating the prognostic significance of threonine tyrosine kinase (TTK).

Source: own author.

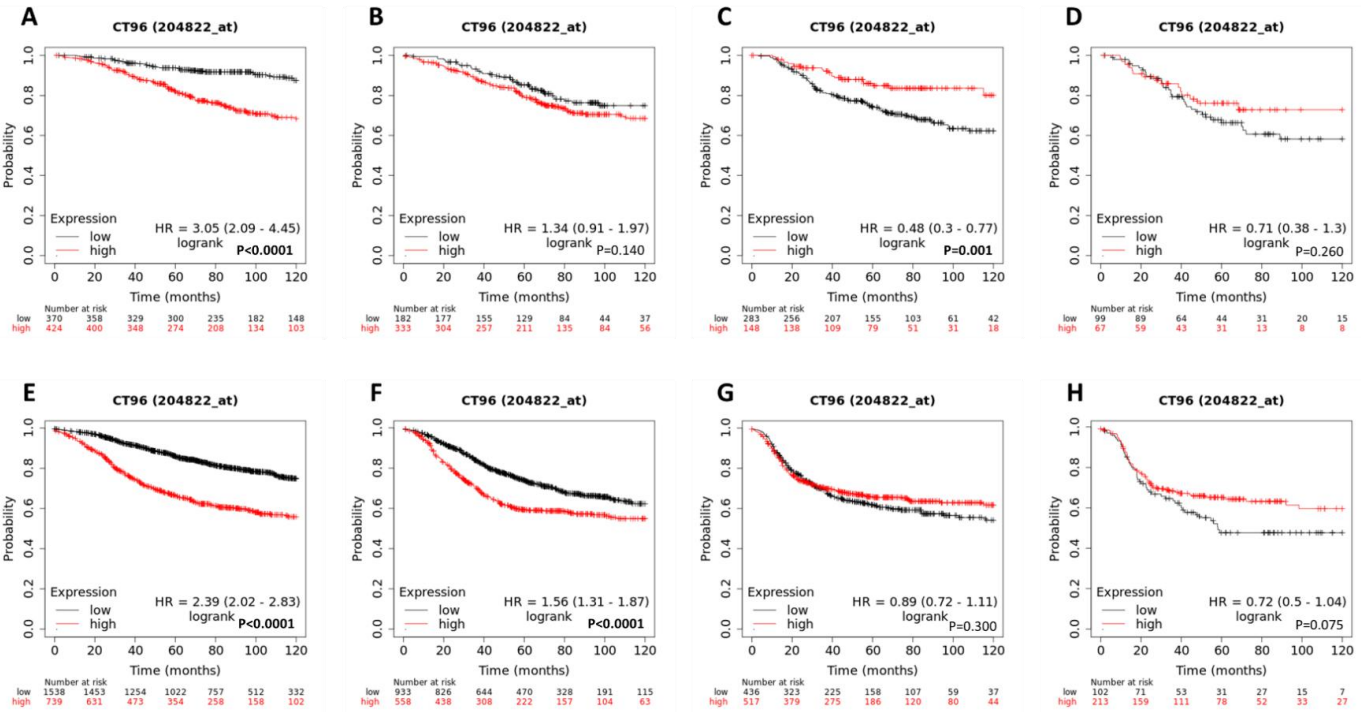


Source: Own authors.

In Figure 2A, we show the overall survival (OS) of breast cancer patients stratified by threonine tyrosine kinase (TTK) expression. In Figure 2B, it is possible to analyze the relapse-free survival (RFS) of patients stratified by threonine tyrosine kinase (TTK) expression. Finally, in Figure 2C, we observe the distant metastasis-free survival (DMFS) of patients stratified by threonine tyrosine kinase (TTK) expression.

We also investigated the prognostic role of threonine tyrosine kinase (TTK) in different intrinsic molecular subtypes. Kaplan-Meier curves indicated that elevated threonine tyrosine kinase (TTK) levels were significantly associated with reduced cumulative rates in the RFS of patients with hormone-dependent tumors (Figures 3E and 3F; Luminal A and Luminal B, $p<0.0001$; respectively) and in OS only in Luminal A tumors (Figure 3A; $p<0.0001$), but not in the Luminal B subtype (Figure 3B; $p=0.140$). Meanwhile, inversely to the effect observed in hormone-dependent tumors, high levels of threonine tyrosine kinase (TTK) demonstrated lower OS in cases of patients with tumors subtyped as TNBC (Figure 3C; $p=0.001$), but there was no statistically relevant difference when evaluating RFS (Figure 3G; $p=0.300$). Finally, the Kaplan-Meier curves did not indicate any type of association between the differential expression of threonine tyrosine kinase (TTK) with OS and RFS in patients with HER2+ tumors (Figures 3D and 3H; $p=0.140$ and $p=0.075$; respectively).

