

Targeted therapies in breast cancer

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Summary

Targeted therapies have improved cure rates and prolonged survival in metastasised breast cancer. The most important new molecular targets in breast cancer therapy are epidermal growth factor receptor (ErbB) family signalling, DNA repair pathways and angiogenesis. Blocking ErbB2 signalling with anti-ErbB2 antibodies or ErbB2 kinase inhibitors is effective in both the adjuvant and the palliative treatment of ErbB2 positive breast cancer. Poly-ADP-ribose polymerase (PARP) inhibitors lead to synthetic lethality in double strand repair deficient tumours. Anti-VEGF antibodies reduce tumour-induced angiogenesis and prolong progression-free survival in breast cancer. The use of both PARP inhibitors and antiangiogenic therapy is currently hampered by a lack of predictive biomarkers. In contrast, predictive markers are available for ErbB family signalling. This review is intended to give a concise summary of recent developments in the therapy of breast cancer with a focus on new, targeted therapies.

Key words: *targeted therapy; breast cancer; predictive marker*

Introduction

Although significant progress in breast cancer diagnosis and therapy has been made in the last two decades, this disease remains one of the leading causes of death in women. This has spurred research on new and more efficient treatments that overcome the limitations of conventional chemotherapy. Recently, the term ‘targeted therapies’ has been coined to describe new drugs that have been designed based on the knowledge of the underlying molecular pathology of the disease.

Generally speaking, targeted therapies come in three “flavours”: (i) hormone receptor antagonists, (ii) monoclonal antibodies, and (iii) inhibitors of catalytic kinase domains. Indeed, the first targeted therapy in oncology was the use of *anti-hormonal compounds* in oestrogen and progesterone positive breast cancer. However, in this review we will forgo the discussion of this topic in order to focus on recent developments of the field. *Monoclonal antibodies* bind with high specificity to their target antigen on the tumour cell and usually induce complement-mediated phagocytosis.

Kinase inhibitors often bind to the ATP-binding pocket of the enzyme and thus inhibit its catalytic reaction.

In a recent review, Hanahan and Weinberg have summarised the most conspicuous features of human tumours: sustaining proliferative signalling, evading growth suppression, avoiding immune destruction, enabling replicative immortality, tumour-promoting inflammation, activating invasion and metastasis, inducing angiogenesis, genome instability and mutation, resisting cell death, and deregulating cellular energetics [1]. Although all the hallmarks of cancer are principally treatable with drugs, only three of them are currently exploited for the therapy of breast cancer: 1. inhibition of proliferative cell signalling, 2. interference with DNA repair, and 3. anti-angiogenic therapy.

In this review, we will give an outline of the hallmarks of cancer currently targeted in breast cancer therapy and discuss some lessons we have learned while using targeted therapies for this disease.

Targeting proliferative cell signalling

Signalling through the epidermal growth factor receptor family (ErbB) seems to be the most important growth stimulator for breast cancer cells although there are some data that suggest a role for insulin-like growth factor receptor signalling and other receptor tyrosine kinases as well. Downstream of the ErbB receptor, signalling is transduced via 2 main pathways: Ras-Raf-MAPK and PI3K-Akt/PKB-mTOR.

ErbB2/Her2-neu

The most important ErbB family member in breast cancer is ErbB2/Her2-neu. ErbB2 can homo- or heterodimerise with other members of the ErbB family and thus initiate downstream signalling. The ErbB2 gene is amplified in 20% of breast cancers. ErbB2 amplification is associated with clinical outcome and in particular predicts response to anti-ErbB2 therapy and in some studies also to anthracycline- and/or taxane-based chemotherapy. Adequate ErbB2 testing is mandatory for every breast cancer patient. Amplification can be detected by either FISH or immunohistochemistry (IHC). An ErbB2/CEP17 ratio (i.e. the number of ErbB2 signals normalised to the number of centromere signals) of more than 2.2 or a IHC graded as 2+ (if FISH is also positive) or 3+ are accepted criteria for defining ErbB2

