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Maria Isabel da Silva Carvalho

Parâmetros que demonstram ter influência na resposta à terapia neoadjuvante em pacientes com cancro da mama: Estado da arte e perspetivas futuras

Parameters that might influence the neoadjuvant therapy response in breast cancer patients: State of the art and future viewpoints

Março, 2021

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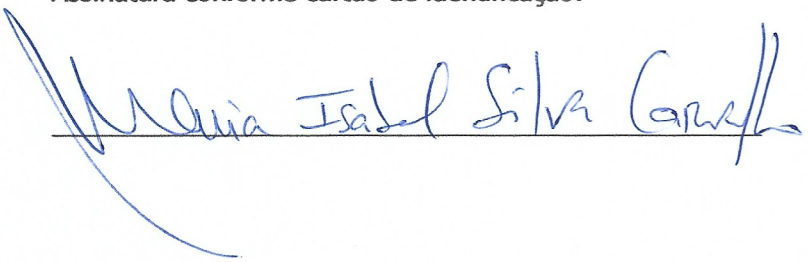

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NOME

Maria Isabel da Silva Carvalho

NÚMERO DE ESTUDANTE	E-MAIL
201301272	mariaisabelsc@hotmail.com

DESIGNAÇÃO DA ÁREA DO PROJECTO

Patologia/Oncologia

TÍTULO ~~DISSERTAÇÃO~~/MONOGRAFIA (riscar o que não interessa)

Parameters that might influence the neoadjuvant therapy response in breast cancer patients: State of the art and future viewpoints

ORIENTADOR

Professor Doutor Fernando Carlos de Lander Schmitt

COORIENTADOR (se aplicável)

Doutora Noémia de Fátima Salgado Afonso

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"If someone loves a flower, of which just one single blossom grows in all the millions and millions of stars, it is enough to make him happy just to look at the stars."

Antoine de Saint-Exupéry, The Little Prince

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Parameters that might influence the neoadjuvant therapy response in breast cancer patients: State of the art and future viewpoints

Authors:

Maria Isabel; Carvalho[1]

Noémia; Afonso[2]

Fernando; Schmitt[1,3]

Author Affiliations:

1. Medical Faculty of the University of Porto, Porto, Portugal.
2. Centro Hospitalar Universitário do Porto - Hospital de Santo António, Porto, Portugal.
3. Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Porto, Portugal.

Corresponding Author:

Maria Isabel da Silva Carvalho

Rua do Cruzeiro nº 182, 1º Dto, 4700-116, Braga

mariaisabelsc@hotmail.com

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Resumo

Objetivos: A extensa heterogeneidade do cancro da mama (CM), associada à grande variabilidade das respostas terapêuticas, fundamenta a necessidade de identificar fatores específicos do tumor que possam estar relacionados com a resistência ao tratamento neoadjuvante (TNA). A presente revisão abrange uma visão global da literatura recente relacionada com esta temática.

Métodos: Foi conduzida uma pesquisa bibliográfica da literatura relevante, na base de dados PubMed, com foco em manuscritos com data de publicação em 2015 ou posterior.

Resultados: A idade mais jovem, o menor tamanho tumoral, a ausência de envolvimento dos gânglios linfáticos axilares, o grau histológico III, o Ki-67 elevado, o ER negativo, o HER2 positivo e os tumores triplo negativos, constituem fatores que predizem uma boa resposta ao TNA. As células imunológicas que infiltram o tumor, a mutação PIK3CA, o status do BRCA e os marcadores agnósticos são parâmetros, ainda sob investigação, que parecem ser candidatos promissores.

Conclusões: Mais do que biomarcadores individuais, um painel combinado de múltiplos parâmetros poderá ser capaz de antecipar a resposta do paciente à terapêutica. No entanto, a utilidade clínica destes novos fatores promissores, ainda requer evidências adicionais em ensaios clínicos multicêntricos.

Termos-chave: Cancro de mama; Biomarcadores; Terapia Neoadjuvante; Resposta patológica completa.

Abstract

Goals: The breast cancer extensive heterogeneity, together with the variability of therapeutic responses, raises the need to distinguish the tumor specific factors of neoadjuvant therapy (NAT) resistance. The present review comprises an overview of the recent literature related with this topic.

Methods: The bibliographic search was conducted in the PubMed database focusing on relevant manuscripts with publication date in 2015 or later.

Outcomes: Younger age, smaller tumor size, absence of axillary lymph node involvement, histological grade III, high Ki-67, ER negative, HER2 positive and triple negative breast cancer are factors that predict a good response to NAT. Tumor infiltrating immune cells, PIK3CA mutation, BRCA status and agnostic markers still under investigation and seems to be promising candidates.

Conclusions: More than individual biomarkers, a panel of combined multilevel parameters may be able to predict patient's therapeutic response. Nevertheless, the clinical utility of these new promising factors still requires additional reliable evidence in multicenter clinical trials.

Key-words: Breast cancer; Biomarkers; Neoadjuvant Therapy; Pathological complete response.

Introduction

Breast cancer (BC) is the most common cancer diagnosed in women, worldwide, every year. Despite all the efforts in early detection and improvements in treatment, BC remains a critical health problem since it is a relevant cause of death [1].

Due to evident clinical, morphological, and molecular heterogeneity exhibited by this type of cancer, its classification into different subtypes is crucial for the treatment decision and prognosis [2,3]. BC classification, based on the anatomopathological examination (WHO histological type, tumor histological grade and TNM system), is complemented with the molecular classification (luminal A and B, HER2-enriched and triple negative) and these four molecular subgroups demonstrate singular clinical course, as well as particular metastatic pattern, treatment requirements and prognostic response [4-6].

It is already well known the important role of neoadjuvant therapy (NAT) in BC treatment. In locally-advanced breast cancer (LABC) and in BC with an aggressive biology, such as triple negative (TN) and HER2 positive tumors, NAT is increasingly indicated and have an additional advantage allowing the assessment of tumor response to a specific treatment with a prognosis improvement in patients which BC cells are sensitive to chemotherapy [7,8]. In fact, the pathological complete response (pCR - absence of tumor cells in the surgical sample, both at the primary tumor site and at regional lymph nodes) is not achieved in all BC patients treated with NAT, and this premise indicates the urgent need in recognize and select the patients that could benefit from a specific treatment, namely in the adjuvant setting [9].

The concept of an individualized therapeutic decision is not totally new in BC field and interesting knowledge about tumor biology, biomarkers, new treatments, as well as patient's quality of life and prognosis are published annually [10,11].

The incorporation of novel diagnostic/tumor classification technologies ("omics") has modified the biological approach of BC, allowing the validation of biomarkers that are already routinely assessed in all cases of invasive carcinomas (hormonal receptors, HER2 overexpression/amplification and Ki-67 expression), and additionally allowing the investigation of new tumor signaling pathways and new biomarkers with promising role as prognostic factors and/or therapeutic targets [8,12].

However, despite all the advances in BC therapy, the intertumoral and intratumoral heterogeneity of this type of tumors, recognized by pathologists and clinicians, remains a real challenge [2,3]. Considering the current scenario where around 70% of patients do not achieve pCR after NAT [9,13] we might conclude that reliable predictive biomarkers and consistent evaluation of clinical benefit from personalized therapy remain limited.

