REVIEW

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Markers of Predicting Response to Neoadjuvant Chemotherapy in Breast Cancer: New in Molecular Oncology

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Abstract

It is known that complete pathomorphological response (pCR) after neoadjuvant therapy (NAC) in patients with breast cancer (BC) correlates with higher rates of recurrence-free and overall survival. In turn, the widespread use of neoadjuvant therapy for the treatment of breast cancer defines the clinical need for prognostic markers of response to ongoing therapy. Currently, some clinicopathological prognostic factors are used to assess the potential benefit of neoadjuvant systemic therapy for female patients, but they have limited applicability. In the era of precision medicine and personalised treatment, a search for new prognostic markers is needed to better tailor patient-specific therapy. To date, novel factors have been proposed to predict response to preoperative treatment in breast cancer patients, but they are either not yet used in routine clinical practice or have limited application. Thus, this review summarises data on both established and proven biomarkers and the latest prognostic factors for response to neoadjuvant treatment in breast cancer patients.

Keywords: Breast cancer- neoadjuvant chemotherapy- predictive biomarkers

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Introduction

Worldwide mortality rates from malignant neoplasms (MN) of various localisations remain at a high level. To date, breast cancer occupies the leading position in the structure of cancer morbidity among women, and predicting the outcome of this oncopathology is still an unsolved problem [1].

The use of chemotherapeutic agents in the preoperative period is a standard treatment option for patients with breast cancer [2-3], especially in aggressive subtypes, such as triple negative breast cancer (TNBC) and HER2+ breast cancer [4-5]. The use of NAC allows to achieve a complete pathomorphological response with a further increase in recurrence-free and overall survival of patients [6-8].

Achieving pCR is one of the main goals for NAC prescription, but it occurs in only a fraction of patients: 30-50% in TNBC, 50-80% in HER2-positive and 5-20% in luminal breast cancer [9]. This is why it is crucial to identify markers for predicting breast cancer patients' pCR, which will be key to identifying patients to whom NAC can maximise the therapeutic benefit [10-11].

To date, several biomolecular markers are known and actively used to predict the efficacy of NAC in the treatment of breast cancer. First of all, surrogate markers of molecular subtypes of breast cancer: ER (estrogen receptor), PR (progesterone receptor) and HER2 (human epidermal growth factor receptor type 2) markers. They are recommended as mandatory for determining the receptor status of breast cancer in order to select the most effective treatment and improve disease prognosis [12].

Another biomolecular marker widely used in the world literature is *Ki-67*, which has been used for a long time as an indicator of tumour cell proliferation and is used to predict the response to NAC [5, 13]. In clinical practice, *Ki-67* is considered to be a reliable indicator of response to treatment, but there are difficulties in its widespread use due to the estimation of threshold values [14]. The importance of *Ki-67* assessment is greatest in luminal A and luminal B HER2- breast cancer [5]. Higher expression of *Ki-67* is generally observed in TNBC [13]. However, the data are still controversial. A study by Chinese scientists showed higher *Ki-67* expression in patients who achieved a complete pathomorphological response [15]. At the same time, in the work of French colleagues, there was no statistically significant difference in Ki-67 expression between the groups of patients who achieved pCR, which was 68.2%, and those who did not achieve pCR which was 63.85% (p=0.48) [16].

Genomic markers of response to NAC have been described in the literature for a long time [6]. DNA mutations are considered as predictors of prognosis, for

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example, mutations in the *BRCA1* and *BRCA2* genes, which contribute to hereditary predisposition to breast cancer [17]. Mutations of these genes are found in 15% of cases in patients with the molecular subtype of TNBC [6, 18]. At the same time, the frequency of pCR in patients with a mutation in *TNBC* under treatment with standard NAC and platinum drugs ranges from 35 to 70% and to 80% [19].

Somatic mutation of the *PIK3CA* gene is also one of the widely studied mutations that is frequently found in breast cancer [10]. In a recent study, the impact of this gene mutation on the outcome of *TNBC* was shown for the first time: patients with *PIK3CA* mutation receiving platinum and paclitaxel-based NAC had a low relapse-free survival rate compared to patients without the mutation [20].

Some biomarkers of response to NAC are currently known and actively used in clinical practice, but they are effective only for a specific subtype of cancer (Figure 1). or do not have 100% significance in practical application.

This is why researchers face the crucial task of finding new biomarkers to predict response to neoadjuvant chemotherapy in breast cancer. The aim of this literature review is to consider potentially useful predictive markers of response to NAC in breast cancer.

The literature search was conducted in PubMed and Google Scholar databases using the keywords “breast cancer”, “neoadjuvant chemotherapy”, “marker of neoadjuvant chemotherapy efficacy in breast cancer”, “markers of response to NAC” in different variations. Full-text articles from 2014 to 2023 were included. The language of the studies was not a barrier to inclusion in this literature review. A total of 69 literature sources were included in the review.

As a result of literature review, we have identified potentially useful candidate markers for predicting response to NAC in breast cancer, with studies nearing the clinical trial stage (Table 1).

We also identified several categories of predictive markers of response to NAC in breast cancer as a result of our literature review.

Biomolecular and biochemical markers

The steroid hormone receptor AR (also known as NR3C4) is prevalent in 90% of all breast cancer cases [28, 29]. However, the question of whether this receptor is a predictive factor for response to NAC in breast cancer and what role it plays in oncogenesis remains open to this day [29]. A recently published study speculated about the nature of this receptor. They found that AR activation has an inhibitory effect on ER. Since ERα is the dominant pathway that promotes tumour growth in ER+ breast cancer, suppression of ERα via AR may slow tumour progression, which may further lead to a positive outcome in patients [30]. AR expression is absent in 80% of TNBC cases. At the same time, TNBC with AR- correlates with a higher rate of achieving complete pathomorphological response than AR+. This proves that AR+ reduces the likelihood of pCR [18]. There is a suggestion that the lower pCR rate for AR-expressing tumours may be due to a lower proliferation rate, making this subgroup more resistant to chemotherapy [13].

For other markers, such as *VEGFR2* (vascular endothelial growth factor receptor 2) and VIM (vimetin) in TNBC there is no association with a high probability of achieving pCR [31]. However, *FGFR4* (fibroblast growth factor receptor 4), NUP98 (nuclear pore complex protein), *Bcl2* (apoptosis regulator 2), *ALDH1* (aldehyde dehydrogenase 1), YAP1 (Yes-associated protein 1) and MMP7 (matrix matalloproteinase-7) have been shown to be associated with poor response to NAC in TNBC [13, 31].