amplification or overexpression [2, 3]. *Trastuzumab* (Herceptin; Genentech/Roche, South San Francisco, CA), the first therapy against ErbB2 over-expressing breast cancer, is exceptionally successful in both the palliative and the adjuvant setting. In the first palliative phase III trial published in 2001, trastuzumab prolonged the OS from 20.3 to 25.1 months [4]. Adjuvant trastuzumab after chemotherapy reduced the one-year relapse risk by 46–52% [5, 6]. The most common side-effect of trastuzumab in these trials was cardiotoxicity, with up to 7% of patients showing a decrease of left ventricular ejection fraction (LVEF). Patients with ErbB2-positive breast cancer used to have a worse outcome compared to their ErbB2 negative counterparts. This has changed nowadays and women with ErbB2 amplified breast cancer receiving trastuzumab have an improved prognosis compared to women with ErbB2 negative disease [7]. Despite these impressive results, more than 50% of ErbB2 over-expressing breast cancers are primarily resistant to trastuzumab [8]. Down-regulation of PTEN was shown to result in trastuzumab resistance [9]. These results were confirmed by an unbiased genome-wide RNA interference screen that identified the PI3K pathway as a major determinant of trastuzumab resistance [10]. In a cohort of 55 breast cancer patients, loss of PTEN and activating PIK3CA mutations were predictive of resistance to trastuzumab therapy. However, only about 50% of trastuzumab resistant breast cancer cells harbour PTEN or PIK3CA mutations. Interestingly enough, in this large-scale RNAi screening, no other signalling pathway contributed significantly to trastuzumab resistance. This raises the question whether at least part of the primary trastuzumab resistance is due to an inherent low efficacy of the trastuzumab-mediated immune response rather than to true resistance.

In an effort to improve trastuzumab efficacy, the antibody was conjugated to the fungal toxin DM1 (trastuzumab-maytansine = *T-DM1*, Genentech/Roche, South San Francisco, CA). DM1 binds to tubulin and inhibits microtubule assembly more efficiently than vincristine or vinblastine. A phase II trial has shown an objective response rate of 25.9% in trastuzumab and chemotherapy pretreated metastatic breast cancer patients [11]. Hypokalaemia and thrombocytopenia were the most frequent toxicities. A phase III trial is running.

Another possibility to tackle trastuzumab resistance is the blockade of PI3K signalling, for example at the level of Akt/PKB. However, isolated inhibition of Akt/PKB or possibly other components of the PI3K signalling pathway leads to feedback activation of multiple receptor tyrosine kinases, such as ErbB3, IGF-1 receptor and insulin receptor [12]. Whether combined inhibition of PI3K, Akt/PKB and ErbB2 is feasible and the side-effects are tolerable, has yet to be tested in the clinical setting. Alternatively, trastuzumab might be combined with an mTOR inhibitor. The mammalian target of rapamycin (mTOR) is a downstream component of the PI3K pathway. A recent phase I/II trial with *everolimus* (Novartis, Basel) and trastuzumab in women who progressed on trastuzumab-based therapy showed a partial response in 7 out of 47 patients and stable disease in an additional 9 patients [13]. It was hypothesised that everolimus-evoked relieve of S6-dependent negative feedback might explain the observed effect. In this and in most

other trials investigating mTOR inhibitors S6 kinase was used as a surrogate marker for mTOR inhibition. However, S6 is but one of many downstream effectors of mTOR signalling, and recent data indicate that other components such as 4EBP-eIF4E might be more important effectors of mTOR signalling in human tumours [14]. Thus, it does not come as a surprise that the phosphorylation of S6 kinase did not correlate with the response rate in this trial [13]. This example vividly underlines the importance of adequate biomarker analyses in trials with targeted agents. When secondary trastuzumab resistance occurs in women pretreated with the anti-ErbB2 antibody, trastuzumab can either be continued in combination with a new therapy or one can switch to another anti-ErbB2 therapy. Although preliminary results of the GBG26/BIG 3-05 trial investigating trastuzumab beyond progression were promising [15], the final analysis of this study was negative for overall survival [16].

