Predictive Factors for Response to Neoadjuvant Therapy in Breast Cancer

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Summary

Within 2 decades, neoadjuvant therapy (NAT) has become a standard treatment option in breast cancer. The advantage of NAT is the ability to monitor the treatment effect. Pathological complete response (pCR) after NAT is a very good predictor for long-term outcome. Clinical factors, such as age and body mass index, as well as recently identified biomarkers can predict the chance of achieving a pCR. Hormone-receptor status, proliferation markers, immune infiltrates and genetic alterations, such as germline *BRCA* and *PIK3CA*, can now be measured almost on a routine basis due to the decreased analysis costs.

Introduction

Neoadjuvant therapy (NAT) is 1 option of standard therapy in breast cancer (BC) [1, 2]. Although the proportion of patients achieving a pathological complete response (pCR) to neoadjuvant (chemo)therapy has increased, 40–80% of the patients, depending on the biological subtype of their tumour, do not achieve a pCR. It has been consistently demonstrated that pCR is very good prognostic factor for long-term benefit from NAT, especially for triple-negative (TNBC) and HER2-positive BC [3–6]. As in the adjuvant setting, the absolute benefit from chemotherapy is smaller for hormone receptor-positive (HR+) tumours, resulting in a lower pCR rate. Nevertheless, HR+ patients who, irrespective of their HER2 status,

achieve a pCR have a better disease-free (DFS) and overall survival (OS) compared to patients who do not attain pCR. Despite pCR holding strong prognostic information, there are patients with pCR who relapse (small number) and patients who do not achieve pCR and never experience a relapse (large number). Therefore, other tools with a more refined separation of the patients into groups with a lower and a higher risk of relapse are needed. The CPS-EG score has been constructed and validated as a prognostic tool, taking into consideration the tumour burden before and after therapy as well as the oestrogen receptor and the grading [7, 8]. Selecting patients with resistance to a given therapy prior to the start of therapy would be the ultimate goal.

Generally, identification of patients with a higher or a lower chance of achieving a pCR can be based on clinical factors and, in the era of translational research, biomarkers.

Clinical Factors

Among the clinical factors, age is an important predictor of response. Young patients seem to achieve higher pCR rates after NAT [9] (fig. 1 [10]). In the GeparTrio study, age was the only independent predictive factor for achieving a pCR in TNBC. The most important factor for survival in TNBC is pCR. In HR+/HER2+ tumours, age also seems to provide prognostic information in patients achieving a pCR [10]. Body mass index (BMI) and age are closely related [11]. Patients with a high BMI have a significantly lower chance of achieving a pCR, and patients with a normal BMI also have the best DFS and OS.

Patients who have an early clinical response to chemotherapy after the first 2–4 cycles have a significantly higher pCR

Fig 1. Age as predictor to neoadjuvant therapy.

Fig 2. Tumour-associated lymphocytes.

rate than those not responding to the first 2–4 cycles. Overall, the responding groups achieve a pCR of about 22%, whereas the non-responding patients have a pCR rate of only about 5% [12, 13]. Several trials have studied whether the adaption of treatment according to early response has an impact on response and/or survival [14–16]. The GeparTrio study [9, 12, 13] investigated whether intensifying therapy at the time of early response is superior to standard therapy. Intensifying the therapy in the responding patients as well as changing the treatment in the non-responding patients resulted in an improved DFS and OS, even though the pCR was not increased. However, this approach seems only to apply for the patients with an HR+ tumour. For patients with a HR-negative (HR-) tumour, the most important prognostic factor is pCR, and adapting the treatment according to response did not have an effect.

Biomarkers

Recently identifying biomarkers for predicting response and resistance has become an important research objective in NAT. The therapeutic landscape has become increasingly complex, and thus the necessity to select the right treatment for the patients is crucial [17]. The HR status is still 1 of the most important biomarkers in BC. In almost all clinical trials investigating neoadjuvant treatment, the HR status was predictive for lower response but better survival [3]. To find additional markers for TNBC, we and others have investigated the androgen receptor (AR). This marker is an independent prognostic marker for DFS and OS in general [18]. Patients with an AR+ tumour had a significantly lower pCR rate than those with an AR- tumour [19]. In TNBC there was no difference in regard to pCR between androgen-receptor positive or negative patients in this study, but others found a difference in favour of higher pCR rate for TNBC patients who were also AR- compared to AR+ TNBC patients [20].