The present review comprises an extensive overview of the recent literature related to several biomarkers/parameters that might indicate the BC patient's response to neoadjuvant therapy. These biomarkers information and evaluation may be useful for outline the individualized initial systemic treatment or to decide whether breast surgery should be applied at the beginning of the therapeutical approach.

Methods

The authors reviewed the topic of parameters that could have a potential role on NAT response in BC patients.

The PubMed search was performed using the following terms, either as MeSH terms or free entries: “neoadjuvant therapy", “breast”, “mammary”, “cancer”, “tumor” and “biomarker” focusing on manuscripts with publication date in 2015 or later. Studies were eligible if: 1) were published in English, Portuguese or Spanish; 2) were performed in women of any age or race; 3) had measured biomarkers expression by immunohistochemistry or molecular quantification in BC and 4) had evaluated biomarkers associated with pCR after NAT.

A total of 281 articles were obtained. Of these 281 articles, we reviewed the title and the abstract and excluded some studies as illustrated on the following flowchart (Figure 1). After this process, it was conducted a detailed revision of the full report of the selected manuscripts.

Our work was designed and written according to the recommendations suggested in SANRA - a scale for the quality assessment of narrative review articles [14].

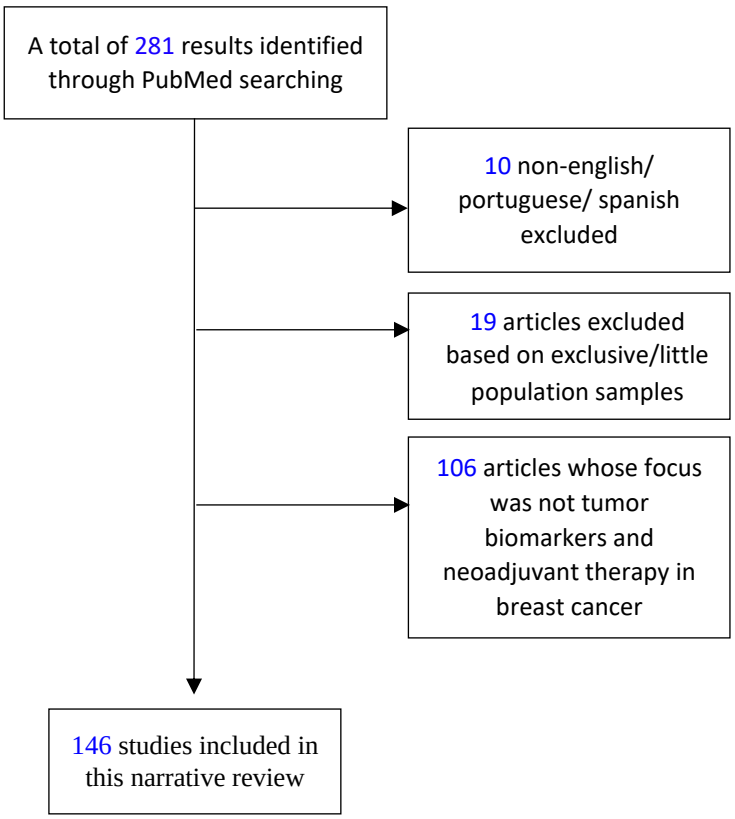

Figure 1 – Flowchart of selected studies.

Predictive Parameters for the Evaluation of Neoadjuvant Therapy Outcomes

In the current BC treatment panorama, the determination of parameters that can differentiate between potential responder and non-responder patients would be extremely important since the initial treatment approach comes up with consequences in survival rates. The potential predictive factors that could be evaluated before NAT are described below and outlined in figure 2 and table I.

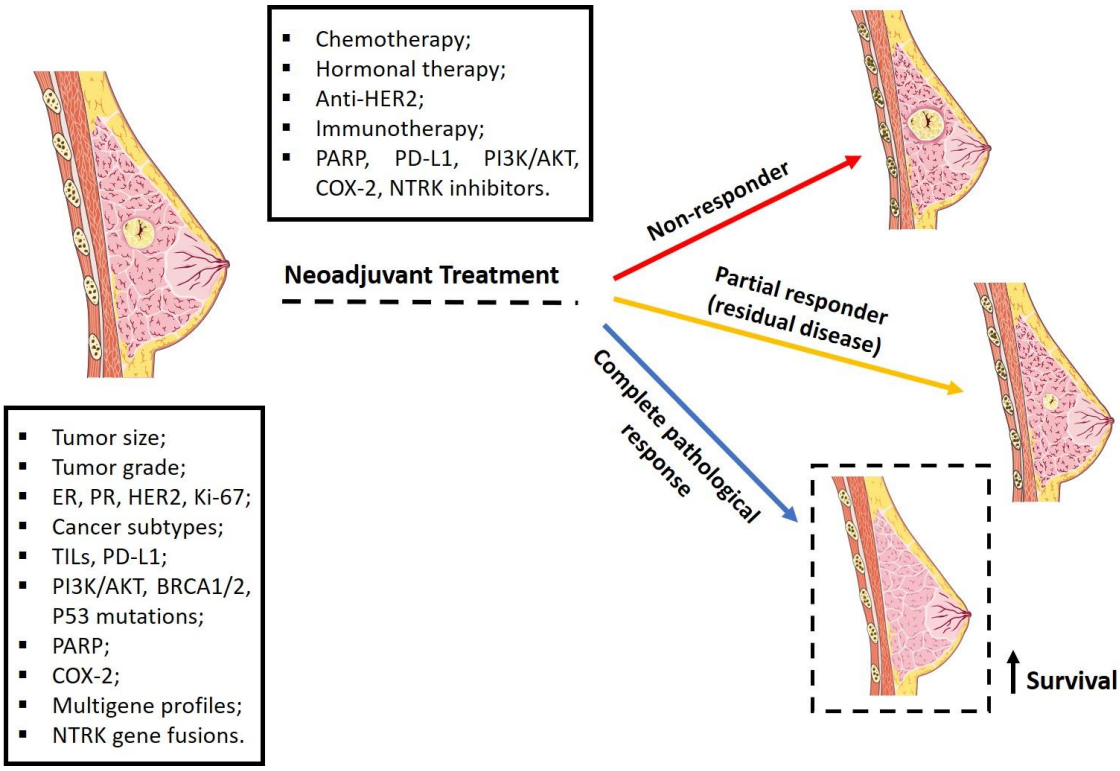

Figure 2 - Potential strategies to predict and manage the neoadjuvant therapy response in breast cancer patients. This figure was created using the Servier Medical Art image portfolio (smart.servier.com).

Clinicopathological parameters

Patient's age seems to be a potential parameter to predict the pCR after NAT. Loibl et al. (2015) suggest a better response in young women. However, the young age appears to have a different significance depending on the tumor biological type. Woman < 40 years, with HER2 negative tumors, seems to achieve higher pCR rates than older ones [15].

Obesity (determined by the body mass index – BMI), has already been associated with an increased risk for the development of BC, worse treatment response and tumor recurrence with lower overall survival [16]. The relationship between obesity and pCR after NAT is a controversial topic. Karatas el al. (2017) suggested that patients with higher BMI have less pCR rates [17].