Figure 3 - Survival curves derived from the Kaplan-Meier Plotter evaluating the prognostic significance of T according to molecular subtype.



Source: Own authors.

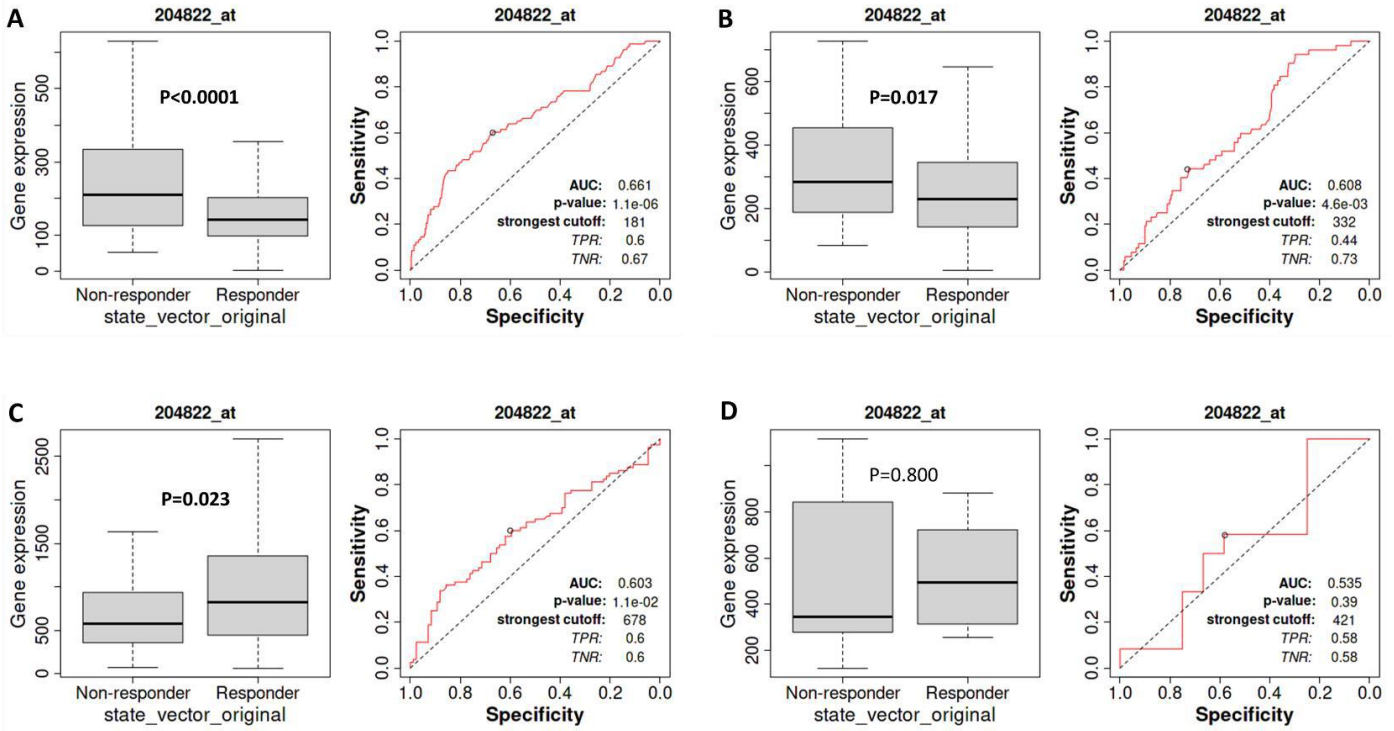
As shown in Figure 3, we analyzed the overall survival (OS) of breast cancer patients stratified by threonine tyrosine kinase (TTK) expression and by Luminal A (3A), Luminal B (3B), TNBC (3C), and HER2+ (3D) subtypes. We also presented the relapse-free survival (RFS) for breast cancer patients stratified by TTK expression and by Luminal A (3E), Luminal B (3F), TNBC (3G), and HER2+ (3H) subtypes.

3.4 Predictive value of TTK for treatment response

Considering the reports of some previous studies indicating threonine tyrosine kinase (TTK) as a potential biomarker for response to different treatments and even an attractive therapeutic target in different neoplasms (J. Chen et al., 2020; Zaman et al., 2017), we performed an analysis with the ROC Plotter web tool.