To date, the FGFR4 protein is known to contribute to

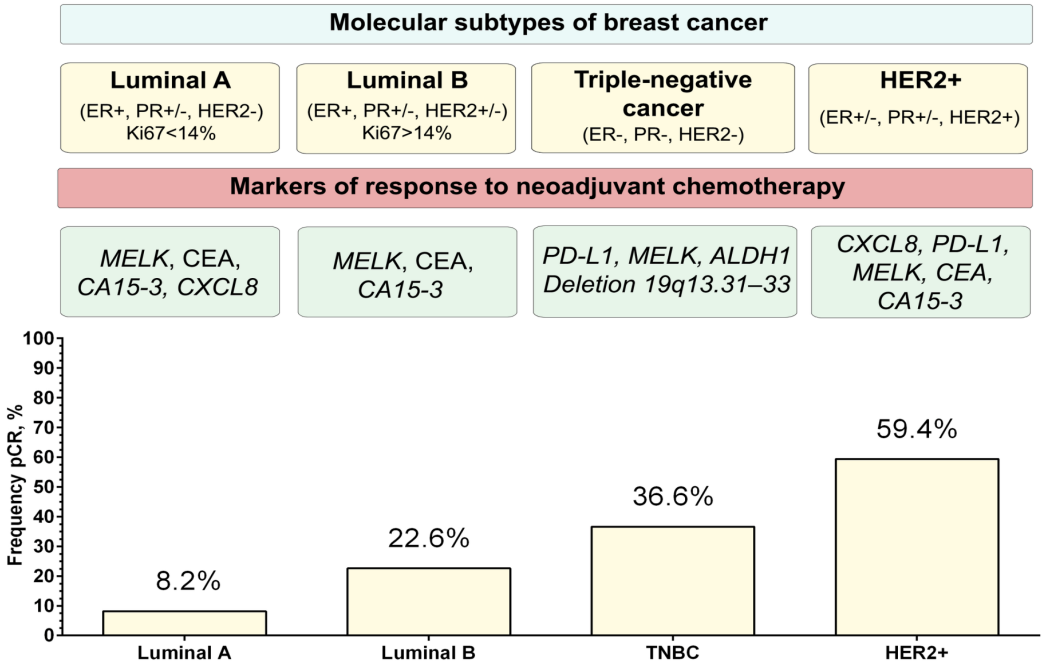

Figure 1. Markers of Response to NAC and Frequency of Complete Pathomorphological Response for Known Molecular Subtypes of Breast Cancer

Table 1. Potential Candidate Biomarkers of Response to NAC in Breast Cancer

Biomarker	Research stage	Methodology	Result
CXCL-8	Continued clinical trials are required.	The study included 303 patients with triple negative breast cancer. The NAC regimen included weekly therapy with paclitaxel and carboplatin for all patients. Serum *CXCL8* levels were measured at baseline and during surgery using enzyme-linked immunosorbent assay (ELISA). Immunohistochemistry was used to detect *CXCR1* and *CXCR2* expression in patients with residual tumours after NAC.	Low expression of *CXCL8* is associated with a positive response to NAC in TNBC patients [21]. Only four of 103 patients who achieved pCR developed disease relapse [21]. High *CXCL8* level is associated with worse outcome in ER/PR+ and HER2+ breast cancer [22]. *CXCL8* receptors (CXCR1 and CXCR2) may be a potentially effective therapeutic target. According to phase I clinical trials, reparixin in combination with paclitaxel, targeting CXCR inhibition, reduced tumour metastasis [21].
PD-L1	Continued clinical trials are required.	The presented review discusses the prognostic aspects of *PD-L1* testing. The method of immunohistochemical staining was used to assess *PD-L1* expression.	PD-L1 expression is found in HER2+ and triple negative breast cancer [23]. Also, PD-L1 can be used as a prognostic marker of the frequency of achieving pCR for TNBC [23].
MELK	Continued clinical trials are required.	A total of 7135 patients with ER+, HER2- and triple negative breast cancer were included. NAC regimens included anthracyclines and taxanes. In order to investigate biological function, groups with low and high MELK expression were compared using gene set enrichment analysis (GSEA) with gene sets from the Molecular Signature Database (MSigDB).	MELK is one of the proliferation markers and is included in clinically used prognostic panels such as MammaPrint and PAM50. When MELK expression is high, achievement of pCR is observed in ER+, HER2- and triple negative breast cancer patients ($p < 0.001$ and $p = 0.027$, respectively, with the following NAC regimen: anthracycline and taxane; $p = 0.006 + p = 0.015$ for cyclophosphamide, doxorubicin, paclitaxel and 5-fluorouracil; $p = 0.003$ and $p = 0.046$, respectively, for cyclophosphamide, doxorubicin, fluorouracil and paclitaxel) [24].
ALDH1	The development stage of therapeutic models.	A total of 40 patients who received 3-6 courses of anthracycline and/or taxane-based NAC were included. Immunohistochemical staining was used to assess *ALDH1* expression.	Treatments targeting ALDH1 inhibition may improve the therapeutic outcome of chemotherapy. To date, therapeutic models targeting surface markers, signalling cascades, microenvironment and ABC transporters have been proposed. Also, therapies that induce apoptosis or differentiation for tumour stem cells have been suggested [25].
Deletion 19q13.31–33	Clinical trials NCT02547987 and NCT02124902	Fifty-nine patients with TNBC who received 6 courses of NAC (carboplatin and docetaxel) were included. Whole exome sequencing (WES) was performed to assess the genetic landscape	Proteogenomic analysis of triple negative breast tumours revealed a complex landscape of chemotherapy response associations, including somatic deletion 19q13.31-33 that encodes genes providing lagging DNA strand synthesis (*LIG1, POLD1* and *XRCC1*), which correlate with non-response and selective resistance to carboplatin [26, 27].

metastasis and chemoresistance in breast cancer, making it a potential target for research [32]. This protein is also resistant to HER2, which is the main reason of inefficient treatment in patients with HER2+ breast cancer [32].

Bcl2 is also involved in oncogenesis and shows resistance to drug therapy [31]. *Bcl2* together with VIM is associated with metastasis to axillary lymph nodes [31]. These proteins are considered as potential markers of response to NAC in breast cancer.

In a recently published study, an association between ALDH1 and response to NAC was shown for the first time [25]. It was found that in the group of those patients who had minimal ALDH1 levels after NAC, overall survival was higher [25]. This suggests that treatment aimed at inhibiting aldehyde dehydrogenase 1 may improve the therapeutic outcome of chemotherapy [25]. Based on these findings, various therapeutic models have been proposed, but the study of this biomarker requires more time [25].