Similarly, Fontanella et al. (2015) described that the BMI ≥ 30 kg/m2 has a negative role on pCR in luminal-like, but not in TN and HER2 positive tumors [18]. Bao et al. (2015) also described that, in triple negative breast cancer (TNBC), obesity was not correlated with pCR [19]. These differences reflect the unresponsive nature of TN and HER2 positive tumors to the hormonal effect of adipose cells on BC proliferation. Additionally, the higher pCR rates in overweight patients with TN or HER2 positive BC compared with hormonal receptor positive tumors may be explained due to the higher chemosensitivity of these aggressive BC subtypes [18,19].

The response to NAT it is possibly related with racial differences. Killelea et al. (2015) demonstrated that black patients have a lower rate of pCR, compared with white patients [20]. However, there are other studies describing no differences in pCR according to race or ethnicity [21]. This controversy proves the need of additional studies to better understand this subject.

Breast imaging technics, such as mammography and/or ultrasonography, fundamental to the routine management of patients with BC, can also contribute to evaluate the NAT response [22]. Recent studies demonstrated that low mammographic breast density was associated with improved pCR rate [23,24]. The mammographic density reflects stromal cells and epithelial components in tumor microenvironment that can contribute to tumor cells initiation and progression [23]. However, Skarping et al. (2020), in a prospective study of BC patients receiving NAT, described no evidence of mammographic density as a predictor of pCR [25].

Whether these clinical features might strongly predict the response to NAT, requires further investigations. The controversial findings demonstrated by the studies described above, suggest that pCR with individually optimized neoadjuvant chemotherapy (NAC) is a function much more dependent of tumor biology and signaling pathways involved in tumor microenvironment, rather than patient characteristics.

Tumor size (cT-stage) and the initial lymph node status have been described, in some recent studies, as a determinant factors of patient's response. In fact, Baron et al. (2016), demonstrated that tumor size > 5 cm was associated with a significantly low probability of pCR on univariate logistic regression analyses. This relationship was statistically significant in TNBC and HER2 positive tumors. However, in multivariate logistic regression analyses, the tumor size was not statistically associated with pCR in any of the molecular subtypes [26]. Additionally, in the same study, the clinical lymph node status was associated with the odds of experiencing pCR both in univariate and multivariate regression analyses. The absence of involvement of axillary lymph nodes seems to be a predictor of good response [26]. Similarly, Prat et al. (2015) and Goorts et al. (2017), also described an association between tumor size and pCR, with the lower cT-stage (cT1-2 vs. cT3-4) correlated with a significantly higher pCR rates [27,28].

Tumor grade seems to be another clinicopathological factor related with therapeutical response. In the neoadjuvant setting, a retrospective recent study demonstrated that patients with grade III TN tumors achieved higher pCR rates after NAT with platinum, suggesting that this therapeutic option should be considered in this subgroup of patients [29]. In addition, Jarzab et al. (2019), demonstrated an increase of pCR rate in patients with high-grade tumors and, interestingly, tumor grading appears as an independent predictor of pCR, which may indicate that should be considered in pre-operative chemotherapy decision-making [30]. Moreover, Diaz-Redondo et al. (2019) showed that tumor grade provides independent predictive information for pCR rate following NAT with trastuzumab plus pertuzumab both in HER2 positive and luminal tumors, of which grade III patients had higher probabilities of pCR [31].

Immunohistochemical breast cancer subtypes

The probability of pCR achievement after NAT and the consequent survival advantage seems to be notably inherited to the specific BC subtype [27].

The luminal-type BC are usually associated with chemoresistance and normally are treated with hormonal therapy. However, recent studies, demonstrated paradoxical results and have been describing significantly higher rates of luminal-like tumoral down-staging with NAC compared with neoadjuvant hormonal therapy alone [32-34]. Despite of the general better prognosis of this tumor subtype, in part due to its indolent development, there is some variability in therapeutical response among luminal-like tumors and the ability to achieve pCR in patients with ER positive BC is significantly lower in comparison with TN or HER2 positive tumors [27,35,36]. In hormonal receptor positive tumors, PR seems to be a useful biomarker to differentiate ER positive patients who fail to achieve pCR. A study performed by Chen et al. (2015) showed that in luminal B subtype, patients with PR negative tumors had a relatively higher pCR rate after NAC with anthracycline-taxane-based regimens. Nevertheless, in patients with residual tumor after NAC, PR negativity was correlated with higher risk of relapse and poor overall survival. These findings indicate that PR status could be used to identify a heterogeneous phenotype of luminal B breast tumors with higher response to NAC that is not properly related with survival benefit in patients with partial pathological response [37]. Patients with ER positive/PR negative BC seem to have a poor prognosis, and the loss of PR has been associated to higher rates of p53 mutation and/or increasing PI3K/mTOR gene signature [38].

Another study performed by Kurozumi et al. (2015) demonstrated additionally that negative hormone receptors and HER2 positivity were correlated with good pathological response. In this study both ER/PR negativity was found to be significantly predictive of higher pCR rates in HER2 positive BC receiving NAC using taxanes followed by fluorouracil, epirubicin, and cyclophosphamide concomitant with trastuzumab [12]. Moreover, Kogawa et al. (2016 and 2020) showed that high HER2 ratio was an independent predictor of high pCR and longer overall survival in patients with stage III HER2 positive BC, treated with NAC ± trastuzumab [39,40].

Patients with TNBC have an increased risk of distant recurrence and death compared with other types of BC. Although the distinct aggressive behavior, TN tumors are particularly sensitive to chemotherapy and approximately 30%–40% of patients achieve pCR after standard anthracycline plus cyclophosphamide and taxane-based NAT [41].

Several recent studies, in univariate and multivariate analyses, consistently proved that luminal A and B patients had significantly lower rates of pCR than HER2-enriched or TN patients [26,27,42]. Gentile et al. (2017) showed that, in LABC, the overall pCR rate was 25% and differed by receptor subtype (HR positive/HER2 negative 7%, TN 23%, HER2 positive 48%) [43]. Similarly, Huober et al. (2019) indicated that 48% of HER2 positive patients treated with NAC plus trastuzumab ± pertuzumab achieved pCR, contrasted with 7% of patients with the HR positive/HER2 negative subtype, or 23% of TN patients treated with NAC alone [44].

Proliferation markers - Ki-67

Ki-67, a well-known proliferation marker, has been widely investigated as a neoadjuvant response predictive factor and, several studies, suggested its role as a good candidate to include in BC patient's decision making [8,45-47].

A retrospective study, including women with stage I to III BC, treated with anthracycline and taxane-based NAC, described that high Ki-67 pre-treatment evaluation showed a significantly association with better pCR rates in luminal B and TN subtypes [48]. Vörös et al. (2015) also demonstrated that the higher Ki-67 in pre-treatment core-biopsy samples of BC patients receiving neoadjuvant docetaxel plus epirubicin ± capecitabine was associated with higher probabilities of pCR [49].

Alba et al. (2016) suggested the Ki-67 cutoff of 50% as predictive of pCR after NAT in ER negative/HER2 negative and ER negative/HER2 positive tumors (pCR rates of 42% and 64% respectively versus 15% and 45% in patients with Ki-67 ≤ 50%) nevertheless, in ER positive tumors this association was not verified. In the same study, the multivariate analyses reinforced the role of Ki-67 as an independent predictive factor for response [50].