Our results showed that among patients with hormone-dependent tumors, those who did not respond to hormonal treatment had significantly increased expression of threonine tyrosine kinase (TTK) both in cases classified as Luminal A (Figure 4A; $p<0.0001$) and in Luminal B type tumors (Figure 4B; $p=0.017$). Contrary to the effect observed for hormone-dependent tumors, patients with TNBC-type tumors that responded to classical chemotherapy showed significantly increased rates of threonine tyrosine kinase (TTK) (Figure 4C; $p=0.023$). Finally, in cases of patients with HER2+ tumors, no relationship was observed between the differential expression of threonine tyrosine kinase (TTK) and the response to monoclonal antibodies directed to this receptor (Figure 4D; $p=0.800$).

Figure 4 - Threonine tyrosine kinase (TTK) expression pattern in breast cancer patients receiving different therapies.



Source: Own authors.

In Figure 4, we analyzed the 5-year recurrence-free survival (RFS) among responders and non-responders to endocrine therapy in patients with tumors classified as Luminal A (4A) and Luminal B (4B). In this figure, we can also observe the 5-year recurrence-free survival among responders and non-responders to chemotherapy in patients with TNBC tumors (4C), and finally, the 5-year recurrence-free survival among responders and non-responders to anti-HER2 therapy in patients with HER2+ tumors (4D). HER2: human epidermal growth factor receptor 2.

4. Discussion

In recent decades, we have witnessed the beginning of the era of modern medicine, also known as precision medicine, thanks to new diagnostic and therapeutic methods (Schick et al., 2021). Diagnosis, for example, was previously focused exclusively on histopathological aspects, which evolved into the investigation of highly accurate prognostic and predictive biomarkers (Afzal et al., 2022). Among the different candidate molecules for biological markers in breast cancer, we can highlight threonine tyrosine kinase (TTK), which is a dual-specificity protein kinase that acts by phosphorylating proteins in tyrosine, serine and threonine, playing a critical role during cell proliferation and division. and essential for the alignment of chromosomes at the centromere during mitosis and centrosome duplication (Combes et al., 2018; Kuijt et al., 2020).

Initially, we analyzed the threonine tyrosine kinase (TTK) expression profile using the UALCAN database. In line with our findings, breast tumor tissue samples showed elevated levels of threonine tyrosine kinase (TTK) compared with adjacent normal tissue samples. A recent study carried out by Albogami, in which The Human Protein Atlas database was accessed to evaluate the protein and gene expression levels of threonine tyrosine kinase (TTK) in different samples, negative regulation of threonine tyrosine kinase (TTK) was observed at both the transcriptional and protein levels in non-cancerous tissues adjacent, but high levels were detected in tumor samples (Albogami, 2022). Additionally, other studies have also demonstrated that threonine tyrosine kinase (TTK) is highly expressed in neoplastic tissues when compared to adjacent healthy or benign tissue, such as in endometrial and bladder cancer (F. Chen et al., 2018; Miao et al., 2023). Regarding epigenetic

mechanisms, we investigated whether methylation could be a gene regulation pathway for threonine tyrosine kinase (TTK). Our work demonstrated that threonine tyrosine kinase (TTK) is hypo methylated compared to adjacent non-cancerous tissue samples. Currently, we know that methylation is a critical biochemical process in the modification of DNA and histones to regulate the functioning of the genome and imbalance in the methylation of specific regions of DNA can lead to the emergence of different neoplasms (Menezo et al., 2020). To date, no study has investigated the gene expression profile of threonine tyrosine kinase (TTK) according to methylation status and, therefore, the present study is pioneering in this regard and paves the way for future investigations into possible mechanisms of epigenetic regulation for threonine tyrosine kinase (TTK). (Chandler et al., 2020; King et al., 2018).

Subsequently, we saw that threonine tyrosine kinase (TTK) was significantly more expressed in breast tumor tissue samples subtyped in TNBC (triple negative breast cancer) when compared to the other molecular subtypes. Corroborating our findings, in two other independent studies, in which data sets from METABRIC (Molecular Taxonomy of Breast Cancer International Consortium) and the other from GOBO (Gene Set Analysis - Tumors) were accessed, overexpression of threonine tyrosine kinase (TTK) was evidenced in the TNBC subtype. compared to all other molecular types of breast cancer (Chandler et al., 2020; King et al., 2018). Finally, our study revealed an intriguing finding, in which threonine tyrosine kinase (TTK) was overexpressed in breast cancer samples from Asian women when compared to Caucasian women, but not in black women. In recent work developed by Rivera's group, a transcriptomic dataset from TCGA (The Cancer Genome Atlas) of 969 women diagnosed with breast cancer was examined for threonine tyrosine kinase (TTK) expression according to self-declared ethnic classification (Rivera-Rivera et al., 2022). It was discovered in this analysis that threonine tyrosine kinase (TTK) was significantly more expressed in black women when compared to white and Hispanic women (Rivera-Rivera et al., 2022). At this time, we cannot directly compare our findings with Rivera's study, firstly, Rivera's work did not incorporate sampling of women with Asian race for the analysis of differential expression of threonine tyrosine kinase (TTK) and secondly, our study has the limitation of having a relatively small sample size in the group of Asian and black women, however, together these studies point to the need for investigations of threonine tyrosine kinase (TTK) as a possible population biomarker.