In the current literature, microRNAs are increasingly being considered as one of the possible ways not only to distinguish cancer subtypes, but also to predict the response to NAC [33]. It is known that microRNA in luminal B HER2-negative breast cancer can be used as a predictive biomarker of response to NAC, but the results to date remain controversial [34]. In particular, a change in miR-34a-5p expression has been shown with taxane-containing and/or anthracycline-containing NAC regimens. Activation of miR-375 and miR-4516 was also observed after neoadjuvant chemotherapy. A marked decrease in miR-125b-5p expression was found in the group of no response to NAC, while miR-125b-5p levels remained relatively stable in the group with complete and/or partial response to treatment [34]. It was found that decreased levels of miR-21 and miR-195 can also be considered as a potential marker of response prognosis to NAC [33]. Correlation of miR-195 with treatment has been performed previously. There is evidence in the literature that high miR-195 levels correlate with poor response to NAC [35]. Based on the data described above, miRNAs have been evaluated as promising prognostic biomarkers, but they still need further validation [36].

The first experience of identifying differentially methylated genes by whole-genome bisulfite DNA sequencing, which can be used as a marker of breast cancer response to NAC, is presented in the literature. The methylation frequencies of 10 informative genes (*SLC9A3, C1QL2, DPYS, IRF4, ADCY8, KCNQ2, TERT, SYNDIG1, SKOR2*, and *GRIK1*) identified in luminal B breast cancer

samples differ between patients responding and non-responding to NAC. Three combinations, (1) IRF4 and C1QL2; (2) IRF4, C1QL2 and ADCY8; (3) IRF4, C1QL2 and DPYS had similar ROC characteristics with AUCs of 0.75, 0.78 and 0.74, respectively. The classifier based on IRF4 and C1QL2 met the requirements of the diagnostic panel with a diagnostic accuracy of 0.75 with a sensitivity of 75% and specificity of 75% [37].

Another interesting marker is GBP5 (guanylate binding protein 5). GBP5 may be a useful biomarker for predicting the therapeutic efficacy of taxane-based chemotherapy in relation to TNBC subtypes. Gene Set Enrichment Analysis computer modelling and cell-based assays showed that GBP5 enhances the cytotoxic efficacy of paclitaxel through activation of the Akt/mTOR signalling axis and suppression of autophagy formation in TNBC cells. It is possible to identify an insensitive population even in the BL1 subtype, which is very sensitive to DNA damaging agents, such as doxorubicin, by the level of GBP5 expression [38].

This year, an interesting study on the role of HIF1α, TWIST1 and ITGB1 as predictive markers of response to neoadjuvant chemotherapy in breast cancer was published. In a prospective study of breast cancer patients receiving NAC, the expression of HIF1α, TWIST1, and ITGB1 was evaluated in biopsy material. These markers were shown to be applicable for predicting a good response to NAC (AUC = 0.81, 0.85, 0.79 for HIF1α, TWIST1, ITGB1, respectively) [39].

Circulating biomarkers

Circulating markers include circulating tumour cells, molecules and exosomal nucleic acids useful for diagnosis, prognosis and real-time therapy monitoring with less cost and better compliance than tumour biopsy due to minimal invasiveness [40].

Elevated levels of circulating tumour cells (CTCs) at the initial stage of treatment are an early independent marker for predicting poor survival, while molecular profiling of CTCs provides prognostic information for assessing the risk of relapse and superior prognostic evaluation of therapeutic regimens [41]. However, to date, the identification and evaluation of CTCs for predicting response to NAC is a challenging task and requires further investigation. The global literature data remain contradictory. One recent meta-analysis found no correlation between circulating tumour cells and response to NAC [42]. However, in another meta-analysis, this correlation was clearly observed, and the authors argue that the amount of CTCs is useful in predicting response to NAC [43]. As for the detection of CTCs during and after treatment, their persistence after treatment has been shown to correlate with a worse outcome [44].

Circulating tumour DNA (cDNA) is a new field in monitoring disease and assessment of response to NAC [38]. The presence of cDNA has been found to be an important predictor of poor response to NAC [45].

To date, it is not easy to identify serum biomarkers that can predict response to chemotherapy. PGRN/GP88 (progranulin), an oncogenesis factor (involved in tumour cell proliferation and survival), is one of the promising biomarkers in breast cancer [14]. According to researchers, increased expression of progranulin is observed in TNBC and shows tumour chemosensitivity [46].

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Another serum biomarker used as a potential marker for predicting response to NAC in patients with TNBC is CXCL8 (chemokine ligand 8) [21]. Low expression of CXCL8 was found to be associated with a positive response to NAC [21]. At the same time, out of 103 patients who achieved complete pathomorphological effect, only four patients developed disease relapse [21]. CXCL8 has been shown to be associated with a worse outcome in ER/PR+ and HER2+ breast cancer [22].

Also, attention has been paid to CEA (carcinoembryonic AG) and CA15-3 (tumour-associated AG) antigens as prognostic factors for breast cancer [47]. The National Comprehensive Cancer Network (NCCN) has given a ban on the use of these markers for clinical evaluation before treatment [47]. However, the European Group on Tumour Markers (EGTM) recommended the use of CEA and CA15-3 for prognosis, early treatment, and treatment monitoring of breast cancer [48]. In one study, the association between these biomarkers and NAC was conducted. After NAC, it was found that CEA had prognostic value in HER2- and HER2+ breast cancer, while CA15-3 had value only in HER2+ breast cancer [49].

In 2023, an interesting prospective work was published to evaluate the relationship of *FTH1* gene-associated CECs (F-CTC) and their dynamic changes with NAC efficacy in patients with non-metastatic breast cancer. *FTH1* gene and EMT markers in CTCs were detected before NAC (T0), after 2 courses of chemotherapy (T1) and before surgery (T2). It was shown that F-CTC in peripheral blood ≥1 at T0 was an independent factor for the incidence of pCR in patients with HER2-positive breast cancer (OR = 0.08, 95%CI 0.01-0.98, p=0.048) [50].

