Breast Cancer Research and Treatment (2019) 178:57–62
https://doi.org/10.1007/s10549-019-05365-y

PRECLINICAL STUDY

Overexpression of TK1 and CDK9 in plasma-derived exosomes is associated with clinical resistance to CDK4/6 inhibitors in metastatic breast cancer patients

Marzia Del Re[1] · Ilaria Bertolini[2] · Stefania Crucitta[1] · Lorenzo Fontanelli[1] · Eleonora Rofi[1] · Claudia De Angelis[2] · Lucrezia Diodati[2] · Diletta Cavallero[2] · Giulia Gianfilippo[1] · Barbara Salvadori[2] · Stefano Fogli[1] · Alfredo Falcone[2] · Cristian Scatena[3] · Antonio Giuseppe Naccarato[3] · Manuela Roncella[4] · Matteo Ghilli[4] · Riccardo Morganti[5] · Andrea Fontana[2] · Romano Danesi[1]

Received: 18 May 2019 / Accepted: 15 July 2019 / Published online: 26 July 2019

Abstract

Purpose Cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) improve progression-free survival (PFS) in patients with hormone receptor-positive (HR+) advanced breast cancer. However, a better knowledge of predictive biomarkers of response and resistance to CDK4/6i is needed. Therefore, the present article addresses the role of the mRNA expression of thymidine kinase 1 (TK1), CDK4, 6 and 9 in plasma-derived exosomes and their relevance in the pharmacologic activity of CDK4/6i.

Methods Blood samples of 40 HR+/HER2- advanced breast cancer patients were collected before (T0) the administration of palbociclib plus hormonal therapy and after 3 months (T1). RNA was isolated from exosomes and analysed for the expression of TK1, CDK 4, 6 and 9 by digital droplet PCR (ddPCR).

Results A higher value of TK1 copies/ml at baseline (T0) was significantly associated with the number of previous lines of chemotherapy ($p=0.009$). In patients with PD, a significant increase was observed in the number of copies/ml of TK1 ($p=0.01$) and CDK9 ($p=0.03$) comparing T1 vs. T0 values. No significant correlations between response to treatment and clinical parameters were found at univariate analysis. High baseline CDK4 expression was significantly correlated with longer PFS in patients treated with fulvestrant + palbociclib (low versus high: 6.45 months vs. not reached, $p=0.01$).

Conclusions The present study demonstrates that, in plasma-derived exosomes, high baseline CDK4 mRNA levels are associated with response to palbociclib plus hormonal therapy, while the increase in TK1 and CDK9 mRNA copies/ml is associated with clinical resistance.

Keywords TK1 · CDK4/6/9 · Metastatic breast cancer · CDK4/6 inhibitors · Plasma-derived exosomes · Predictive biomarkers

Background

Breast cancer has been widely studied with respect to its aetiology [8, 14]; however, little is known about the mechanisms underlying its progression as a systemic disease. Loss

Marzia Del Re and Ilaria Bertolini equally contributed to this work.

✉ Romano Danesi
romano.danesi@unipi.it

[1] Unit of Clinical Pharmacology and Pharmacogenetics, Department of Clinical and Experimental Medicine, University of Pisa, 55, Via Roma, 56126 Pisa, Italy

[2] Unit of Medical Oncology, Department of Translational Research and New Technologies in Medicine, University of Pisa, Pisa, Italy

[3] Unit of Pathology, Department of Translational Research and New Technologies in Medicine, University of Pisa, Pisa, Italy

[4] Unit of Breast Surgery, Breast Cancer Center, University Hospital of Pisa, Pisa, Italy