In HER2 positive BC receiving NAC using taxanes followed by fluorouracil, epirubicin, and cyclophosphamide concomitant with trastuzumab, pCR rates were also significantly higher in patients whose tumors had a high Ki-67 index [12]. Moreover, Sánchez-Muñoz et al. (2015) showed, in patients with HER2 positive BC treated with trastuzumab-based chemotherapy, a role of proliferation rate determined by Ki-67 ≥ 50% as an independent predictive factor of pCR [51].

Although Ki-67 staining is not currently standardized as a predictive biomarker for routine clinical use in TNBC, Javanovic et al. (2017) showed that higher levels of Ki-67 expression prior to NAT with cisplatin, paclitaxel ± everolimus in patients with stage II/III TNBC were significantly correlated with higher ratio of pCR [52]. Similarly, a recent study performed by Gluz et al. (2020) in a cohort of TNBC patients treated with nab-paclitaxel + carboplatin or with nab-paclitaxel + gemcitabine, proposed the high Ki-67 index as an important predictor of higher pCR rates in both treatment regimens [53].

The studies described above demonstrated that achieving a pCR after NAT is more common in tumors with high proliferative rates as TN and HER2 positive BC, suggesting that cell proliferation is a phenomenon closely related to chemosensitivity [12,50-53].

Immune cells in tumor microenvironment

Within the BC microenvironment, immune cells, namely T-lymphocytes appears to be key effectors during the several steps of tumor development. Besides, the tumor-infiltrating lymphocytes (TILs) seems to be important contributors to the BC therapy response [54,55].

Carbognin et al. (2016), in a retrospective study, demonstrated that the presence of higher levels of TILs were associated with a 29.5% increase in pCR rate after NAT. This association was significantly more consistent in patients with TN and HER2 positive BC, suggesting that, in these tumor subtypes, the presence of TILs may represent a robust predictive factor of treatment response [56]. Similar results were described by Denkert et al. (2018), also with HER2 positive and TNBC appearing as the subtypes more likely to benefit from the presence of higher expression of TILs (48% and 50% of pCR, respectively). In this study, increased TILs were associated with better response to NAT in all BC molecular subtypes, however, the survival benefit of this association was verified in HER2 positive and TN tumors, but not in luminal ones [57].

Furthermore, Cerbelli et al. (2020) confirmed the predictive value of TILs evaluation in TNBC (at univariate analysis, 76.5% of patients with higher TILs achieved pCR after standard NAT) [58] and

Ruan et al. (2018) proposed a 20% threshold for stromal TILs evaluated in pre-NAT core needle biopsy of this tumor subtype as a reasonable predictive value [59].

In accordance with previous described studies, Liu et al. (2015) and Salgado et al. (2015) confirmed that the presence of TILs, at the time of diagnosis, was an independent, positive, predictive marker for pCR endpoint, in HER2 positive BC treated with neoadjuvant anti-HER2 agents and chemotherapy [60,61] and Yang et al. (2018) also recommended the 20% threshold for stromal TILs as the best cutoff to predict pathological response to trastuzumab-based NAT in HER2 positive patients [62].

Concerning the role of the different T-lymphocyte subsets, Asano et al. (2016) described that, in HER2 positive and TNBC, the pCR rate was significantly higher in tumors with high CD8+/FOXP3+ ratio. This association was also verified in a multivariate analysis suggesting the independent prognostic value of this ratio to predict response in these aggressive BC subtypes [63]. Furthermore, Rao et al. (2017) demonstrated that, in TNBC patients treated with anthracycline-taxane-based NAT, both high values of CD4+ and CD8+ TILs were associated with higher probabilities of pCR in univariate analyses. CD4+ T cells are critical for the priming of tumor specific CD8+ T cells and for its secondary expansion and memory, which indicate that the effective anti-tumor immune response requires the involvement of both CD4+ and CD8+ T cells [64].

The expression of co-inhibitory immune checkpoint proteins namely the programmed death-ligand 1 (PD-L1), has been described to be a critical reason for T-cell cytotoxic function unresponsiveness against the tumor development and progression. Apart from their prognostic or predictive value, not only the stromal TILs, but also the expression of PD-L1 are strong markers of immune activation in BC and could be implicated in the response to preoperative systemic treatment [65]. Regarding this topic, Wimberly et al. (2015) demonstrated, in univariate and multivariate analyses, that beyond TILs evaluation, also PD-L1 measurement in the epithelium or stroma of hormonal receptor negative and TNBC, can predict pCR [66]. Moreover, Cerbelli et al. (2017) and Loibl et al. (2019) also confirmed, in TNBC, a significant association between PD-L1 expression and pCR rates [67,68] with the use of durvalumab ± conventional NAT [68].

The results of the studies described above, validate the role of immune influence on systemic therapy response, suggesting that, some subtypes of BC, might benefit from immunotherapy. In fact, recent trials are ongoing and demonstrated that pCR rates were improved with the combination of pembrolizumab and standard NAT in hormonal receptor positive/HER2 negative and TNBC [69,70] however, despite of the revolutionary use of pembrolizumab in the treatment of some different cancers [71], more studies are needed in order to better understand its utility in BC patients in this context.

PI3K signaling axis

PI3K/AKT/mTOR is a major intracellular signaling pathway and was well established to play a very significant role in breast tumor cells proliferation and resistance to apoptosis [72]. The central role in this pathway is played by the PI3K heterodimer and the activation of this axis is mainly performed by the declining of PTEN inhibitory functions or by PIK3CA activating mutations on the p110α catalytic subunit [72]. PIK3CA mutation seems to have an important role in BC therapeutic response being in the focus of several recent studies as another promising marker to predict NAT response [73-75].

Overall, BC patients with PIK3CA mutations exhibited a lower pCR rate than wild-type counterparts [74]. Furthermore, patients who retain their initial PIK3CA mutations after NAC seems to have an unfavorable survival [74].

Particularly in the HER2 positive tumors, Seo et al. (2018) and Lobl et al. (2019), demonstrated that mutated PIK3CA was associated with a reduced pCR rate [73,76]. In addition, Guarneri et al. (2015) and Rimawi et al. (2018) showed that, in this tumor subtype, PIK3CA mutations predict lower response to dual blockade NAT with trastuzumab and lapatinib [77,78] and, a study performed by Majewski et al. (2015), also reported that patients treated with a combination of trastuzumab and lapatinib who had wild-type PIK3CA obtained a pCR rate of 53.1%, which decreased to 28.6% in patients with tumors that carried PIK3CA activating mutations [79]. Nevertheless, contradictory results are achieved by Toomey et al. (2017) that generally described no significant difference in pCR rates between HER2 positive patients with PIK3CA mutated tumors and patients without PI3K activation, who received neoadjuvant HER2-targeted therapies [80], justifying the need for more studies to clarify this topic.

Preliminary evidence about the targetability of AKT mutations (a key component of the PI3K signaling network) was demonstrated in a phase I multi-histology basket study. AZD5363 (a pan-AKT kinase inhibitor) was tested in a cohort of 58 patients with different solid cancers including 20 AKT-mutant BC patients along with a response rate of 19% [81].

Although the considerable fewer number of studies about this subject, in TNBC, the FAIRLANE phase II trial demonstrated that pCR rate in the PIK3CA/AKT1/PTEN-altered subgroup was 39% with neoadjuvant ipatasertib (AKT inhibitor) plus paclitaxel versus 9% with placebo. These results may indicate that PI3K/AKT axis seems to be clinically relevant in TN subtype and support further evaluation of the ipatasertib–paclitaxel targeted therapy effectiveness [82].