In this study, we analyzed the relevance of threonine tyrosine kinase (TTK) expression for different clinicopathological characteristics of breast cancer patients. We revealed that threonine tyrosine kinase (TTK) was associated as a possible predictor of worse prognosis in breast cancer, showing higher transcriptional expression of threonine tyrosine kinase (TTK) in samples of neoplastic breast tissues from patients with lymph node involvement and with the highest grade in the SBR (Scarff-Bloom-Richardson) classification. To date, no work has sought to investigate the differential expression of threonine tyrosine kinase (TTK) in patients with breast cancer, without stratification by intrinsic molecular subtypes. Additionally, we showed high expression of threonine tyrosine kinase (TTK) in samples from patients with breast cancer tissues carrying mutations in TP53. Corroborating our findings, an elegant study developed by Győrffy's group revealed that threonine tyrosine kinase (TTK) protein expression was consistently higher in breast cancer lines that have mutations in TP53 (T47D and MDA-MB-231) when compared to wild lines. -type (Gyorffy et al., 2014). Furthermore, reduction of threonine tyrosine kinase (TTK) expression by siRNA caused increased apoptosis induction of TP53 mutant cells, particularly the T47D line that expresses the estrogen receptor (ER) (Gyorffy et al., 2014). Therefore, these results suggest that threonine tyrosine kinase (TTK) expression may predict an unfavorable prognosis of breast cancer, especially in hormone-dependent tumors. Interestingly, our work revealed that threonine tyrosine kinase (TTK) was consistently upregulated in tissue samples from patients with breast tumors with negative expression for ER (estrogen receptor) and PR (progesterone receptor). Corroborating our study, King and colleagues identified higher threonine tyrosine kinase (TTK) expression in tumors with low ER expression in a set of 1,620 samples from breast cancer patients deposited in GOBO (King et al., 2018).

Subsequently, the prognostic significance of threonine tyrosine kinase (TTK) in breast cancer was investigated using the public Kaplan-Meier Plotter database. It was found that elevated threonine tyrosine kinase (TTK) mRNA expression was associated with decreased overall survival (OS), recurrence-free survival (RFS), and distant metastasis-free survival (DMFS) rates in breast cancer patients. Supporting our results, another study using a TCGA dataset with 302 samples from breast cancer patients revealed that high threonine tyrosine kinase (TTK) gene expression was correlated with lower OS and RFS rates when compared to patients who had tumors with low expression of this marker (J. Tang et al., 2019). Furthermore, in an elegant work conducted by Tang and collaborators, differentially expressed genes were investigated in metastatic brain samples from breast cancer patients accessed from the GEO (Gene Expression Omnibus) platform, among them, threonine tyrosine kinase (TTK) was one of the 10 core genes (D. Tang et al., 2019). Taken together, these works shed light on a possible critical role of threonine tyrosine kinase (TTK) in promoting metastasis and worse prognosis in breast cancer.

Regarding the prognostic impact of threonine tyrosine kinase (TTK) in different molecular subtypes, we identified that high transcriptional expression of threonine tyrosine kinase (TTK) was associated with significantly reduced OS and RFS rates in patients with Luminal A type tumors and only with RFS in the Luminal B subtype. Corroborating with our findings, Gao and colleagues found that elevated threonine tyrosine kinase (TTK) gene expression was a determining factor for lower OS and RFS in patients carrying TPBC (triple positive breast cancer) tumors (Gao et al., 2022). Furthermore, the group also performed threonine tyrosine kinase (TTK) knockdown in BT474 cells that mimic the Luminal molecular profile, leading to a significant reduction in cell survival, colony formation and invasive potential (Gao et al., 2022). In summary, the expression of threonine tyrosine kinase (TTK) in Luminal tumors is revealed as a potential predictive marker for a worse prognosis.