The role of Gal-3 (galectin-3) as a marker of chemotherapy efficacy in breast cancer patients was investigated in a prospective study in 2020. A total of 88 patients with newly diagnosed cancer without prior treatment were included. Gal-3 levels in stroma and plasma were measured in each patient at the time of diagnosis and then throughout treatment. Patients were followed up for 84 months to analyse recurrence-free survival. Elevated plasma (adjuvant) and stromal (neoadjuvant) Gal-3 levels were found to be markers of chemotherapy efficacy. Patients with a chemotherapy-induced increase in extracellular Gal-3 had a longer relapse-free period and a significantly lower relapse rate during the 84-month follow-up in comparison with patients who had unchanged or decreased secretion. The findings support the possibility of using Gal-3 in plasma as a marker of chemotherapy efficacy when residual tumour is not visible on imaging. In addition, stromal levels in any residual tumours after

chemotherapy can also be used for predicting long-term prognosis in patients [51].

Immunological markers

Because the tumour is transformed from normal tissues, it induces innate immune responses in order to eliminate nascent tumour cells through immunoreduction [41].

The tumour microenvironment plays an important role in response to ongoing treatment and prognosis in patients with breast cancer and includes immune cells or molecules, blood vessels, fibroblasts, mesenchymal cells, adipocytes and extracellular matrix [52, 53]. Immune cells that contribute to tumour immunoreduction include TILS (tumour infiltrating lymphocytes), TAMS (tumour associated macrophages), Tregs (regulatory T cells), NKT (natural killer cells) and MDSCs (myeloid derived suppressor cells) [54]. The main microenvironmental components can be considered as potential biomarkers of response to antitumour therapy [41].

Infiltrated regulatory T cells have been shown to decline more strongly during chemotherapy than normal T cells, it is suggested that Tregs are more sensitive to the chemotherapy regimen [55].

It was found that in TNBC and HER2+ breast cancer, a high ratio of CD8+/FOXP3+ TILs can be considered as a valuable biomarker for assessing response to NAC [56]. TILs are also prognostic markers for TNBC, where high TIL density is associated with better survival [18]. However, it is essential to take into account TIL density, TIL phenotype and location for consideration of TIL as a prognostic marker [57].

PD-L1 (ligand of programmed cell death receptor-1) has been known to scientists for a long time, as well as its role in oncogenesis. However, only recently scientists have started to actively study the influence of this gene expression on prognosis after neoadjuvant chemotherapy [23]. In breast cancer, *PD-L1* expression together with increased TIL density plays an important role in predicting response to NAC in HER2+ and triple negative breast cancer [23]. It is believed that PD-L1 can be used as a prognostic marker of pCR achievement rate for TNBC [23]. Meanwhile, the use of PD-L1+ (positive expression) to predict pathological response to NAC in breast cancer has shown obvious accuracy (OR = 2.01; 95% CI 1.35-3.01; P<0.05) [58].

The expression of immune checkpoint receptors (ICRs) on TILs, where PD-L1, TIM-3 (T-cell immunoglobulin-3), LAG-3 (lymphocyte activating gene 3) and *CTLA-4* (cytotoxic T-lymphocyte glycoprotein 4) are included, has long attracted the attention of researchers because of its positive correlation with immunotherapy in breast cancer [57]. It was found that *PD-L1, LAG-3* and *CTLA-4* were associated with a positive response to chemotherapy among 61 patients with TNBC, most of whom received NAC with anthracyclines and taxanes, whereas TIM-3 expression was associated with a worse response to NAC [59].

Recently, FKBP12 (FK506-binding protein 12) was found to be a prognostic biomarker of anthracycline-based NAC efficacy in TNBC [60]. Deletion of FKBP12 leads to poor prognosis and increased resistance to anthracycline-based chemotherapy [60].

MELK (maternal embryo leucine zip kinase), which plays a significant role in cell cycle and proliferation, has been actively studied since 2021 [24]. At high expression of this gene, it has been observed to achieve complete pathomorphological response during NAC in patients with ER+, HER2- breast cancer and TNBC [24]. In this regard, MELK can also be considered as a potential predictive biomarker.

Tumour stem cells and plasticity markers

Tumour stem cells (TSCs) are undifferentiated cells with drug and radioresistance. According to researchers, TSCs persist after therapy and cause recurrence and metastasis, which makes them a good therapeutic target [61]. At the same time, it is difficult to prove NAC efficacy in relation to TSCs in non-luminal breast cancer, but to date there is evidence that the expression of TSC markers in tumour cells is significantly altered by NAC [62]. For example, *ALDH* biomarker described above and CD24-/+ are actively used in clinical practice as TSC markers [61]. Today, new stem cell markers are actively searched for to effectively predict the response to NAC [63].

Wright et al. first demonstrated the role of CD133 as a biomarker on cell lines from *BRCA1*-associated tumours [64]. There are data that CD133+ expression has a positive correlation with poor survival in PR-, ER-, and HER2+ breast cancer [65]. At the same time, this biomarker may be useful in predicting the response to NAC, and its decrease will indicate in favour of NAC efficacy [65].

Amplifications of stemness gene loci are considered as markers of response to NAC in breast cancer patients. According to our own previous studies, ectopic expression of stemness genes (*OCT3, SOX2, KLF4, MYC, NOTCH1, NANOG*, etc.) is caused by the presence of amplifications in the following chromosomal regions: 3q, 5p, 6p, 7p, 7q, 8q, 13q, 9p, 9q, 10p, 10q21.1, 16p, 18chr, 19p. The occurrence of amplifications in the regions of stemness gene localisation during NAC (22% of cases) in residual tumours was associated with a very high incidence of metastasis (91% of cases). Deletion of tumour clones with stemness gene amplification under NAC (42% of cases) resulted in 100% metastasis-free survival [66]. In other words, elimination of clones with amplifications may be a good measure of NAC efficacy. In the following prospective study, a new strategy of neoadjuvant chemotherapy prescription depending on the presence of stemness gene amplifications in the tumour before treatment was tested.

If there were two or more amplifications, patients were treated with NAC according to a personalised regimen (Group 1); if there was no stemness gene amplification in the tumour, patients were not treated with NAC, and treatment was started with surgery (Group 2). Group 3 served as historical controls. The objective response rate to NAC in Groups 1 and 3 was 79%. Metastatic-free survival was noted in 100% of cases in Group 2 patients. The metastasis rate in Group 1 patients was 10% (4/41), in Group 3 patients it was 47% (14/30). It was shown that NAC treatment was most appropriate in patients

with the presence of stemness gene amplifications in the primary tumour, while in the absence of amplification NAC resulted in a sharp decrease in the metastasis-free survival rate [67].

Thus, tumour stem cells and stemness gene amplifications can be useful for predicting the efficacy of NAC.