The activation of the PI3K/AKT/mTOR pathway seems to also be a mechanism of resistance to endocrine therapy [83]. On the other hand, the LORELEI phase II trial, showed global low pCR rate and no differences between groups of postmenopausal women with ER positive/HER2 negative BC treated with letrozole plus taselisib (selective PI3K inhibitor) versus letrozole plus placebo, nor in the overall study population, neither in patients with PIK3CA mutations [84].

Based on the different studies described above, PIK3CA mutations could be a major mediator of therapy resistance in BC, mainly in HER2 positive subtype patients, appearing as an important marker to be evaluated on the way to expect treatment response to neoadjuvant anti-HER2 therapies.

DNA repair defects – BRCA and PARP

Double-strand DNA breaks are repaired by the products of BRCA1 and BRCA2 through homologous recombination. Single-strand DNA breaks are repaired by PARP1 and nucleotide exon repair mechanisms via the base-excision pathway [85].

BRCA mutation is a predisposing factor for BC initiation and development by impairing homologous recombination and triggering genomic instability and, in BC emerge as an independent factor for poor prognosis [86,87]. Beyond BRCA1/2 mutations, other BRCA defects (BRCAness), including BRCA1 promoter methylation, low expression and copy number deletions, probably seems to have similar phenotypic characteristics comparing with tumors carrying germline mutations [88].

In addition to higher risk to develop BC in BRCA mutation carriers, understanding the impact on NAT response will also be important to practice guidelines. The loss of function of the BRCA1/BRCA2 proteins in BC cells may be responsible for the high sensitivity to some agents such as anthracyclines and platinum salts, which induce double-strand breaks in the DNA [89-91]. Indeed, some recent studies demonstrated that BRCA-mutated BC and particularly TN subtype, treated with neoadjuvant anthracycline- or platinum-based regimens are more likely to achieve higher pCR rates [90,92-96]. The TNT trial demonstrated that, in TN metastatic tumors with BRCA mutations, first line therapy with carboplatin had double probability of objective response rate versus docetaxel (68% and 33%, respectively). However, this benefit was not observed in tumors with BRCA1 methylation or low BRCA1 mRNA (BRCAness tumors) [97]. Although these promising results with neoadjuvant platinum-based regimens, a recent study performed by Hahnen et al. (2017) reported that, in *BRCA1* and *BRCA2* mutated TNBC, the pCR rate observed (66.7%) was not further increased in carboplatin arm (65.4%) [98] and, in a recent meta-analysis, was also demonstrated that combining platinum in BC treatment has no differences in pCR rate between BRCA mutated or BRCA wild-type patients [99]. These results showed that the use of BRCA mutations to predict the response to platinum in NAT is quite controversial and further large-scale randomized control trials are required for more robust evidence.

In the line of the possible role of BRCAness in BC treatment response, and similarly with the explained above, in TNBC treated with neoadjuvant taxanes-containing regimens, non-BRCAness tumors was significantly associated with higher pCR rates comparing with BRCAness tumors (77.8% vs. 14.3%). Furthermore, after NAT, the clinical response rates were significantly lower for BRCAness than for non-BRCAness tumors [100]. Other studies demonstrated no significant differences in pCR rate, recurrence and survival between the BRCA1-like breast tumors and sporadic type [101,102], suggesting that patients with LABC would benefit from characterization of BRCA1/2 mutations, but not BRCA1 methylation or low BRCA1 mRNA as indicators of chemotherapy sensitivity and better therapeutic response.

Poly(adenosine diphosphate-ribose) polymerase (PARP) is a key biomarker in DNA repair and cellular stress response, and the PARP inhibitors has being described as a potential controllers in BRCA mutation positive tumors. In fact, in cancer cells with BRCA mutation (abnormal homologous recombination DNA repair), the base excision repair pathway is important for cell survival and some studies suggest that PARP inhibitors are effective in causing BRCA-mutant cells apoptosis (synthetic lethality) [103,104].

A recent study demonstrated that olaparib induced *in vitro* BC cells death and ROS production and *in vivo* caused a reduction of volume and weight of the xenografted breast tumors [105]. Another study described that higher nuclear PARP expression was correlated with increased *in vitro* chemosensitivity to docetaxel and epirubicin. Additionally, *in vivo* chemotherapy sensitivity test, demonstrated that tumors with high nuclear PARP were more sensitive to anthracycline-taxane-based regimens and were associated with pathologic responses to NAT both in univariate and multivariate analyses [106].

Likewise, in the I-SPY 2 trial, was showed that, in TNBC with or without BRCA mutation, the combination of veliparib (a PARP inhibitor) with carboplatin increased the pCR rate comparatively with the control group (51% and 26% respectively), however with a concomitant increase in hematologic toxicity [107]. The BrighTNess trial described, in TNBC patients, an overall increased proportion of pCR in the paclitaxel, carboplatin, and veliparib group (53%) versus paclitaxel alone group (31%) with no additional treatment toxicities, but this difference was not verified in relation to the group of patients receiving paclitaxel plus carboplatin (58% of pCR) [108].

Furthermore Litton et al. (2020), demonstrated that the NAT with oral talazoparib alone (during 6 months after surgery) was associated with 53% of pCR in BRCA1/2-mutated TN and hormonal receptor positive BC [109].

To summarize, we might conclude that, although the potential role of PARP inhibitors as a new anticancer agents in BC, additional research clinical trials are necessary to clarify this proposition.

Multigene expression profiles

Biomarker assays such as Oncotype DX, PAM50 and MammaPrint are known as important tools used to improve the prediction of clinical outcomes, in BC, compared to standard clinical and pathological markers [110-112].

There have been some attempts to identify a molecular signature that could be applied in the neoadjuvant setting and the ability of gene expression profiles to predict NAT response in BC patients [113-115].

In hormonal receptors positive/HER2 negative tumors the Oncotype DX recurrence score (RS) can be used to guide decisions on systemic chemotherapy versus hormonal therapy based on the probability of late recurrence [111,112]. Nevertheless, the possible application of this assay in neoadjuvant setting is less well-studied, with controversial results among different studies. Two neoadjuvant hormonal trials have shown that low RS was associated with higher tumor response to neoadjuvant hormonal therapy than high RS [116,117]. In fact, Bear et al. (2017), described that, in hormonal receptor positive BC, successful breast conserving surgery, after NAT, was performed in 75% and 72% of tumors with RS <11 and 11-25 respectively, receiving hormonal treatment versus 64% and 57% of tumors with RS 11-25 and >25, respectively, receiving chemotherapy. The results of this study suggest that for patients with RS <25, neoadjuvant hormonal therapy is a potentially effective strategy [116]. Furthermore, Iwata et al. (2019) showed that, in postmenopausal patients with ER positive, HER2 negative and clinically node-negative breast tumors, an Oncotype DX recurrence score <18 was significantly associated to a better clinical response after NAT with letrozole [117]. Despite the promising results of these studies, Soran at al. (2016), revealed, in ER positive BC, no differences in pCR after NAT among low, intermediate, and high Oncotype DX RS [118]

A phase II trial of ixabepilone and cyclophosphamide as NAT for patients with LABC demonstrated that, only patients with high RS in baseline Oncotype DX assay achieved pCR, while no pCR rates occurred in the 36 patients with low or intermediate RS. These results were verified in the entire study group but mostly in ER negative patients that had higher scores at baseline and after NAT. The strong positive correlation between the higher rates of pCR and the baseline RS suggests that patients with greatest risk of recurrence are more likely to benefit from NAC [113].