[5] Section of Statistics, Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy

of cell-cycle control is a classical hallmark of cancer and it has been demonstrated to be an effective target in metastatic breast cancer patients resistant to hormonal therapies. In particular, cyclin-dependent kinases (CDKs) play a key role in promoting cell-cycle progression, and a significant proportion of breast cancers exhibit dysregulation of the CDK4 pathway [20]. Cyclin-dependent kinases 4 and 6 (CDK4/6), which are activated by type D cyclins, promote cell-cycle entry to initiate the transition from the G1 to the S phase. Evidence suggests that breast cancer patients who develop resistance to endocrine therapy remain dependent on cyclin D1 and CDK4 for cell proliferation [9]. In this regard, CDK4/6 inhibitors (CDK4/6i), such as palbociclib, ribociclib and abemaciclib, have demonstrated to be highly active in ER+/HER2- metastatic breast cancer patients progressed to hormonal therapy. However, despite their efficacy, predictive biomarkers of response to these agents are still under investigation. Recent published data report that primary resistance to palbociclib may be mediated by loss of the Rb1 gene or appearance of Rb mutations [6]. Other cyclines are involved in the cell-cycle progression, growth, proliferation, differentiation and apoptosis, i.e. CDK9 [22]. Moreover, the serum activity of thymidine kinase 1 (TK1) seems to be correlated to response to CDK4/6i [2]. TK1 is a cytosolic cell-cycle-regulated enzyme important for nucleotide metabolism during DNA synthesis, as it catalyses the conversion of thymidine to deoxythymidine monophosphate, which is further phosphorylated to di- and triphosphates before its use for DNA synthesis [21].

Exosomes are microvesicles actively released from cancer cells; they have a size range of 40–150 nm and they carry proteins, RNA and DNA of cells from which they are originated. Exosomes are involved in the exchange of information between cells, promoting intercellular communication without cell-to-cell contact. They are involved in tumorigenesis, and able to influence tumour microenvironment in favour of immune escape, therapy resistance, tumour growth and metastasis [19].

Our hypothesis is to assess if changes in the exosomal levels of targets relevant to CDK4/6i, including TK1, CDK4, 6 and 9, may be predictive of response/resistance to CDK4/6i.

Patients and methods

Patient selection

The ECLIPS study (Exosome Cdk cLInical PatientS), is a prospective, pharmacogenetic study, which enrolled mBC patients treated with palbociclib in association with hormonal therapy (letrozole or fulvestrant) as per approved label. The study was approved by the Ethics Committee of Pisa University Hospital and conducted in accordance with the principles of the Declaration of Helsinki. All patients gave their signed informed consent before blood collection and data analysis.

Exosome isolation, RNA extraction and analysis of TK1 and CDKs

Serial blood samples were obtained at baseline (prior to palbociclib, T0) and after 3 months of treatment (T1). 6 ml of blood were collected in EDTA tubes and centrifuged at 1900×*g* for 10 min at 4 °C within 2 h after drawing to collect plasma which was stored at − 80 °C until analysis. Plasma samples were then centrifuged again at 1900×*g* for 15 min to remove cellular debris. Exosome isolation and RNA extraction was performed using the exoRNeasy kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

The mRNA analysis of TK1 and CDK4, 6 and 9 was performed by the QX100 ddPCR (Bio-Rad, Hercules, CA) using the One-Step RT-ddPCR kit. The PrimePCR Expression Probe Assays for human TK1 and CDK4, 6 and 9 were used to assess their levels, while the human β-actin ddPCR assay was used as internal control. Fluorescence signal quantification was performed by the droplet reader and the QuantaSoft software (Bio-Rad). The ratio of positive vs negative droplets was used to determine the number of mRNA copies/ml of the target molecule in the input reaction. Droplets with a fluorescence intensity threshold higher than 4000 were considered positive.

Statistical analysis

Categorical variables, including ECOG performance status, tumour burden, lines of treatment, hormone sensitivity, type of previous hormonotherapy, pre- versus peri-menopausal status, visceral versus bone disease, number of tumour sites and previous chemotherapy were described by absolute and relative frequencies. To evaluate the normality of the quantitative data distributions, the Kolmogorov–Smirnov test was performed. Quantitative assessments of TK1, CDK4, 6, 9 were performed by the Mann–Whitney test (two tailed). The assessment of the paired data (matched and repeated) was performed by Wilcoxon test (two tailed). Progression-free survival (PFS) curves were created by the Kaplan–Meier method, log-rank test was used to evaluate differences between curves and hazard ratio was calculated to compare cumulative risks. Differences were considered significant at $p < 0.05$. All statistical analyses, descriptive and inferential, were performed using SPSS version 24 and R version 3.5.2.