The first drug approved by the FDA for trastuzumab resistant breast cancer was the oral ErbB2 and ErbB1 kinase inhibitor *lapatinib*. Lapatinib bioavailability is highly influenced by concomitant food intake. In particular, high-fat food increases the uptake of lapatinib [17, 18]. The pivotal trial that led to lapatinib approval compared lapatinib plus capecitabine with capecitabine monotherapy. The trial was closed prematurely, because the first interim analysis showed that the combined treatment with lapatinib reduced the risk of disease progression by 51% [19]. Diarrhoea, dyspepsia, and rash were more frequent in the group with lapatinib. There were no differences in the mean LVEF values between the two cohorts. The combination of lapatinib and capecitabine is particularly attractive in the therapy of ErbB2 positive brain metastasis [20]. In general, the benefit of lapatinib is probably larger in ER and PR negative breast cancer [21]. As first line palliative therapy, combination of lapatinib with either letrozol or paclitaxel improved time to progression and the clinical benefit rate if compared to endocrine or chemotherapy alone [22, 23]. Combining lapatinib with trastuzumab in trastuzumab-refractory metastatic breast cancer increased PFS and clinical benefit rate versus lapatinib alone [24]. Lapatinib is also active as monotherapy in ErbB2 positive inflammatory or non-inflammatory breast cancer that progressed on trastuzumab therapy [25, 26]. In the neoadjuvant setting, lapatinib and paclitaxel were effective against ErbB2 positive inflammatory breast cancer [27]. First results from the phase III neoadjuvant NeoAltto trial confirm the superiority of a combined trastuzumab/lapatinib treatment with regard to pathological complete response. However, the other large phase III trial running in the neoadjuvant setting, GepearQuinto, raised some concerns about increased toxicity when combining lapatinib with epirubicin/cyclophosphamide (EC) and docetaxel chemotherapy [28]. For adjuvant use, lapatinib is currently being evaluated in the Altto trial. Intriguingly, an interim analysis led to the premature closure of arm B (lapatinib alone).

Of note, preliminary data indicate that (in contrast to trastuzumab) low PTEN expression does not predict resistance to lapatinib [29]. Intriguingly, there are preclinical data that lapatinib is also active in ErbB2 negative breast cancer cells. Expression of neuregulin-1 and ErbB3 (but not Er-

bb1) seems to predict response to lapatinib in ErbB2 non-amplified cancer cells [30]. These surprising results argue that an amplification of ErbB2 or activation of ErbB1 are not a *conditio sine qua non* for lapatinib activity. However, this hypothesis has not yet been tested in a clinical trial and the currently available clinical data still suggest that ErbB2 expression is necessary.

A new approach to circumvent trastuzumab resistance is the combination therapy with *pertuzumab*. Pertuzumab is a monoclonal antibody which binds to the ErbB2 dimerisation domain and inhibits heterodimerisation with ErbB1, ErbB3 and ErbB4. Thus, pertuzumab can serve as a paradigm for a new class of targeted drugs, which are aimed at inhibiting protein-protein interactions rather than inhibiting catalytic domains or inducing an immune response. The efficacy of a combined pertuzumab and trastuzumab therapy in patients with disease progression during prior trastuzumab-based therapy was assessed in an open-label, single arm trial [31]. The objective response rate was 24.2% and the clinical benefit rate 50%. The neoadjuvant Neosphere Trial confirmed the benefit of combining trastuzumab with pertuzumab [32]. Importantly, the palliative phase III CLEOPATRA trial has tested the combination of pertuzumab with trastuzumab and docetaxel against placebo, trastuzumab and docetaxel. Pertuzumab increased the PFS from 12.4 to 18.5 months [33]. This underlines the efficacy of a dual inhibition of the ErbB2 signalling pathway. An adjuvant trial with pertuzumab and trastuzumab (APHINITY) is running.