Conclusions and perspectives

Today, *HER2, ER, PR, PD-L1, ALDH, CD44, CD24,* as well as CEA and CA15-3 are widely used biomarkers of NAC efficacy in clinical practice. To date, there are a number of agents whose action is related to the need and importance of determining previously identified biomarkers of response to NAC. Anastrazole is one such agent, a non-steroidal compound that reduces estrogen levels and is based on the response of the circulating biomarker CA 15-3. A decrease in CA 15-3 levels indicates a positive therapeutic effect. At the same time, an increase in CA 15-3 concentration during the course of anastrazole therapy, which may be associated with disease progression [68]. Another example is the complex of everolimus (associated with the biomarker FKBP12) and exemestane. Despite the high risk of adverse events, this combination therapy is useful for the treatment of patients with HER2-negative and ER-positive tumours with good tolerability [69, 17]. It should be noted that in the previous chapter, amplifications of stemness gene loci were described, and nowadays they are used to determine the appropriateness of NAC and further personalised treatment of breast cancer. Moreover, interesting results on the newest markers of preoperative treatment efficacy are presented in the literature (Figure 2).

However, work aimed at finding potentially useful biomarkers for predicting NAC efficacy in breast cancer is actively ongoing. Many biomarkers are only at the research stage, and only a small proportion of them are close to clinical trials. Response assessment to NAC therapy of the biomarkers presented in this review may be useful for predicting the therapeutic response to different anticancer agents, which may further improve treatment strategies and reduce side effects from ineffective therapy. The prediction of response to NAC in breast cancer still requires continued further study, as most of the work done so far has limited efficacy.

In order to find effective markers of response to NAC, a sufficient number of successful clinical trials leading to a complete pathomorphological response to preoperative treatment and a high survival rate among breast cancer patients should be conducted.

Author Contribution Statement

Writing the text of the article: Ekaterina Kravtsova. Preparation of illustrations, editing of the article text: Matvey Tsyganov. Information search: Irina Tsydenova, Daria Dolgasheva, Ksenia Gaptulbarova. Text correction and final editing of the article: Marina Ibragimova, Nikolai Litviakov. All authors have read and agreed to the published version of the manuscript.

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Approval by scientific body: This article has not been approved by any academic body and is not part of an approved student thesis.

Data Availability Statement

The datasets created and analyzed during this study are publicly available due to their availability. They can be obtained from the corresponding author upon request.

Ethical approvals

All studies included in the literature review with human participants met the ethical standards developed

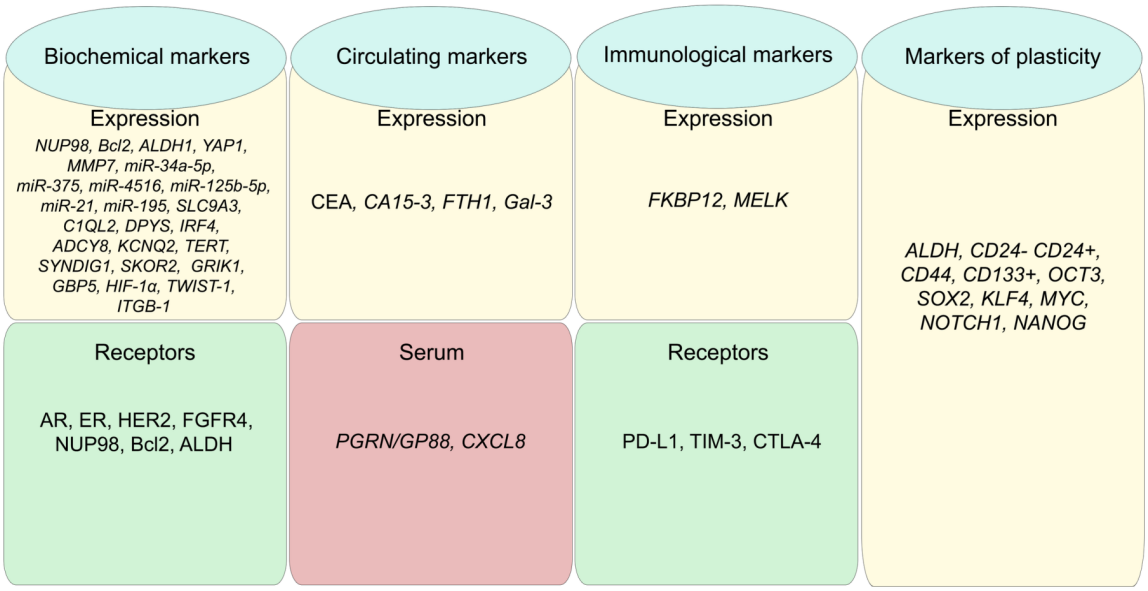

Figure 2. Markers of Preoperative Treatment Efficacy Presented in the World Literature

in accordance with the World Medical Association's Declaration of Helsinki "Ethical Principles for Scientific Medical Research Involving Human Subjects".