Results

Forty patients were enrolled in the ECLIPS study. All patients received palbociclib plus hormonal therapy (letrozole or fulvestrant) for metastatic disease. Hormonal

treatment was performed according to clinical indication: palbociclib in combination with letrozole as initial treatment for advanced disease, and in association with fulvestrant in women with disease progression following endocrine therapy. Clinical data of patients are reported in Table 1. The objective response rate among all patients enrolled was 20% and the clinical benefit was 80%; in particular, 20% of patients achieved a partial response (PR), 60% stable disease (SD) and 20% had a progression of disease (PD).

The biomarker analysis showed that higher TK1 copies/ml at baseline were significantly associated with the number of previous lines of chemotherapy ($p = 0.009$). The analysis of T1 versus T0 in patients with PD demonstrated a significant increase in TK1 ($p = 0.01$) and CDK9 ($p = 0.03$), but not in patients with PR + SD (Fig. 1). Median values (copies/ml) of TK1, CDK4, 6 and 9 according to clinical response to CDK4/6i are reported in Table 2. No other significant association was found comparing the baseline levels of TK1, CDK4, 6 or 9 in the overall population with respect to the type of response, PFS, tumour burden, line of treatment, hormone sensitivity, type of previous hormonotherapy, ECOG, pre/peri-menopausal status, visceral versus bone disease, number of tumour sites ($p \geq 0.05$).

Since the follow-up was completed, a correlative analysis of baseline biomarker values and PFS was performed in patients treated with the association of fulvestrant + palbociclib. A significant correlation was found between higher baseline amount (> 5050 copies/ml) of CDK4 and longer PFS (high versus low CDK4: not reached vs. 6.45 months, $p = 0.01$; Fig. 2).

Table 1 Clinical characteristics of patients

Clinical data	*N* (*n* = 40)
Age, years (range)	54.4 (39–72)
ECOG PS	
0	31 (77%)
1	9 (23%)
Stage at initial diagnosis	
I	7 (17%)
II	11 (28%)
III	10 (25%)
IV	12 (30%)
Menopausal status	
Pre-menopausal	12 (30%)
Post-menopausal	28 (70%)
Previous (neo)adjuvant therapy	
Neoadjuvant chemotherapy	8 (20%)
Adjuvant chemotherapy	17 (43%)
Adjuvant endocrine therapy	27 (67%)
Sites of metastases	
Visceral*	30 (75%)
Non-visceral	10 (25%)
Lines of treatment for advanced disease	
0	19 (47%)
1 or greater	21 (53%)
Lines of chemotherapy for advanced disease	
0	23 (57%)
1	12 (30%)
2	1 (3%)
3 or greater	4 (10%)
Endocrine therapy for advanced disease	
0	23 (57%)
1	13 (32%)
2	3 (8%)
3	1 (3%)
Previous sensitivity to endocrine therapy[a]	
Yes	11 (28%)
No	29 (72%)

*Visceral refers to lung, liver, brain, pleural, and peritoneal involvement

[a]Sensitivity to previous hormonal therapy was defined as a documented clinical benefit from at least one previous endocrine therapy in the metastatic setting or treatment with at least 24 months of adjuvant therapy before disease recurrence