Concerning the role of PAM50 in neoadjuvant setting, a recent study that includes 124 patients with ER positive BC treated with paclitaxel followed by a combination of 5-FU + epirubicin + cyclophosphamide, showed that luminal A tumors, subtyped by PAM50, were less sensitive to NAC and were very unlikely to achieve pCR. Furthermore, PAM50 gene profile also revealed that tumors with low ER expression (1–9%) should present a behavior like ER negative tumors and were more sensitive to NAC than those with high ER levels (≥10%). These low ER expression tumors should therefore benefit from the treatment with chemotherapy [114]. The added value of PAM50 subtyping in the prediction of pCR is most relevant for HER2 positive breast tumors. The HER2-enriched subtype has been associated to a higher pCR rate with the use of transtuzumab and pertuzumab in NAT [31,119,120]. PAM50 signature seems to be useful also for separating

potential responders to NAT among TNBC. A recent study, derived from the WSG-ADAPT-TN trial, was designed to identify predictive markers of pCR in a TN cohort treated with different combinations (nab-paclitaxel + carboplatin or nab-paclitaxel + gemcitabine). The results of this study showed that basal-like PAM50 was independently associated with pCR (38% basal-like vs. 20% non-basal-like) [53].

Some neoadjuvant trials applied the 70-gene assay (MammaPrint) as an integral biomarker on core biopsies for research purposes. In I-SPY 2, a platform trial with multiple experimental-therapy groups, cancers were categorized into eight biomarker subtypes regarding the status of HER2, hormone receptors and risk according to a 70-gene profile. Data demonstrated that veliparib-carboplatin added to standard therapy resulted in higher rates of pCR than standard therapy alone specifically in TNBC [107]. Furthermore, neratinib added to standard therapy was associated to higher rates of pCR than standard chemotherapy with trastuzumab among patients with HER2 positive/hormonal receptor negative BC [121].

The lack of further studies and the limited data available concerning this topic, demonstrated that the benefit of proceed these multigene expression assays to decide NAT requires further clarifications.

Apoptosis-related markers

Many chemotherapeutic agents destroy cancer cells by inducing apoptosis. Therefore, some studies have been evaluating the role of proteins such as p53 and Bcl-2, which are involved in the apoptotic pathway, in predicting response to NAT [122-125].

A recent study, with 247 BC patients receiving anthracycline-taxane-based NAC, demonstrated an association between p53 mutation and chemosensitivity [124]. Particularly, in HER2 positive BC, a trial demonstrated that p53 mutation signature was independently associated with higher pCR rates after NAT with paclitaxel plus trastuzumab alone or with the addition of lapatinib (OR=2.06 95% CI 1.17 to 3.70, p<0.0119) [122]. Furthermore, in patients with TNBC, treated with taxane-based NAC, a significant association between p53 immunoexpression (cutoff 25%), higher objective response and pCR rates was observed (OR=3.961; p=0.003). In this study, Bcl-2 immunoexpression was not significantly associated with pCR [123].

Even though the promising findings of some studies about the role of apoptosis-related markers, specially p53, in predicting NAT response, other studies confirm that, despite high p53 mutation levels in HER2 positive and TNBC, this mutation status did not predict the response to anthracycline-taxane NAC [125].

COX2

COX2 overexpression has been observed in different malignant tumors, including BC [126]. In breast tumor microenvironment COX2 overexpression promote cancer angiogenesis and proliferation, prompting cell invasiveness and metastases [127,128]. Consequently, selective COX2 inhibitors, such as celecoxib, have been explored as a therapeutic or chemosensitizer agents in neoadjuvant setting [129,130].

A recent study, based on a series of 156 HER2 negative BC samples from patients of the celecoxib-treated arm included in the REMAGUS-02 randomized phase II trial, demonstrated

that the effect of celecoxib in addition to NAC was different according to COX2 expression level in terms of pCR rates. Tumors with the highest expression of COX2 demonstrated higher histological grade and hormonal receptor negativity, and pCR rates were significantly higher in patients with COX2-overexpressing tumors receiving NAC plus celecoxib [129]. However, there are other works with some contradictory findings, describing that celecoxib use during chemotherapy adversely affect survival in patients with BC [130].

If the previous results are taken under the same perspective, these findings justify the need for additional trials using COX2 inhibitors in combination with chemotherapy to elucidate the role of COX2 as a predictive biomarker.

Agnostic markers - microsatellite instability, tumor mutational burden and NTRK fusions

The perception that tumors are propelled by their genomic heterogeneity and complexity, rather than just by the tissue of origin, has allowed for a new paradigm in cancer treatment. The tumor-agnostic approach can be used when the tumor retains a very specific molecular alteration, targeted by a particular drug, independently of the tumor histological type. FDA has already approved some drugs based in agnostic markers, such as pembrolizumab in malignancies with microsatellite instability (MSI)/high tumor mutational burden (TMB), and larotrectinib/entrectinib for tumors with NTRK gene fusions [131,132].

The incidence of MSI (hypermutability of short repetitive sequences in cancer genome due to impairment of DNA mismatch repair) in BC is around 1% [133]. Although the low incidence, in TNBC, this parameter could be a potential focus for targeted therapy based in immune checkpoint inhibitors [134]. The TMB also seems to be a potential biomarker for cancer therapy. Tumors with high mutational burden may present more neoantigens that can be recognized by T cells, inciting an anti-tumor response (intensified with the use of immune checkpoint inhibitors) [135,136]. The TMB is variable according to the BC subtype, with TN tumors appeared as the main hypermutated subtype, followed by HER2 positive and luminal-like tumors [137]. Although the lack of studies demonstrating the NAT response predictive role of this marker, recently, Karn et al. (2020), described that, in TNBC, mutational burden independently predicts pCR after NAT (odds ratios for pCR were 2.06 among all patients, 1.77 in the durvalumab treatment arm, and 2.82 in the placebo treatment arm, respectively) [138]. Despite of these positive reports, in BC, additional trials should be developed.

NTRK gene fusions lead to the expression of a chimeric TRK proteins characterized by ligand-independent kinase activation, which consequently drives oncogenesis [139]. A recent basket trial that also include a little percentage of BC patients (3%), demonstrated that larotrectinib (a selective NTRK inhibitor) had marked and durable antitumor activity in NTRK fusion–positive cancers (~79% of patients with an objective response and ~16% demonstrating a complete response to the treatment) [140]. Similarly, in the LOXO-101 trial, a total of 55 patients were treated with larotrectinib, including 1 patient with BC (2%) and the overall response was 75% [141]. On top of that, in another trial, was demonstrated an antitumor activity of entrectinib (tyrosine kinase TRK inhibitor) in a cohort of patients with a large range of solid tumors, including mammary carcinoma, harboring NTRK gene fusions [142]. Highest NTRK gene fusion frequencies were reported in rare cancers like secretory breast carcinoma (92.87% vs 0.60% in non-secretory tumors) [143] and the strong nuclear pan-TRK staining emerge as a sensitive and specific marker for this type of tumor [144,145]. Secretory breast carcinomas are negative for ER, PR and HER2 being considered as a specific subtype of TNBC, probably under-recognized, that could take benefit

from this type of specific therapy [146]. Indeed, the results of these preliminary studies, might open perspectives for further studies in the field of molecularly targeted therapies specially in secretory breast carcinoma subtype.