Surprisingly, in TNBC tumors we observed the opposite role, since high threonine tyrosine kinase (TTK) expression promoted higher OS rates for patients. In the work conducted by Maire and colleagues, it was also observed that high expression of threonine tyrosine kinase (TTK) was associated with a favorable outcome in the OS of patients with TNBC (Maire et al., 2013). However, in in vitro experiments it was confirmed that depletion of threonine tyrosine kinase (TTK) expression by siRNA in three TNBC cell lines (MDA-MB-468, MDA-MB-231 and HCC70) was sufficient to compromise cell viability and cell capacity. to form colonies in an anchorage-independent manner (Maire et al., 2013). In another study, Xu's group recruited 164 patients with TNBC tumors and subsequently performed IHC (immunohistochemistry) evaluation of these samples (Xu et al., 2016). High expression of threonine tyrosine kinase (TTK) was associated with a significant reduction in OS and RFS rates (Xu et al., 2016). Finally, the work of King's group revealed different results regarding the impact of threonine tyrosine kinase (TTK) expression in TNBC when compared to those of our group, Xu and Maire. In the study by King's group, a significant cohort of 2,091 samples of TNBC tumors was used in which it was revealed that high transcriptional expression of threonine tyrosine kinase (TTK) resulted in a decrease in patients' OS rates (King et al., 2018). Furthermore, genomic silencing of threonine tyrosine kinase (TTK) in MDA-MB-231 cells led to increased expression of E-cadherin (epithelial marker) and decreased expression of vimentin (mesenchymal marker), suggesting that increased expression of threonine tyrosine kinase (TTK) facilitates mesenchymal signaling in TNBC cells (King et al., 2018). Therefore, we can infer that the impact of threonine tyrosine kinase (TTK) expression still needs to be better elucidated in TNBC tumor samples and more in vitro experiments are necessary to understand the more precise biological role of this marker in TNBC.

In the last molecular subtype, our study revealed that the differential expression of threonine tyrosine kinase (TTK) had no statistically significant impact on the survival of patients with HER2+ subtyped breast tumors. To date, no study has sought to understand the role of threonine tyrosine kinase (TTK) expression in HER+ breast cancer and, therefore, our work is pioneering in this sense. However, Lee and colleagues performed functional experiments to evaluate the biological impact of threonine tyrosine kinase (TTK) expression in HER2+ breast cancer cell lines (HCC1954, JIMT-1 and SKBR3) (Lee et al., 2014). In this work, it was revealed that knockdown of threonine tyrosine kinase (TTK) by siRNA can significantly decrease

the percentage of centrosome amplification of HER2+ breast cancer cell lines, indicating that aberrant expression of threonine tyrosine kinase (TTK) can result in genomic instability and greater tumor malignancy (Lee et al., 2014).

Finally, threonine tyrosine kinase (TTK) has been identified as a potential therapeutic target in cancer. In the work of Mason and collaborators, the group identified the compound CFI-402257 as a potent and selective inhibitor for threonine tyrosine kinase (TTK), preventing its auto phosphorylation (Mason et al., 2017). Furthermore, the compound CFI-402257 revealed potent growth inhibitory activity in several breast cancer cell lines (SKBr-3, CAL51, T-47D, MDA-MB-436, MDA-MB-361 and HCC202) (Mason et al., 2017). Therefore, we sought to investigate the role of threonine tyrosine kinase (TTK) to predict therapeutic response in breast cancer patients receiving different antineplastic therapies and according to molecular subtype.

Our results demonstrated that high threonine tyrosine kinase (TTK) expression was significantly correlated with resistance to endocrine therapy in patients subtyped as Luminal. Corroborating our findings, in recent work by Zhang's group it was discovered that threonine tyrosine kinase (TTK) overexpression can make breast cancer cells (MCF-7 and T47-D) resistant to tamoxifen (Zhang et al., 2023). Mechanistically, co-immunoprecipitation and proximity ligation assays revealed that threonine tyrosine kinase (TTK) can interact with ERα, stimulating its transactivation activity in a kinase activity-dependent manner (Zhang et al., 2023). Therefore, it is suggested that threonine tyrosine kinase (TTK) may contribute to resistance to hormone therapy and as a potential therapeutic target in hormone-dependent breast cancer.

Unlike the effect observed for hormone-dependent tumors, patients with TNBC-type tumors that responded to classical chemotherapy showed significantly increased rates of threonine tyrosine kinase (TTK). Several studies have sought to understand the role of the threonine tyrosine kinase (TTK) molecule as a therapeutic target in TNBC. Elango's group demonstrated that threonine tyrosine kinase (TTK) knockdown, when combined with paclitaxel exposure, has deleterious effects on colony-forming unit potential in the BT-549 model (Elango et al., 2021). Furthermore, the mechanistic synergy between paclitaxel and the potent and highly selective threonine tyrosine kinase (TTK) kinase inhibitor (BOS172722) in MDA-MB-231 cells was able to prevent chromosomal alignment, drastically reducing mitotic time and culminating in severe chromosomal abnormalities and non-viable cells (Anderhub et al., 2019). Maia and colleagues demonstrated that the combination of the threonine tyrosine kinase (TTK) inhibitor (NTRC 0066-0) with a therapeutic dose of docetaxel resulted in a significant prolongation of survival in mice with TNBC breast tumor cell xenografts (MDA-MB-231) and in prolonged tumor remission, without limiting toxic effects (Maia et al., 2015). Therefore, many studies have strived to elucidate the mechanistic role of threonine tyrosine kinase (TTK) in TNBC tumor lines, however, no work to our knowledge has sought to investigate the role of threonine tyrosine kinase (TTK) in the response to treatment of patients undergoing traditional chemotherapy and our work provides some clues of its potential value as a biomarker predicting response to treatment.