Discussion

Circulating nucleic acids are being recognized as potential predictive biomarkers of response to therapies due to their specificity and lack of invasiveness of sampling procedures [3]. We investigated whether exosomal mRNA expression of TK1, CDK4, 6, and 9 could be used as biomarker of resistance to CDK4/6i in patients with mBC ER+/HER2- enrolled in the ECLIPSE biomarker study. The results demonstrate that TK1 and CDK9 mRNA expression in plasma-derived exosomes is associated with resistance to palbociclib, since their levels were significantly increased after 3 months of treatment in patients with PD. Moreover, high CDK4 levels at baseline are significantly associated with longer PFS in patients treated with fulvestrant + palbociclib. It was already shown that high TK1 activity and expression levels are associated with malignant lesions [11, 13] and higher risk or recurrence [10, 16]. Moreover, additional studies demonstrated that TK1 activity was associated with PFS and OS in patients with advanced and metastatic breast cancer [4]. The association found in the NeoPalAna study between TK1 variations and response to palbociclib provided the basis to further investigate the potential predictive value of TK1 on response to CDK4/6i in mBC patients. Our study demonstrates that the increase in TK1 mRNA expression is significantly associated with PD in patients treated with palbociclib and provides an internal validation of the CDK6/9 increase in resistant patients, being concordant with their changes in exosomes.

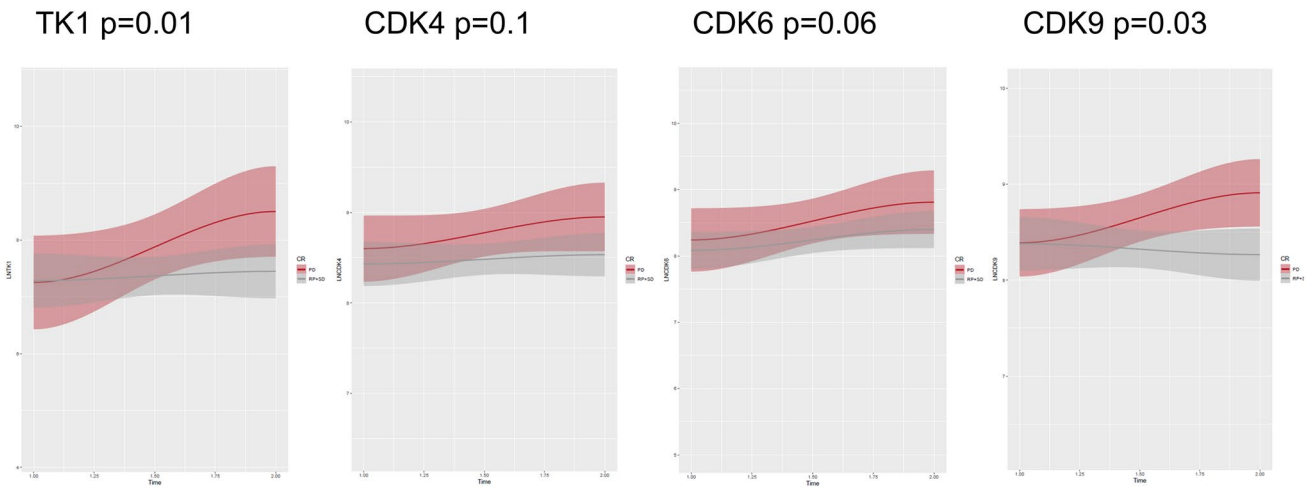

Fig. 1 Plots representing the average trend (T0 versus T1) of TK1 and CDK4, 6, 9 according to response (RP + SD as grey; PD as red) to treatment in the overall population

Table 2 Median values (copies/ml) of TK1, CDK4, 6 and 9 according to clinical response to CDK4/6i

Biomarker	Clinical response	N	T0 median copies/ml	T1 median copies/ml	*p*-Value
TK1	RP + SD	25	1300	1800	0.7
	PD	15	1200	3350	0.01
CDK4	RP + SD	25	5100	5600	0.9
	PD	15	4550	8200	0.1
CDK6	RP + SD	25	3800	3900	0.2
	PD	15	2800	6900	0.06
CDK9	RP + SD	25	4340	4900	0.9
	PD	15	3800	7500	0.03