Proliferation is probably the most important factor predicting a pCR after NAT, and different surrogates for proliferation have been put forward. Tumour grade has been shown to be an independent predictor for a pCR at surgery with grade 3 tumours being most favourable [9]. The highest pCR rates were detected in young patients (< 40 years) with undifferentiated tumours. Subgroup analysis revealed that grading remained predictive in luminal A and B tumours. Surrogate intrinsic subtyping using tumour grading has even been shown to outperform intrinsic subtyping in terms of response prediction [21]. The proliferation marker Ki67 is a valuable tool that provides predictive information regarding NAT response and prognosis across molecular subgroups [22]. Being a continuous variable, numerous cut-off points have been defined for Ki67 to try and discern those with a better from those with a worse prognosis [23, 24]. An issue that remains under debate is the different performance of the marker regarding its predictive and prognostic value in different molecular subgroups. In HR+ tumours, the Ki67 cut-off point of 14% has been validated as distinguishing prognostically good (luminal A) and prognostically poor (luminal B) subtypes [25], and has been used for recommendations [26]. However, the cut-off point is rather arbitrary, and a cut-off of 20% has been put forward instead [27].

The genomic grade index (GGI) is a gene expression signature that has been shown to outperform the classic histological grade assessment in terms of prediction, and classifies tumour specimen into low or high risk instead of grades 1, 2, or 3. In the HER2-normal setting, high GGI was significantly and independently predictive for increased chemotherapy response regardless of the HR status [28]. However, despite higher response rates, patients with high GGI had a worse prognosis in terms of distant DFS if they were also oestrogen positive, a relationship that had already been seen when using histological grading as a predictor.

The recurrence score (RS) is a panel of 21 genes that was set up to quantify recurrence risk in oestrogen-positive, node-

negative patients [29]. The RS has also been described to correlate with pCR, such that patients with high RS, and therefore high recurrence risk, are also those most likely to benefit from chemotherapy [30]. Similarly, the ROR (risk of relapse score), which is based on the PAM 50 (the prediction analysis of microarray 50) assay, has been investigated within the NOAH study and was found to be highly predictive of response to neoadjuvant chemotherapy. As shown for the RS, patients with high ROR showed higher pCR rates than those with low or intermediate ROR [31]. Another approach to assess probability of response to treatment is the use of immune markers, due to the suggested effect of the immune system to enhance chemotherapy effectiveness [32].

Tumour-infiltrating lymphocytes (TILs) have been proposed to provide information on pCR prediction in a number of studies. TILs can be grouped into intratumoural (i.e. those with direct contact to tumour cells), stromal (i.e. those between the tumour cells) and LPBC (lymphocyte predominant BC, i.e. if there are more lymphocytes than tumour cells). Lymphocyte infiltration used as a continuous factor (fig. 2) is predictive of neoadjuvant chemotherapy response, and tumour specimens classified as LPBC had significantly increased pCR rates compared to non-LPBC [33, 34]. Moreover, an independent association between TILs and higher responses to trastuzumab and chemotherapy was confirmed in primary HER2+ disease, and an underlying correlation between TILs and immune genes has been found [35]. TILs have also shown to be indicative of good prognosis after chemotherapy, particularly in TNBC [36]. The role of LPBC was also investigated within the translational research programme of the recent GeparSixto study, in which previous results on the predictive value of LPBC in TNBC and HER2+ were validated [37]. There was a significant difference in pCR rates between no LPBC and LPBC groups in the overall cohort. This was even more pronounced in patients treated with carboplatin, indicating a real predictive effect for carboplatin in HER2+ BC. This needs to be validated. In addition, it was shown that the up-regulation of immune genes (immune-suppressing as well as immune-stimulating genes) predict pCR [38].

Molecular Subgroup-Specific Predictors

TNBC is heterogeneous and lacks the possibility of targeted therapies. However, TNBC is also special in that, with the use of more sophisticated and combined therapies, pCR rates have continuously increased in recent years [9, 39–42]. TNBC has been subcategorized into 6 specific and 1 unknown group, and all of them lead to different responses to neoadjuvant chemotherapy [20, 43], the basal-like 1 subgroup showing the highest pCR rate with 52% and basal-like 2 and luminal AR group the lowest rate with only 0% and 10%, respectively. This classification warrants prospective validation but has already set hopes high for distinct response prediction in TNBC.

BRCA mutations have been linked with higher risk for TNBC and seem to be a good predictor of response to NAT for BC. It has been shown that carriers of the *BRCA1* mutation had significantly higher pCR rates compared to *BRCA2* carriers or non-carriers, irrespective of the NAT administered [44]. The GeparSixto study recently demonstrated not only a higher pCR rate for mutation carriers and those with a positive family history, but also a stronger benefit from carboplatin, still to be confirmed in larger cohorts [45].

In HER2+ disease pCR rates have increased dramatically over the years due to the advancement in treatment possibilities. The use of trastuzumab, longer chemotherapy regimens and lately the dual anti-HER2 therapy have helped to continuously raise the pCR rate to as much as 50–60%. The recent Neo-ALTTO study showed a significantly higher pCR rate in the dual anti-HER2 treatment group (51.3%) compared with trastuzumab (29.5%) or lapatinib (24.7%) alone [46] and, furthermore, that pCR correlated with better survival [47].