5. Conclusion

In summary, our study showed that threonine tyrosine kinase (TTK) is transcriptionally more expressed in breast tumors compared to adjacent tissue. Furthermore, among tumor subtypes, TNBC showed higher levels of threonine tyrosine kinase (TTK). Notably, elevated threonine tyrosine kinase (TTK) gene expression was associated with worse survival in breast cancer patients. Finally, threonine tyrosine kinase (TTK) proved to be a potential biomarker for predicting response in different molecular subtypes of breast cancer.

Based on the results of our study, there are several interesting directions for future research that can deepen the understanding of the role of the TTK gene in breast tumors and expand clinical applications. Some research suggestions include investigating the molecular mechanisms by which TTK influences breast cancer progression; identifying specific targets regulated by TTK that contribute to tumor aggressiveness; and exploring the possible association between TTK

expression and other known biomarkers of breast cancer.

References

Afzal, S., Hassan, M., Ullah, S., Abbas, H., Tawakkal, F., & Khan, M. A. (2022). Breast Cancer; Discovery of Novel Diagnostic Biomarkers, Drug Resistance, and Therapeutic Implications. *Frontiers in Molecular Biosciences*, 9(2), 1–10. https://doi.org/10.3389/fmolb.2022.783450

Albogami, S. (2022). Comprehensive analysis of gene expression profiles to identify differential prognostic factors of primary and metastatic breast cancer. *Saudi Journal of Biological Sciences*, 29(7), 1–18. https://doi.org/10.1016/j.sjbs.2022.103318

Anderhub, S. J., Mak, G. W. Y., Gurden, M. D., Faisal, A., Drosopoulos, K., Walsh, K., Woodward, H. L., Innocenti, P., Westwood, I. M., Naud, S., Hayes, A., Theofani, E., Filosto, S., Saville, H., Burke, R., van Montfort, R. L. M., Raynaud, F. I., Blagg, J., Hoelder, S., & Linardopoulos, S. (2019). High proliferation rate and a compromised spindle assembly checkpoint confers sensitivity to the MPS1 inhibitor BOS172722 in triple-negative breast cancers. *Molecular Cancer Therapeutics*, 18(10), 1696–1707. https://doi.org/10.1158/1535-7163.MCT-18-1203

Bhushan, A., Gonsalves, A., & Menon, J. U. (2021). Current state of breast cancer diagnosis, treatment, and theranostics. *Pharmaceutics*, 13(5), 1–24. https://doi.org/10.3390/pharmaceutics13050723

Chandler, B. C., Moubadder, L., Ritter, C. L., Liu, M., Cameron, M., Wilder-Romans, K., Zhang, A., Pesch, A. M., Michmerhuizen, A. R., Hirsh, N., Androsiglio, M., Ward, T., Olsen, E., Niknafs, Y. S., Merajver, S., Thomas, D. G., Brown, P. H., Lawrence, T. S., Nyati, S., … Speers, C. (2020). TTK inhibition radiosensitizes basal-like breast cancer through impaired homologous recombination. *Journal of Clinical Investigation*, 130(2), 958–973. https://doi.org/10.1172/JCI130435

Chandrashekar, D. S., Karthikeyan, S. K., Korla, P. K., Patel, H., Shovon, A. R., Athar, M., Netto, G. J., Qin, Z. S., Kumar, S., Manne, U., Crieghton, C. J., & Varambally, S. (2022). UALCAN: An update to the integrated cancer data analysis platform. *Neoplasia*, 25(1), 18–27. https://doi.org/10.1016/j.neo.2022.01.001

Chen, F., Wu, P., Hu, H., Tian, D., Jiang, N., & Wu, C. (2018). Protein kinase TTK promotes proliferation and migration and mediates epithelial-mesenchymal transition in human bladder cancer cells. *International Journal of Clinical and Experimental Pathology*, 11(10), 4854–4861. http://www.ncbi.nlm.nih.gov/pubmed/31949560%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC6962905

Chen, J., Wu, R., Xuan, Y., Jiang, M., & Zeng, Y. (2020). Bioinformatics analysis and experimental validation of TTK as a biomarker for prognosis in non-small cell lung cancer. *Bioscience Reports*, 40(10), 1–14. https://doi.org/10.1042/BSR20202711