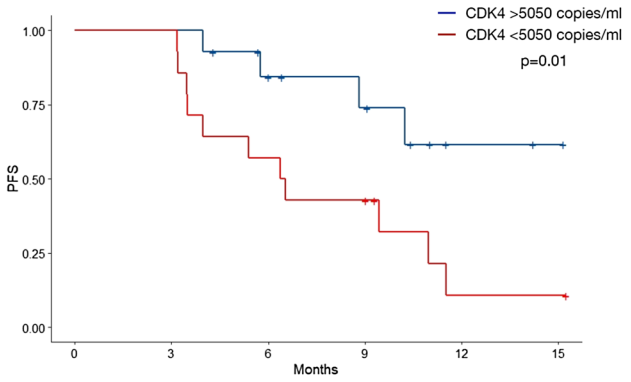

Fig. 2 Progression-free survival (PFS) of patients treated with fulvestrant + palbociclib based on baseline CDK4 expression

It has been already reported that cyclin E/CDK2 mediates resistance to palbociclib in breast cancer [12]; moreover, high CDK6 expression has been already described as involved in resistance to fulvestrant in breast cancer cells and high CDK6 expression levels in tissues have been associated with shorter PFS in patients with metastatic ER+ breast cancer treated with fulvestrant [1]. However, our data further expands the knowledge about the involvement of levels of drug targets in treatment outcome by showing that the overexpression of CDK9 is detected in patients not responding to palbociclib. In a study on 84 breast cancer patients who had undergone surgical resection after neoadjuvant chemotherapy, high CDK9 expression was significantly associated with an increase in OS ($p = 0.046$) after 3 years; however, Kaplan–Meier curves for OS, loco-regional recurrence-free survival (LRRFS), distant failure-free survival (DFFS), recurrence-free survival (RFS), and event-free survival (EFS) did not reach statistical significance [17]. Conversely, to the best of our knowledge, this is the first report that finds an association between CDK9 levels and progression to palbociclib. CDK9 phosphorylates a serine residue of RNA polymerase II carboxy-terminal domain; CDK9 is not cell-cycle regulated, but it acts preferentially in differentiation and transcription processes [22]. CDK9 was found elevated in a MCF7-derived panel of ERα+ breast cancer cells which can proliferate in the absence of oestrogen, and displays reduced sensitivities to anti-oestrogen therapies [18]. Indeed, inhibition of CDK9 reduces the protein levels of cMYC in MCF7 cells as well as selectively inhibits the oestrogen-independent growth of resistant cell lines, suggesting that CDK9 is a potential drug target in endocrine-resistant breast cancer [18]. For these reasons, CDK9 is in constant development in cancer therapy, and CDK9 inhibitors have demonstrated good antitumour activity in vitro [7, 15].

This is the first study reporting that baseline CDK4 expression may be a predictive biomarker of response in patients treated with palbociclib. This is not surprising, since

CDK4 is the target of the CDK4/6i, and it is expected that the hyper-expression of a drug target influences treatment outcome.

Conclusions

Thus, the findings of the present study suggest that strategies aimed at a broader suppression of CDKs may reduce the mechanisms of resistance to pharmacological inhibition [5]. Even if our study is limited by its small and heterogeneous cohort of patients, it nonetheless provides the first evidence that the early increases in the expressions of TK1, CDK4 and 9 in exosomes are significantly correlated with worse treatment response and disease progression in HR+/HER2- mBC patients and can be used to monitor treatment outcome.

Author contributions MDR, IB, SC, ER, CDA, LD, DC, GG, LF, BS, SF, MG, CS, AGN, MR, AF, RM, AF, and RD made substantial contributions to conception and design. MDR, SC, ER, LF, GG, SF, and RD made substantial contributions to the laboratory analysis and interpretation of data. IB, CDA, LD, DC, BS, MG, CS, AGN, MR, AF, and AF made substantial contributions to the acquisition of clinical data and their interpretation. RM, SC, and LF performed the statistical analysis. MDR, IB, SC, ER, LF, GG, AF, and RD have been involved in drafting the manuscript. MDR, IB, MG, CS, AGN, MR, AF, GG, RM, AF, and RD have been involved in revising the paper critically for important intellectual content. All authors gave their final approval of the version to be published. Each author participated sufficiently in the work to take public responsibility for appropriate portions of its content. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. RD is responsible for the financial support for the project leading to this publication.