In the light of these results the importance of detecting the target correctly becomes obvious. Prediction of pCR can only be reliable if patients are treated adequately according to their true HER2 status. Analysis of the GeparQuattro data revealed relevant differences between local and central HER2 testing and the consequences in terms of pCR prediction. Centrally HER2+ patients showed the largest benefit from anti-HER2 therapy, with a pCR rate of 46.8%, whereas patients that were locally HER2+ but negative in the central evaluation had only a 20.3% probability of obtaining a pCR [48].

Within the HER2+ study population, HR status has been identified as a predictor for pCR after NAT. Subgroup analysis of the NeoALTTO and the NeoSphere trial data showed that the benefit in terms of pCR rate was higher in HR– patients compared with HR+ patients, independent of the anti-HER2 therapy (lapatinib, trastuzumab or combination) given [47, 49]. The same effect was observed in the NSABP B-41 trial in which even higher pCR rates could be achieved using the combination of weekly paclitaxel and targeted therapy (lapatinib, trastuzumab or combination) following standard doxorubicin plus cyclophosphamide treatment [50].

With p95HER2 (p95), a promising biomarker has been detected that might predict response to NAT for BC in HER2+ patients. P95 is the C-terminal fragment of the full-length HER2 and can be detected by an immunohistochemical assay using a monoclonal antibody that specifically recognizes the 611 C-terminal fragment. A translational effort using samples from the GeparQuattro study has shown that tumours staining positive for p95 (20% cut-off) showed a higher pCR rate following chemotherapy plus trastuzumab than p95– tumours (59% vs 24%, $p = 0.001$). The predictive value of p95 appeared more pronounced in HR+ tumours and was independent from age, tumour and nodal stage, grade and HR status [51]. These intriguing results were, however, not supported by the data from the smaller CHER-LOB study, in which p95 was not found to be predictive for pCR in any treatment group [52].

More recently, the *PIK3CA* gene has been in the focus as another promising marker [53]. A landmark study by Samuels et al. [54] found frequent tumour-specific (somatic) mutations in *PIK3CA*, the gene encoding p110 α 62, in various human cancers, with > 90% of the mutations affecting exon 9 and 20 [55]. *PIK3CA* has been found to be the second most frequently mutated gene in BC, with unequal distribution amongst the different biological subtypes [56]. Mutations in *HER2*-overexpressing cells have been shown to predict resistance to trastuzumab but not to lapatinib therapy [57]. In the same study, a loss of the phosphatase and tensin homolog (PTEN) predicted for response to lapatinib. The predictive value of *PIK3CA* was confirmed using much larger sample sizes within the GeparQuattro, GeparQuinto and GeparSixto trials. *PIK3CA* was mutated in 20.8% of *HER2*+ tumours [58]. The pCR rate was significantly lower in the *PIK3CA* mutant compared to the wild-type cohort in *HER2*+ tumours, with the difference being largest when a double *HER2* blockade was present [58]. The lowest pCR rate of only 6.3% was found within an *HER2*+/*HR*+ cohort harbouring a *PIK3CA* mutation, identifying *HR* status in addition to *PIK3CA* mutation as an independent predictor of pCR in *HER2*+ BC. Those results are in concordance with data from the Neo-ALTTO [59], Neosphere [60] and the TBCRC 006 [61] studies, and the concept has paved the way for the development of new treatment options with *PI3K*-targeting agents. In the phase II Neophoebe study (NCT01816594), *HER2*-+ patients are stratified according to their *PIK3CA* mutation status and randomized within each cohort to neoadjuvant trastuzumab versus trastuzumab + BKM120 (a potent and highly specific oral pan-class I *PI3K*

inhibitor) in combination with weekly paclitaxel. Results for the primary endpoint pCR will reveal if the *PIK3CA* mutant population benefits more from inhibition of the *PI3K* pathway.

Another putative predictive marker, TP53, which is associated with DNA damage response, has been evaluated in the NAT setting. In a study using a taxane-based regimen, TP53 did not offer reliable prediction of a pCR after 2 chemotherapy cycles [62]. Likewise, an evaluation of whether taxanes confer a greater advantage than anthracyclines in tumours with mutated TP53 compared with wild-type TP53 found that, despite being prognostic for OS, TP53 was not predictive of preferential sensitivity to taxanes [63].

In summary, several factors have been identified that can be used to predict response following systemic NAT and this is reflected in the current AGO guidelines (www.ago-online.de). Promising new candidates, such as TILs, immune markers, *PIK3CA* mutation or the Vanderbilt signature, as well as the germline *BRCA* status are still under investigation. However, there is still a lack of predictors when bevacizumab, dose-dense regimens or PARP inhibitors are being used, and further research will be needed in these areas.

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