Conflicts of Interest

The authors declare no conflict of interests.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424. https://doi.org/10.3322/caac.21492.
2. Sella T, Weiss A, Mittendorf EA, King TA, Pilewskie M, Giuliano AE, et al. Neoadjuvant endocrine therapy in clinical practice: a review. JAMA Oncol. 2021;7(11):1700-8. https://doi.org/10.1001/jamaoncol.2021.2132.
3. Soliman H, Wagner S, Flake DD, Robson M, Schwartzberg L, Sharma P, et al. Evaluation of the 12-gene molecular score and the 21-gene recurrence score as predictors of response to neo-adjuvant chemotherapy in estrogen receptor-positive, HER2-negative breast cancer. Ann Surg Oncol. 2020;27:765-771. https://doi.org/10.1245/s10434-019-08039-7.
4. Leon-Ferre RA, Hieken TJ, Boughey JC. The landmark series: neoadjuvant chemotherapy for triple-negative and HER2-positive breast cancer. Ann Surg Oncol. 2021;28:2111-9. https://doi.org/10.1245/s10434-020-09480-9.
5. Holanek M, Selingerova I, Fabian P, Coufal O, Zapletal O, Petrakova K, et al. Biomarker dynamics and long-term treatment outcomes in breast cancer patients with residual cancer burden after neoadjuvant therapy. Diagnostics. 2022;12(7):1740. https://doi.org/10.3390/diagnostics12071740.
6. Freitas AJAD, Causin RL, Varuzza MB, Hidalgo Filho CMT, Silva VDD, Souza CDP, et al. Molecular biomarkers predict pathological complete response of neoadjuvant chemotherapy in breast cancer patients. Cancers. 2021;13(21):5477. https://doi.org/10.3390/cancers13215477.
7. Choi HJ, Ryu JM, Kim I, Nam SJ, Kim SW, Yu J, et al. Prediction of axillary pathologic response with breast pathologic complete response after neoadjuvant chemotherapy. Breast Cancer Res Treat. 2019;176:591-6. https://doi.org/10.1007/s10549-019-05214-y.
8. Orsaria P, Grasso A, Ippolito E, Pantano F, Sammarra M, Altomare C, et al. Clinical outcomes among major breast cancer subtypes after neoadjuvant chemotherapy: Impact on breast cancer recurrence and survival. Anticancer Res. 2021;41(5):2697-2709. https://doi.org/10.21873/anticanres.15051.
9. Harbeck N, Penault-Llorca F, Cortes J, Gnant M, Houssami N, Poortmans P. Breast cancer. Nat Rev Dis Primers. 2019;5(1):1-31. https://doi.org/10.1038/s41572-019-0111-2.
10. Oshi M, Gandhi S, Angarita FA, Kim TH, Tokumaru Y, Yan L, et al. A novel five-gene score to predict complete pathological response to neoadjuvant chemotherapy in ER-positive/HER2-negative breast cancer. Am J Cancer Res. 2021;11(7):3611-27.
11. Chica-Parrado MR, Godoy-Ortiz A, Jiménez B, Ribelles N, Barragan I, Alba E. Resistance to neoadjuvant treatment in breast cancer: clinicopathological and molecular predictors. Cancers. 2020;12(8):2012. https://doi.org/10.3390/cancers12082012.
12. Fang D, Li Y, Li Y, Chen Y, Huang Q, Luo Z, et al. Identification of immune-related biomarkers for predicting neoadjuvant chemotherapy sensitivity in HER2 negative breast cancer via bioinformatics analysis. Gland Surg. 2022;11(6):1026. https://doi.org/10.21037/gs-22-234.
13. van den Ende NS, Nguyen AH, Jager A, Kok M, Debets R, van Deurzen CH. Triple-Negative Breast Cancer and Predictive Markers of Response to Neoadjuvant Chemotherapy: A Systematic Review. Int J Mol Sci. 2023;24(3):2969. https://doi.org/10.3390/ijms24032969.
14. Davey MG, Hynes SO, Kerin MJ, Miller N, Lowery AJ. *Ki-67* as a prognostic biomarker in invasive breast cancer. Cancers. 2021;13(17):4455. https://doi.org/10.3390/cancers13174455.
15. Zuo K, Yuan X, Liang X, Sun X, Liu S, Connell PP, et al. qRT-PCR-based DNA homologous recombination-associated 4-gene score predicts pathologic complete response to platinum-based neoadjuvant chemotherapy in triple-negative breast cancer. Breast Cancer Res Treat. 2022;19(12):1-10. https://doi.org/10.1007/s10549-021-06442-x.
16. Bignon L, Fricker JP, Nogues C, Mouret-Fourme E, Stoppa-Lyonnet D, Caron O, et al. Efficacy of anthracycline/taxane-based neo-adjuvant chemotherapy on triple-negative breast cancer in BRCA 1/BRCA 2 mutation carriers. Breast J. 2018;24(3):269-277. https://doi.org/10.1111/tbj.12887.
17. Sueta A, Yamamoto-Ibusuki M, Tomiguchi M, Fujiki Y, Goto-Yamaguchi L, Iwase H, et al. Predictive and prognostic significance of BRCAness in HER2-negative breast cancer. Breast Cancer. 2022;29(2):368-376. https://doi.org/10.1007/s12282-021-01319-9.
18. Mohammed AA, Elsayed FM, Algazar M, Rashed HE, Anter AH. Neoadjuvant chemotherapy in triple negative breast cancer: correlation between androgen receptor expression and pathological response. Asian Pac J Cancer Prev. 2020;21(2):563. https://doi.org/10.31557/APJCP.2020.21.2.563.
19. Huang L, Lang GT, Liu Q, Shi JX, Shao ZM, Cao AY. A predictor of pathological complete response to neoadjuvant chemotherapy in triple-negative breast cancer patients with the DNA repair genes. Ann Transl Med. 2021;9(4). https://doi.org/10.21037/atm-20-4852.
20. Li Z, Han Y, Wang J, Xu B. Prognostic Factors for Triple-Negative Breast Cancer with Residual Disease after Neoadjuvant Chemotherapy. J Pers Med. 2023;13(2):190. https://doi.org/10.3390/jpm13020190.
21. Wang RX, Ji P, Gong Y, Shao ZM, Chen S. Value of CXCL8–CXCR1/2 axis in neoadjuvant chemotherapy for triple-negative breast cancer patients: A retrospective pilot study. Breast Cancer Res Treat. 2020;181:561-570. https://doi.org/10.1007/s10549-020-05660-z.
22. Milovanovic J, Todorović-Raković N, Radulovic M. Interleukin-6 and interleukin-8 serum levels in prognosis of hormone-dependent breast cancer. Cytokine. 2019;118:93-8. https://doi.org/10.1016/j.cyto.2018.02.019.
23. Vranic S, Cyprian FS, Gatalica Z, Palazzo J. PD-L1 status in breast cancer: Current view and perspectives. Semin Cancer Biol. 2021;72:46-154. https://doi.org/10.1016/j.semcancer.2019.12.003.
24. Oshi M, Gandhi S, Huyser MR, Tokumaru Y, Yan L, Yamada A, et al. MELK expression in breast cancer is associated with infiltration of immune cell and pathological compete response (pCR) after neoadjuvant chemotherapy. Am J Cancer Res. 2021;11(9):4421.
25. Lee A, Won KY, Lim SJ, Cho SY, Han SA, Park S, et al. *ALDH1* and tumor infiltrating lymphocytes as predictors for neoadjuvant chemotherapy response in breast cancer. Pathol Res Pract. 2018;214(5):619-24. https://doi.org/10.1016/j.prp.2018.04.006.
26. Anurag M, Jaehnig EJ, Krug K, Lei JT, Bergstrom EJ, Kim BJ, et al. Proteogenomic markers of chemotherapy resistance