Final considerations

Despite the efforts to identify novel biomarkers that can be used to predict response after systemic NAT, current guidelines only recommend, besides tumor and histological grading, routinely assessment of ER, PR, HER2 and Ki-67. Since about 70% of patients do not achieve pCR after NAT, there is an urgent need of new feasible biomarkers that could contribute for the construction of a strategy that allows an individualized treatment. This strategy is mandatory for the improvement of the prognosis in BC patients with a consequent decrease in mortality.

Promising new candidates, such as TILs, immune markers, PIK3CA mutation, as well as the germline BRCA status are still under investigation. Nonetheless, the clinical utility of these parameters still requires additional reliable evidence in multicenter clinical trials.

Table I - Outline of the potential predictors of BC patient's response to NAT.

Clinicopathological parameters	
Patient's age	○ Better response to NAT in young women [15]. ○ Woman < 40 years, with HER2 negative tumors - higher pCR rates [15].
Obesity	○ Higher BMI has a negative role on pCR in luminal-like, but not in TN and HER2 positive tumors [17-19].
Breast imaging technics	○ Low mammographic breast density was associated with improved pCR rate [23,24].
Tumor size	○ In basal-like and HER2 positive tumors, the lower cT-stage was correlated with a significantly higher pCR rates [26-28].
Lymph node status	○ Absence of axillary lymph nodes involvement seems to be a predictor of good response to NAT [26].
Tumor grade	○ Grade III TN tumors achieved higher pCR rates after NAC with platinum [29]. ○ Grade III HER2 positive tumors had higher probabilities of pCR following NAT with trastuzumab ± pertuzumab [31].
Immunohistochemical breast cancer subtypes	
Hormonal receptor positive	○ ER positive/PR positive tumors - low pCR [27]. ○ ER positive/PR negative tumors - higher pCR (with anthracycline-taxane-based regimens) [37]. ○ PR negative patients with residual tumor after NAC - higher risk of relapse and poor overall survival [37].
HER2 positive	○ High HER2 expression was an independent predictor of pCR and overall survival [39,40]. ○ ~50% of patients presented elevated probabilities of pCR after NAC ± trastuzumab [43].
Triple negative	○ ~30%–40% of patients achieve pCR after standard anthracycline plus cyclophosphamide and taxane-based NAC [41].
Proliferation markers	
Ki-67	○ High Ki-67 - better pCR rates in all BC subtypes, manly in HER2 positive and TN [49-53].
Immune cells in tumor microenvironment	
TILs	○ High levels of TILs were associated with a 29.5% increase in pCR rate. This association was significantly more consistent in patients with TN and HER2 positive BC [56, 57]. ○ High CD8+/FOXP3+ ratio - higher pCR rates in HER2 positive and TNBC [63]. ○ High values of CD4+ and CD8+ TILs - higher probabilities of pCR in TNBC treated with anthracycline-taxane-based NAC [64].
PD-L1	○ TNBC treated with durvalumab ± conventional NAC - improved response [66-68].
PI3K/AKT signaling axis	
○ HER2 positive tumors - PIK3CA mutations predict lower response to dual blockade NAT with trastuzumab and lapatinib (53.1% pCR in wild-type PIK3CA vs 28.6% in tumors with PIK3CA activating mutations) [77,79]. ○ TN tumors - pCR rates in the PIK3CA/AKT1/PTEN-altered subgroup was 39% with neoadjuvant ipatasertib plus paclitaxel versus 9% with placebo [82].	
DNA repair defects	
BRCA mutation	○ Loss of function of the BRCA1/BRCA2 proteins in BC cells - high sensitivity to anthracycline and platinum salt agents, which induce double-strand breaks in the DNA [89-91]. ○ BRCA-mutated BC and particularly TN subtype, treated with neoadjuvant anthracycline- or platinum-based regimens are more likely to achieve higher pCR rates [90, 92, 95, 96].

PARP	○ BRCA-mutated TN tumors - combination of PARP inhibitors (veliparib or talazoparib) with standard NAC - increased pCR rates [107-109].
Multigene expression profiles	
Oncotype	○ Hormonal receptor positive BC - Oncotype DX low RS was associated with higher tumor response to neoadjuvant hormonal therapy than high RS [116,117]. ○ Locally advanced ER negative BC treated with ixabepilone and cyclophosphamide - only patients with high RS in baseline Oncotype DX assay achieved pCR [113].
PAM50	○ PAM50 gene profile revealed that tumors with low ER expression (1–9%) should present a behavior like ER negative tumors and were more sensitive to NAC than those with high ER levels (≥10%) [114]. ○ The HER2-enriched subtyped by PAM50 - higher pCR rate with the use of transtuzumab and pertuzumab in NAT [119,120]. ○ Basal-like PAM50 was independently associated with pCR after different neoadjuvant combinations (nab-paclitaxel + carboplatin or nab-paclitaxel + gemcitabine) (38% basal-like vs. 20% non-basal-like) [53].
Apoptosis-related markers	
P53 mutation	○ HER2 positive tumors - p53 mutation was independently associated with higher pCR rates after NAT with paclitaxel plus trastuzumab alone or with the addition of lapatinib [122]. ○ In TNBC treated with taxane-based NAC, a significant association between p53 immunoexpression (cutoff 25%), higher objective response and pCR rates was observed [123].
COX2	
○ HER2 positive subtype - pCR rates seems to be significantly higher in patients with COX2-overexpressing tumors receiving NAC plus celecoxib [130].	
Agnostic markers	
MSI/TMB	○ In TNBC, MSI could be a potential focus for targeted therapy based in immune checkpoint inhibitors and, TMB independently predicts pCR after NAT [134, 138].
NTRK gene fusions	○ Basket trials that also include a little percentage of BC patients demonstrated that larotrectinib and entrectinib were associated with higher pCR rates in NTRK fusion-positive cancers [140-142]. ○ Highest NTRK gene fusion frequencies were reported in rare cancers like secretory breast carcinoma (92.87% vs 0.60% in non-secretory tumors) and the strong nuclear pan-TRK staining emerge as a sensitive and specific marker for this type of tumor [143-145]. ○ Secretory breast carcinomas are negative for ER, PR and HER2, being considered as a specific subtype of TNBC, probably under-recognized, that could take benefit from the NTRK inhibitors specific therapy [146].

Abbreviations: BC, breast cancer; BMI, body mass index; MSI, microsatellite instability; NAC, neoadjuvant chemotherapy; NAT, neoadjuvant therapy; pCR, pathological complete response; RS, recurrence score; TILs, tumor infiltrating lymphocytes; TN, triple negative; TNBC, triple negative breast cancer; TMB, tumor mutational burden.

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APPENDIX I

REVISTA SPO – Sociedade Portuguesa de Oncologia

The **Portuguese Journal of Oncology** is the official journal of the Portuguese Society of Oncology and is a scientific publication in oncology area (clinical and investigation). Publishes original articles, reviews articles, clinical cases, oncology images, pharmacoeconomics studies, investigation in health services, special articles and letters to the editor.