Funding RD's Institutional fundings

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Alves CL, Elias D, Lyng M, Bak M, Kirkegaard T, Lykkesfeldt AE, Ditzel HJ (2016) High CDK6 protects cells from fulvestrant-mediated apoptosis and is a predictor of resistance to fulvestrant in estrogen receptor-positive metastatic breast cancer. Clin Cancer Res 22:5514–5526. https://doi.org/10.1158/1078-0432.CCR-15-1984
2. Bagegni N, Thomas S, Liu N, Luo J, Hoog J, Northfelt DW, Goetz MP, Forero A, Bergqvist M, Karen J, Neumuller M, Suh EM, Guo Z, Vij K, Sanati S, Ellis M, Ma CX (2017) Serum thymidine kinase 1 activity as a pharmacodynamic marker of cyclin-dependent kinase 4/6 inhibition in patients with early-stage breast cancer receiving neoadjuvant palbociclib. Breast Cancer Res 19:123. https://doi.org/10.1186/s13058-017-0913-7
3. Berghuis AM, Koffijberg H, Prakash J, Terstappen LW, IJzerman M (2017) Detecting blood-based biomarkers in metastatic breast cancer: a systematic review of their current status and clinical utility. Int J Mol Sci. https://doi.org/10.3390/ijms18020363
4. Bjohle J, Bergqvist J, Gronowitz JS, Johansson H, Carlsson L, Einbeigi Z, Linderholm B, Loman N, Malmberg M, Soderberg M, Sundquist M, Walz TM, Ferno M, Bergh J, Hatschek T (2013) Serum thymidine kinase activity compared with CA 15-3 in locally advanced and metastatic breast cancer within a randomized trial. Breast Cancer Res Treat 139:751–758. https://doi.org/10.1007/s10549-013-2579-x
5. Chen P, Lee NV, Hu W, Xu M, Ferre RA, Lam H, Bergqvist S, Solowiej J, Diehl W, He YA, Yu X, Nagata A, VanArsdale T, Murray BW (2016) Spectrum and degree of CDK drug interactions predicts clinical performance. Mol Cancer Ther 15:2273–2281. https://doi.org/10.1158/1535-7163.MCT-16-0300
6. Condorelli R, Spring L, O'Shaughnessy J, Lacroix L, Bailleux C, Scott V, Dubois J, Nagy RJ, Lanman RB, Iafrate AJ, Andre F, Bardia A (2018) Polyclonal RB1 mutations and acquired resistance to CDK 4/6 inhibitors in patients with metastatic breast cancer. Ann Oncol 29:640–645. https://doi.org/10.1093/annonc/mdx784
7. de Falco G, Giordano A (1998) CDK9 (PITALRE): a multifunctional cdc2-related kinase. J Cell Physiol 177:501–506. https://doi.org/10.1002/(SICI)1097-4652(199812)177:4%3c501:AID-JCP1%3e3.0.CO;2-4
8. Fernandez SV, Russo J (2010) Estrogen and xenoestrogens in breast cancer. Toxicol Pathol 38:110–122. https://doi.org/10.1177/0192623309354108
9. Finn RS, Crown JP, Lang I, Boer K, Bondarenko IM, Kulyk SO, Ettl J, Patel R, Pinter T, Schmidt M, Shparyk Y, Thummala AR, Voytko NL, Fowst C, Huang X, Kim ST, Randolph S, Slamon DJ (2015) The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study. Lancet Oncol 16:25–35. https://doi.org/10.1016/S1470-2045(14)71159-3
10. He Q, Fornander T, Johansson H, Johansson U, Hu GZ, Rutqvist LE, Skog S (2006) Thymidine kinase 1 in serum predicts increased risk of distant or loco-regional recurrence following surgery in patients with early breast cancer. Anticancer Res 26:4753–4759
11. He Q, Zou L, Zhang PA, Lui JX, Skog S, Fornander T (2000) The clinical significance of thymidine kinase 1 measurement in serum of breast cancer patients using anti-TK1 antibody. Int J Biol Markers 15:139–146
12. Herrera-Abreu MT, Palafox M, Asghar U, Rivas MA, Cutts RJ, Garcia-Murillas I, Pearson A, Guzman M, Rodriguez O, Grueso J, Bellet M, Cortes J, Elliott R, Pancholi S, Baselga J, Dowsett M, Martin LA, Turner NC, Serra V (2016) Early adaptation and acquired resistance to CDK4/6 inhibition in estrogen receptor-positive breast cancer. Cancer Res 76:2301–2313. https://doi.org/10.1158/0008-5472.CAN-15-0728
13. Mao Y, Wu J, Wang N, He L, Wu C, He Q, Skog S (2002) A comparative study: immunohistochemical detection of cytosolic thymidine kinase and proliferating cell nuclear antigen in breast cancer. Cancer Invest 20:922–931
14. Mazzanti CM, Lessi F, Armogida I, Zavaglia K, Franceschi S, Al Hamad M, Roncella M, Ghilli M, Boldrini A, Aretini P, Fanelli G,