Combes, G., Barysz, H., Garand, C., Gama Braga, L., Alharbi, I., Thebault, P., Murakami, L., Bryne, D. P., Stankovic, S., Eyers, P. A., Bolanos-Garcia, V. M., Earnshaw, W. C., Maciejowski, J., Jallepalli, P. V., & Elowe, S. (2018). Mps1 Phosphorylates Its N-Terminal Extension to Relieve Autoinhibition and Activate the Spindle Assembly Checkpoint. *Current Biology*, 28(6), 872–883. https://doi.org/10.1016/j.cub.2018.02.002

Elango, R., Vishnubalaji, R., Shaath, H., & Alajez, N. M. (2021). Molecular subtyping and functional validation of TTK, TPX2, UBE2C, and LRP8 in sensitivity of TNBC to paclitaxel. *Molecular Therapy - Methods and Clinical Development*, 20(3), 601–614. https://doi.org/10.1016/j.omtm.2021.01.013

Fekete, J. T., & Győrffy, B. (2019). ROCplot.org: Validating predictive biomarkers of chemotherapy/hormonal therapy/anti-HER2 therapy using transcriptomic data of 3,104 breast cancer patients. *International Journal of Cancer*, 145(11), 3140–3151. https://doi.org/10.1002/ijc.32369

Gao, Y.-H., QU, S.-S., Cao, L.-Q., & Yao, M. (2022). TTK predicts triple positive breast cancer prognosis and regulates tumor proliferation and invasion. *Neoplasma*, 69(2), 274–282. https://doi.org/10.4149/neo

Győrffy, B. (2023). Discovery and ranking of the most robust prognostic biomarkers in serous ovarian cancer. *GeroScience*, 45(3), 1889–1898. https://doi.org/10.1007/s11357-023-00742-4

Gyorffy, B., Bottai, G., Lehmann-Che, J., Kéri, G., Orfi, L., Iwamoto, T., Desmedt, C., Bianchini, G., Turner, N. C., de Thè, H., André, F., Sotiriou, C., Hortobagyi, G. N., Di Leo, A., Pusztai, L., & Santarpia, L. (2014). TP53 mutation-correlated genes predict the risk of tumor relapse and identify MPS1 as a potential therapeutic kinase in TP53-mutated breast cancers. *Molecular Oncology*, 8(3), 508–519. https://doi.org/10.1016/j.molonc.2013.12.018

Jézéquel, P., Gouraud, W., Ben Azzouz, F., Guérin-Charbonnel, C., Juin, P. P., Lasla, H., & Campone, M. (2021). Bc-GenExMiner 4.5: New mining module computes breast cancer differential gene expression analyses. *Database*, 27(3), 1–10. https://doi.org/10.1093/database/baab007

King, J. L., Zhang, B., Li, Y., Li, K. P., Ni, J. J., Saavedra, H. I., & Dong, J. T. (2018). TTK promotes mesenchymal signaling via multiple mechanisms in triple negative breast cancer. *Oncogenesis*, 7(69), 1–13. https://doi.org/10.1038/s41389-018-0077-z

Kuijt, T. E. F., Lambers, M. L. A., Weterings, S., Ponsioen, B., Bolhaqueiro, A. C. F., Staijen, D. H. M., & Kops, G. J. P. L. (2020). A Biosensor for the Mitotic Kinase MPS1 Reveals Spatiotemporal Activity Dynamics and Regulation. *Current Biology*, 30(19), 3862–3870. https://doi.org/10.1016/j.cub.2020.07.062

Lee, M. Y., Marina, M., King, J. L., & Saavedra, H. I. (2014). Differential expression of centrosome regulators in Her2+ breast cancer cells versus non-tumorigenic MCF10A cells. *Cell Division*, 9(1), 1–14. https://doi.org/10.1186/1747-1028-9-3

Maia, A. R. R., De Man, J., Boon, U., Janssen, A., Song, J. Y., Omerzu, M., Sterrenburg, J. G., Prinsen, M. B. W., Willemsen-Seegers, N., De Roos, J. A. D. M., Van Doornmalen, A. M., Uitdehaag, J. C. M., Kops, G. J. P. L., Jonkers, J., Buijsman, R. C., Zaman, G. J. R., & Medema, R. H. (2015). Inhibition of the spindle assembly checkpoint kinase TTK enhances the efficacy of docetaxel in a triple-negative breast cancer model. *Annals of Oncology*, 26(10), 2180–2192. https://doi.org/10.1093/annonc/mdv293