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PAGE 1

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PAGE 2

- Title;
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Journal:
Surname and initials of the authors. Title of the article. Name of journal. Year, Volume: Pages.

Ex.: Pao W, Girard N. New driver mutations in non-small-cell lung cancer. Lancet Oncol. 2011; 12(2):175-80.

Book Chapter:
Name and initials of the chapter authors. Chapter title. In: name(s) and initials of the editors. Title of book, City: Name the publishing company, year of publication: first to last page of the chapter.

Ex.: Remy J, Remy-Jardin M, Voisin C. Endovascular management of bronchial bleeding. In: Butler J (ed). The Bronchial Circulation. New York: Dekker, 1992:667-723.

Book:
Name and initials of the authors. Title of the Book. City: Name of publishing company, Year of publication: consulted pages (if applicable).

Ex.: Vainio H, Bianchini F, eds. IARC handbook of cancer prevention. Vol 7. Breast cancer screening. Lyon, France: IARC Press, 2002.

Electronic Document:
Surveillance Epidemiology and End Results (SEER) Program of the National Cancer Institute. SEER. Stat Fact Sheets. http://www.seer.cancer.gov/statfacts/html/all.html (10 May 2011, date last accessed).

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Sending format - JPEG or TIFF - 300 dpi.
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APPENDIX II

Scale for the Assessment of Narrative Review Articles – SANRA

Please rate the quality of the narrative review article in question, using categories 0–2 on the following scale. For each aspect of quality, please choose the option which best fits your evaluation, using categories 0 and 2 freely to imply general low and high quality. These are not intended to imply the worst or best imaginable quality.

1) Justification of the article's importance for the readership

The importance is not justified. — 0
The importance is alluded to, but not explicitly justified. — 1
The importance is explicitly justified. — 2

2

2) Statement of concrete aims or formulation of questions

No aims or questions are formulated. — 0
Aims are formulated generally but not concretely or in terms of clear questions. — 1
One or more concrete aims or questions are formulated. — 2

2

3) Description of the literature search

The search strategy is not presented. — 0
The literature search is described briefly. — 1
The literature search is described in detail, including search terms and inclusion criteria. — 2

2

4) Referencing

Key statements are not supported by references. — 0
The referencing of key statements is inconsistent. — 1
Key statements are supported by references. — 2

2

5) Scientific reasoning

(e.g., incorporation of appropriate evidence, such as RCTs in clinical medicine)

The article's point is not based on appropriate arguments. — 0
Appropriate evidence is introduced selectively. — 1
Appropriate evidence is generally present. — 2

2

6) Appropriate presentation of data

(e.g., absolute vs relative risk; effect sizes without confidence intervals)

Data are presented inadequately. — 0
Data are often not presented in the most appropriate way. — 1
Relevant outcome data are generally presented appropriately. — 2

2

Sumscore 12

Item no 1. Justification of the article's importance for the readership

The importance is explicitly justified:

Pag. 3

"… despite all the advances in BC therapy, the intertumoral and intratumoral heterogeneity of this type of tumors, recognized by pathologists and clinicians, remains a real challenge [2,3]. Considering the current scenario where around 70% of patients do not achieve pCR after NAT [9,13] we might conclude that reliable predictive biomarkers and consistent evaluation of clinical benefit from personalized therapy remain limited".

Item no 2. Statement of concrete/specific aims or formulation of questions

One or more concrete aims or questions are formulated:

Pag. 3

"The present review comprises an extensive overview of the recent literature related to several biomarkers/parameters that might indicate the BC patient's response to neoadjuvant therapy. These biomarkers information and evaluation may be useful for outline the individualized initial systemic treatment or to decide whether breast surgery should be applied at the beginning of the therapeutical approach".

Pag. 5

"In the current BC treatment panorama, the determination of parameters that can differentiate between potential responder and non-responder patients would be extremely important since the initial treatment approach comes up with consequences in survival rates. The potential predictive factors that could be evaluated before NAT are described below and outlined in figure 2 and table I".

Item no 3. Description of the literature search

The literature search is described in detail, including search terms and inclusion criteria:

Pag. 4

"The PubMed search was performed using the following terms, either as MeSH terms or free entries: "neoadjuvant therapy", "breast", "mammary", "cancer", "tumor" and "biomarker" focusing on manuscripts with publication date in 2015 or later. Studies were eligible if: 1) were published in English, Portuguese or Spanish; 2) were performed in women of any age or race; 3) had measured biomarkers expression by immunohistochemistry or molecular quantification in BC and 4) had evaluated biomarkers associated with pCR after NAT.

A total of 281 articles were obtained. Of these 281 articles, we reviewed the title and the abstract and excluded some studies as illustrated on the following flowchart (Figure 1). After this process, it was conducted a detailed revision of the full report of the selected manuscripts".

Item no 4. Referencing

Key statements are supported by references:

Check along the manuscript.

Item no 5. Scientific reasoning

Appropriate evidence is generally present:

Pag.8

"A retrospective study, including women with stage I to III BC, treated with anthracycline and taxane-based NAC, described that high Ki-67 pre-treatment evaluation showed a significantly association with better pCR rates in luminal B and TN subtypes [48]. Vörös et al. (2015) also demonstrated that the higher Ki-67 in pre-treatment core-biopsy samples of BC patients receiving neoadjuvant docetaxel plus epirubicin ± capecitabine was associated with higher probabilities of pCR [49]".

Pag. 11

"… in the I-SPY 2 trial, was showed that, in TNBC with or without BRCA mutation, the combination of veliparib (a PARP inhibitor) with carboplatin increased the pCR rate comparatively with the control group (51% and 26% respectively), however with a concomitant increase in hematologic toxicity [107]. The BrighTNess trial described, in TNBC patients, an overall increased proportion of pCR in the paclitaxel, carboplatin, and veliparib group (53%) versus paclitaxel alone group (31%) with no additional treatment toxicities, but this difference was not verified in relation to the group of patients receiving paclitaxel plus carboplatin (58% of pCR) [108]".

Pag. 13 - 14

"A recent study, based on a series of 156 HER2 negative BC samples from patients of the celecoxib-treated arm included in the REMAGUS-02 randomized phase II trial, demonstrated that the effect of celecoxib in addition to NAC was different according to COX2 expression level in terms of pCR rates. Tumors with the highest expression of COX2 demonstrated higher histological grade and hormonal receptor negativity, and pCR rates were significantly higher in patients with COX2-overexpressing tumors receiving NAC plus celecoxib [129]. However, there are other works with some contradictory findings, describing that celecoxib use during chemotherapy adversely affect survival in patients with BC [130]".

Item no 6. Appropriate presentation of data

Relevant outcome data are generally presented appropriately:

Pag. 13

"A recent study, with 247 BC patients receiving anthracycline-taxane-based NAC, demonstrated an association between p53 mutation and chemosensitivity [124]. Particularly, in HER2 positive BC, a trial demonstrated that p53 mutation signature was independently associated with higher pCR rates after NAT with paclitaxel plus trastuzumab alone or with the addition of lapatinib (OR=2.06

95% CI 1.17 to 3.70, p < 0.0119) [122]. Furthermore, in patients with TNBC, treated with taxane-based NAC, a significant association between p53 immunoexpression (cutoff 25%), higher objective response and pCR rates was observed (OR=3.961; p=0.003). In this study, Bcl-2 immunoexpression was not significantly associated with pCR [123]".