Marchetti I, Scatena C, Hochman J, Naccarato AG, Bevilacqua G (2015) Human saliva as route of inter-human infection for mouse mammary tumor virus. Oncotarget 6:18355–18363. https://doi.org/10.18632/oncotarget.4567

15. Morales F, Giordano A (2016) Overview of CDK9 as a target in cancer research. Cell Cycle 15:519–527. https://doi.org/10.1080/15384101.2016.1138186
16. Nisman B, Allweis T, Kadouri L, Mali B, Hamburger T, Baras M, Gronowitz S, Peretz T (2013) Comparison of diagnostic and prognostic performance of two assays measuring thymidine kinase 1 activity in serum of breast cancer patients. Clin Chem Lab Med 51:439–447. https://doi.org/10.1515/cclm-2012-0162
17. Schlafstein AJ, Withers AE, Rudra S, Danelia D, Switchenko JM, Mister D, Harari S, Zhang H, Daddacha W, Ehdaivand S, Li X, Torres MA, Yu DS (2018) CDK9 expression shows role as a potential prognostic biomarker in breast cancer patients who fail to achieve pathologic complete response after neoadjuvant chemotherapy. Int J Breast Cancer 2018:6945129. https://doi.org/10.1155/2018/6945129
18. Sengupta S, Biarnes MC, Jordan VC (2014) Cyclin dependent kinase-9 mediated transcriptional de-regulation of cMYC as a critical determinant of endocrine-therapy resistance in breast cancers. Breast Cancer Res Treat 143:113–124. https://doi.org/10.1007/s10549-013-2789-2
19. Tickner JA, Urquhart AJ, Stephenson SA, Richard DJ, O'Byrne KJ (2014) Functions and therapeutic roles of exosomes in cancer. Front Oncol 4:127. https://doi.org/10.3389/fonc.2014.00127
20. Vasan N, Yelensky R, Wang K, Moulder S, Dzimitrowicz H, Avritscher R, Wang B, Wu Y, Cronin MT, Palmer G, Symmans WF, Miller VA, Stephens P, Pusztai L (2014) A targeted next-generation sequencing assay detects a high frequency of therapeutically targetable alterations in primary and metastatic breast cancers: implications for clinical practice. Oncologist 19:453–458. https://doi.org/10.1634/theoncologist.2013-0377
21. Welin M, Kosinska U, Mikkelsen NE, Carnrot C, Zhu C, Wang L, Eriksson S, Munch-Petersen B, Eklund H (2004) Structures of thymidine kinase 1 of human and mycoplasmic origin. Proc Natl Acad Sci USA 101:17970–17975. https://doi.org/10.1073/pnas.0406332102
22. Yu DS, Cortez D (2011) A role for CDK9-cyclin K in maintaining genome integrity. Cell Cycle 10:28–32. https://doi.org/10.4161/cc.10.1.14364