and response in triple-negative breast cancer. Cancer Discov. 2022;12(11):2586-2605. https://doi.org/10.1158/2159-8290.CD-22-0200.
27. Ademuyiwa FO, Chen I, Luo J, Rimawi MF, Hagemann IS, Fisk B, et al. Immunogenomic profiling and pathological response results from a clinical trial of docetaxel and carboplatin in triple-negative breast cancer. Breast Cancer Res Treat. 2021;189(1):187-202. https://doi.org/10.1007/s10549-021-06307-3.
28. Tan MH, Li J, Xu HE, Melcher K, Yong EL. Androgen receptor: structure, role in prostate cancer and drug discovery. Acta Pharmacol Sin. 2015;36(1):3-23. https://doi.org/10.1038/aps.2014.18.
29. Gerratana L, Basile D, Buono G, De Placido S, Giuliano M, Minichillo S, et al. Androgen receptor in triple negative breast cancer: A potential target for the targetless subtype. Cancer Treat Rev. 2018;68:102-10. https://doi.org/10.1016/j.ctrv.2018.06.005.
30. Hickey TE, Selth LA, Chia KM, Laven-Law G, Milioli HH, Roden D, et al. The androgen receptor is a tumor suppressor in estrogen receptor–positive breast cancer. Nat Med. 2021;27(2):310-20. https://doi.org/10.1038/s41591-020-01168-7.
31. Guestini F, Ono K, Miyashita M, Ishida T, Ohuchi N, Nakagawa S, et al. Impact of Topoisomerase IIα, PTEN, ABCC1/MRP1, and KI67 on triple-negative breast cancer patients treated with neoadjuvant chemotherapy. Breast Cancer Res Treat. 2019;173:275-88. https://doi.org/10.1007/s10549-018-4985-6.
32. Zou Y, Zheng S, Xie X, Ye F, Hu X, Tian Z, et al. N6-methyladenosine regulated FGFR4 attenuates ferroptotic cell death in recalcitrant HER2-positive breast cancer. Nat Commun. 2022;13(1):2672. https://doi.org/10.1038/s41467-022-30217-7.
33. McGuire A, Casey MC, Waldron RM, Heneghan H, Kalinina O, Holian E, et al. Prospective assessment of systemic microRNAs as markers of response to neoadjuvant chemotherapy in breast cancer. Cancers. 2020;12(7):1820. https://doi.org/10.3390/cancers12071820.
34. Zhang Z, Zhang H, Li C, Xiang Q, Xu L, Liu Q, et al. Circulating microRNAs as indicators in the prediction of neoadjuvant chemotherapy response in luminal B breast cancer. Thorac Cancer. 2021;12(24):3396-406. https://doi.org/10.1111/1759-7714.14219.
35. Baxter DE, Allinson LM, Al Amri WS, Poulter JA, Pramanik A, Thorne JL, et al. MiR-195 and its target SEMA6D regulate chemoresponse in breast cancer. Cancers. 2021;13(23):5979. https://doi.org/10.3390/cancers13235979.
36. Rodrigues-Ferreira S, Nahmias C. Predictive biomarkers for personalized medicine in breast cancer. Cancer Lett. 2022;215828. https://doi.org/10.1016/j.canlet.2022.215828.
37. Sigin VO, Kalinkin AI, Kuznetsova EB, Simonova OA, Chesnokova GG, Litviakov NV, et al. DNA methylation markers panel can improve prediction of response to neoadjuvant chemotherapy in luminal B breast cancer. Sci Rep. 2020;10(1):9239. https://doi.org/10.1038/s41598-020-66197-1.
38. Cheng SW, Chen PC, Ger TR, Chiu HW, Lin YF. GBP5 serves as a potential marker to predict a favorable response in triple-negative breast cancer patients receiving a taxane-based chemotherapy. J Pers Med. 2021;11(3):197. https://doi.org/10.3390/jpm11030197.
39. Zhang J, Zhang S, Gao S, Ma Y, Tan X, Kang Y, et al. HIF-1α, TWIST-1 and ITGB-1, associated with tumor stiffness, as novel predictive markers for the pathological response to neoadjuvant chemotherapy in breast cancer. Cancer Manag Res. 2020;2209-22. https://doi.org/10.2147/CMAR.S246349
40. Ravelli A, Reuben JM, Lanza F, Anfossi S, Cappelletti MR, Zanotti L. Solid Tumor Working Party of European Blood and Marrow Transplantation Society (EBMT). Breast cancer circulating biomarkers: advantages, drawbacks, and new insights. Tumor Biol. 2015;36:6653-5. https://doi.org/10.1007/s13277-015-3944-7.
41. Tan W, Yang M, Yang H, Zhou F, Shen W. Predicting the response to neoadjuvant therapy for early-stage breast cancer: tumor-, blood-, and imaging-related biomarkers. Cancer Manag Res. 2018;4333-47. https://doi.org/10.2147/CMAR.S174435
42. Bidard FC, Michiels S, Riethdorf S, Mueller V, Esserman LJ, Lucci A, et al. Circulating tumor cells in breast cancer patients treated by neoadjuvant chemotherapy: a meta-analysis. J Natl Cancer Inst. 2018;110(6):560-7. https://doi.org/10.1093/jnci/djy018.
43. Yan WT, Cui X, Chen Q, Li YF, Cui YH, Wang Y, et al. Circulating tumor cell status monitors the treatment responses in breast cancer patients: a meta-analysis. Sci Rep. 2017;7(1):43464. https://doi.org/10.1038/srep43464.
44. Banys-Paluchowski M, Krawczyk N, Fehm T. Liquid biopsy in breast cancer. Geburtshilfe Frauenheilkd. 2020;80(11):1093-104.
45. Magbanua MJM, Swigart LB, Wu HT, Hirst GL, Yau C, Wolf DM, et al. Circulating tumor DNA in neoadjuvant-treated breast cancer reflects response and survival. Ann Oncol. 2021;32(2):229-39. https://doi.org/10.1016/j.annonc.2020.11.007.
46. Guha R, Yue B, Dong J, Banerjee A, Serrero G. Anti-progranulin/GP88 antibody AG01 inhibits triple negative breast cancer cell proliferation and migration. Breast Cancer Res Treat. 2021;186:637-53. https://doi.org/10.1007/s10549-021-06120-y.
47. Nam SE, Lim W, Jeong J, Lee S, Choi J, Park H, et al. The prognostic significance of preoperative tumor marker (CEA, CA15-3) elevation in breast cancer patients: data from the Korean Breast Cancer Society Registry. Breast Cancer Res Treat. 2019;177:669-78. https://doi.org/10.1007/s10549-019-05357-y.
48. Molina R, Barak V, van Dalen A, Duffy MJ, Einarsson R, Gion M, et al. Tumor markers in breast cancer–European Group on Tumor Markers recommendations. Tumor Biol. 2005;26(6):281-93. https://doi.org/10.1159/000089260.
49. Kim JY, Jeon E, Kwon S, Jung H, Joo S, Park Y, et al. Prediction of pathologic complete response to neoadjuvant chemotherapy using machine learning models in patients with breast cancer. Breast Cancer Res Treat. 2021;189:747-57. https://doi.org/10.1007/s10549-021-06310-8.
50. Jia S, Yang Y, Zhu Y, Yang W, Ling L, Wei Y, et al. Association of FTH1-expressing circulating tumor cells with efficacy of neoadjuvant chemotherapy for patients with breast cancer: a prospective cohort study. Oncologist. 2023. https://doi.org/10.1093/oncolo/oyad195.
51. Shafiq A, Moore J, Suleman A, Faiz S, Farooq O, Arshad A, et al. Elevated soluble galectin-3 as a marker of chemotherapy efficacy in breast cancer patients: A prospective study. Int J Breast Cancer. 2020;2020.
52. Choi J, Gyamfi J, Jang H, Koo JS. The role of tumor-associated macrophage in breast cancer biology. Histol Histopathol. 2018;33(2):133-145.
53. García-Torralba E, Ivars-Rubio A, Perez-Ramos M, Navarro Manzano E, Blaya-Boluda N, de la Morena Barrio P, et al. Correlation of neutrophil-to-lymphocyte ratio and stromal tumor infiltrating lymphocytes across early breast cancer subtypes. 2023.
54. Anani W, Shurin MR. Targeting myeloid-derived suppressor cells in cancer. Tumor Immune Microenvironment in Cancer