Maire, V., Baldeyron, C., Richardson, M., Tesson, B., Vincent-Salomon, A., Gravier, E., Marty-Prouvost, B., De Koning, L., Rigaill, G., Dumont, A., Gentien, D., Barillot, E., Roman-Roman, S., Depil, S., Cruzalegui, F., Pierré, A., Tucker, G. C., & Dubois, T. (2013). TTK/hMPS1 Is an Attractive Therapeutic Target for Triple-Negative Breast Cancer. *PLoS ONE*, 8(5), 1–15. https://doi.org/10.1371/journal.pone.0063712

Mason, J. M., Wei, X., Fletcher, G. C., Kiarash, R., Brokx, R., Hodgson, R., Beletskaya, I., Bray, M. R., & Mak, T. W. (2017). Functional characterization of CFI-402257, a potent and selective Mps1/TTK kinase inhibitor, for the treatment of cancer. *PNAS*, 114(12), 3127–3132. https://doi.org/10.1073/pnas.1700234114

Menezo, Y., Clement, P., Clement, A., & Elder, K. (2020). Methylation: An ineluctable biochemical and physiological process essential to the transmission of life. *International Journal of Molecular Sciences*, 21(23), 1–13. https://doi.org/10.3390/ijms21239311

Miao, Y., Konno, Y., Wang, B., Zhu, L., Zhai, T., Ihira, K., Kobayashi, N., Watari, H., Jin, X., Yue, J., Dong, P., & Fang, M. (2023). Integrated multi-omics analyses and functional validation reveal TTK as a novel EMT activator for endometrial cancer. *Journal of Translational Medicine*, 21(1), 1–23. https://doi.org/10.1186/s12967-023-03998-8

Rivera-Rivera, Y., Vargas, G., Jaiswal, N., Núñez-Marrero, A., Li, J., Chen, D. T., Eschrich, S., Rosa, M., Johnson, J. O., Dutil, J., Chellappan, S. P., & Saavedra, H. I. (2022). Ethnic and racial-specific differences in levels of centrosome-associated mitotic kinases, proliferative and epithelial-to-mesenchymal markers in breast cancers. *Cell Division*, 17(6), 1–14. https://doi.org/10.1186/s13008-022-00082-3

Schick, J., Ritchie, R. P., & Restini, C. (2021). Breast Cancer Therapeutics and Biomarkers: Past, Present, and Future Approaches. Breast Cancer: *Basic and Clinical Research*, 15(3), 1–19. https://doi.org/10.1177/1178223421995854

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*, 71(3), 209–249. https://doi.org/10.3322/caac.21660

Tang, D., Zhao, X., Zhang, L., Wang, Z., & Wang, C. (2019). Identification of hub genes to regulate breast cancer metastasis to brain by bioinformatics analyses. *Journal of Cellular Biochemistry*, 120(6), 9522–9531. https://doi.org/10.1002/jcb.28228

Tang, J., Lu, M., Cui, Q., Zhang, D., Kong, D., Liao, X., Ren, J., Gong, Y., & Wu, G. (2019). Overexpression of ASPM, CDC20, and TTK Confer a Poorer Prognosis in Breast Cancer Identified by Gene Co-expression Network Analysis. *Frontiers in Oncology*, 9(4), 1–14. https://doi.org/10.3389/fonc.2019.00310

Xie, Y., Wang, A., Lin, J., Wu, L., Zhang, H., Yang, X., Wan, X., Miao, R., Sang, X., & Zhao, H. (2017). Mps1/TTK: A novel target and biomarker for cancer. *Journal of Drug Targeting*, 25(2), 112–118. https://doi.org/10.1080/1061186X.2016.1258568

Xu, Q., Xu, Y., Pan, B., Wu, L., Ren, X., Zhou, Y., Mao, F., Lin, Y., Guan, J., Shen, S., Zhang, X., Wang, C., Zhong, Y., Zhou, L., Liang, Z., Zhao, H., & Sun, Q. (2016). TTK is a favorable prognostic biomarker for triple-negative breast cancer survival. *Oncotarget*, 7(49), 81815–81829. https://doi.org/10.18632/oncotarget.13245

Zaman, G. J. R., De Roos, J. A. D. M., Libouban, M. A. A., Prinsen, M. B. W., De Man, J., Buijsman, R. C., & Uitdehaag, J. C. M. (2017). TTK inhibitors as a targeted therapy for CTNNB1 (β-catenin) mutant cancers. *Molecular Cancer Therapeutics*, 16(11), 2609–2617. https://doi.org/10.1158/1535-7163.MCT-17-0342

Zhang, X., Huang, L., Sun, J., Liu, J., & Zong, Y. (2023). Monopolar spindle 1 contributes to tamoxifen resistance in breast cancer through phosphorylation of estrogen receptor α. *Breast Cancer Research and Treatment*, 202(9), 595–606. https://doi.org/10.1055/s-0037-1608898