Progression and Cancer Therapy. 2017;105-28. https://doi.org/10.1007/978-3-319-67577-0_8.
55. Mao Y, Qu Q, Zhang Y, Liu J, Chen X, Shen K. The value of tumor infiltrating lymphocytes (TILs) for predicting response to neoadjuvant chemotherapy in breast cancer: a systematic review and meta-analysis. PloS One. 2014;9(12):e115103. https://doi.org/10.1371/journal.pone.0115103.
56. Asano Y, Kashiwagi S, Goto W, Kurata K, Noda S, Takashima T, et al. Tumour-infiltrating CD8 to FOXP3 lymphocyte ratio in predicting treatment responses to neoadjuvant chemotherapy of aggressive breast cancer. Br J Surg . 2016;103(7):845-54. https://doi.org/10.1002/bjs.10127.
57. El Bairi K, Haynes HR, Blackley E, Fineberg S, Shear J, Turner S, et al. The tale of TILs in breast cancer: a report from the international immuno-oncology biomarker working group. NPJ Breast Cancer. 2021;7(1):150. https://doi.org/10.1038/s41523-021-00346-1.
58. Du Q, Che J, Jiang X, Li L, Luo X, Li Q. PD-L1 acts as a promising immune marker to predict the response to neoadjuvant chemotherapy in breast cancer patients. Clin Breast Cancer. 2020;20(1):e99-e111. https://doi.org/10.1016/j.clbc.2019.06.014.
59. Cabioglu N, Onder S, Oner G, Karatay H, Tukenmez M, Muslumanoglu M, et al. TIM3 expression on TILs is associated with poor response to neoadjuvant chemotherapy in patients with locally advanced triple-negative breast cancer. BMC cancer. 2021;21(1):1-13. https://doi.org/10.1186/s12885-021-08054-6.
60. Xing M, Wang J, Yang Q, Wang Y, Li J, Xiong J, et al. FKBP12 is a predictive biomarker for efficacy of anthracycline-based chemotherapy in breast cancer. Cancer Chemother Pharmacol. 2019;84:861-72. https://doi.org/10.1007/s00280-019-03923-1.
61. Kim SL, Choi HS, Kim JH, Jeong DK, Kim KS, Lee DS. Dihydrotanshinone-induced NOX5 activation inhibits breast cancer stem cell through the ROS/Stat3 signaling pathway. Oxid Med Cell Longev. 2019;2019. https://doi.org/10.1155/2019/9296439.
62. Leng X, Huang G, Zhang L, Ding J, Ma F. Changes in tumor stem cell markers and epithelial-mesenchymal transition markers in nonluminal breast cancer after neoadjuvant chemotherapy and their correlation with contrast-enhanced ultrasound. Biomed Res Int. 2020;2020:1-12. https://doi.org/10.1155/2020/3869538.
63. Ibragimova M, Tsyganov M, Litviakov N. Tumour stem cells in breast cancer. Int J Mol Sci. 2022;23(9):5058 https://doi.org/10.3390/ijms23095058.
64. Wright MH, Calcagno AM, Salcido CD, Carlson MD, Ambudkar SV, Varticovski L. Brca1 breast tumors contain distinct CD44+/CD24-and CD133+ cells with cancer stem cell characteristics. Breast Cancer Res. 2008;10(1):1-16. https://doi.org/10.1186/bcr1855.
65. Brugnoli F, Grassilli S, Al-Qassab Y, Capitani S, Bertagnolo V. CD133 in breast cancer cells: more than a stem cell marker. J Oncol. 2019; 2019. https://doi.org/10.1155/2019/7512632.
66. Litviakov N, Ibragimova M, Tsyganov M, Kazantseva P, Deryusheva I, Pevzner A, et al. Amplifications of stemness genes and the capacity of breast tumors for metastasis. Oncotarget. 2020;11(21):1988. https://doi.org/10.18632/oncotarget.27608.
67. Litviakov NV, Ibragimova MK, Tsyganov MM, Kazantseva PV, Doroshenko AV, Garbukov EY, et al. Amplifications of Stemness Gene Loci—New Markers for the Determination of the Need for Neoadjuvant Chemotherapy for Patients with Breast Cancer. A Prospective Study. J Pers Med. 2021;11(5):397. https://doi.org/10.3390/jpm11050397.
68. Abed SN, Mahdi HS, Sahib AS, Abo Almaali MHM, AL-Haydar M, Mohsin KK. Serum levels of cancer antigen 15.3 and estrogen in a samples of iraqi women with breast cancer treated with anastrazole. J Pharm Res Int. 2020;12:1604-8. https://doi.org/10.31838/ijpr/2020.SP1.246.
69. Dhillon S. Everolimus in combination with exemestane: a review of its use in the treatment of patients with postmenopausal hormone receptor-positive, HER2-negative advanced breast cancer. Drugs. 2013;73:475-85. https://doi.org/10.1007/s40265-013-0034-2.