In addition, there are interesting data with *neratinib*, an oral, irreversible, ErbB1, -2 and -4 inhibitor. In a phase II trial, 16 weeks PFS rates were 59% for trastuzumab-pretreated and 78% for trastuzumab-naïve patients [34]. No phase III data are available yet. Finally, there is evidence that the HSP90 chaperones play a role in degrading the ErbB2 receptor. Inhibiting HSP90 therefore increases ErbB2 expression at the cell surface. Tanespimycin binds to the ATP pocket of HSP90 thus inhibiting its function. In a phase II trial, the objective response rate in trastuzumab-refractory patients was 22% and the clinical benefit rate was 59% [35]. Unfortunately, no further trial with tanespimycin is on-going at this time (cf. [36]).

ErbB1/EGFR

EGFR over-expression has been observed in about 15% of unselected breast cancers. In triple negative breast cancer, up to 50% of tumours were found to over-express EGFR [37]. It is not clear whether EGFR over-expression is predictive for anti-EGFR-therapy. Phase II trials investigating single-agent gefitinib or erlotinib showed only a minimal activity. However, an exploratory analysis of gefitinib therapy with tamoxifen or anastrozole compared to endocrine therapy alone indicated a possible benefit in terms of PFS for the gefitinib arm [38]. Although overexpression seems to be a frequent phenomenon, EGFR mutation is not. About 11% of triple negative breast cancers were positive for EGFR mutations in a recent analysis [39]. At this time we do not know if an EGFR mutation predicts response to EGFR blocking agents in breast cancer.

mTOR inhibitors

mTOR inhibitors such as *everolimus* and *temsirolimus* have not only been tested in combination with ErbB2 inhibitors (see above) but also with endocrine therapy. A phase III trial investigated letrozol with or without temsirolimus in ER positive, metastatic breast cancer [40]. The trial was terminated early because of increased toxicity and lack of efficacy. However, a more recent phase II trial assessing letrozol with or without everolimus in the neoadjuvant setting showed a marginally significant increase of the response rate in the combination arm [41]. More importantly yet, the phase III BOLERO-2 trial has investigated the combination of the aromatase inhibitor exemestane plus everolimus in patients with advanced breast cancer. The PFS was 6.9 months in the interventional and 2.8 months in the control group [42]. Taken together, the clinical results obtained with mTOR inhibitors are strongly dependent on the chosen concomitant therapy. This dependence on combination therapy is a feature often observed in targeted therapies.

Src

Src is another kinase involved in proliferative signalling in breast cancer cells. Membranous src is detected in almost 80% of triple negative breast cancers but in only 40% of the other breast cancer subtypes [43]. The results of a phase II trial with the combined src/abl inhibitor dasatinib were disappointing [44]. Another phase II trial with bosutinib (again a src/abl inhibitor) showed evidence of activity [45], but the utility of these compounds in breast cancer therapy has yet to be formally established.

Interference with DNA repair

In mammalian cells, the most important mechanism for repair of DNA double strand breaks is homologous recombination. Blocking homologous recombination and thus DNA double strand break repair results in severe chromosomal aberrations that usually lead to cell death. In familial breast cancer, BRCA1 and -2 genes are frequently mutated. Since BRCA is a crucial component of intracellular homologous recombination, double strand break repair is defective in affected cells.

In contrast, both single strand and base excision repair are controlled by mechanisms independent of BRCA. Single strand break repair definitively depends on poly-ADP-ribose polymerase (PARP), while the role of PARP in base excision repair has remained elusive (reviewed by [46]). The observation that PARP inhibitors trigger cell death in BRCA deficient cells has sparked the interest for PARP inhibitors in breast cancer therapy.

PARP inhibitors

Synthetic lethality is a concept that describes cell death as a consequence of blocking two complementing intracellular pathways. PARP inhibitors induce synthetic lethality in BRCA deficient cells by inhibiting single strand repair in an already double strand repair deficient cell. By which molecular mechanism this is achieved is still a matter of debate (reviewed by [46]). An unresolved issue around PARP inhibitors is the question whether only BRCA mutated can-

cers or rather all triple negative cancers are targets for PARP inhibition. Triple negative and basal-like breast cancer share many clinico-pathological features with BRCA mutated tumours. To describe the similarity between BRCA mutated and BRCA wild-type cancers resembling BRCA deficient tumours, the term 'BRCA-ness' has been coined [47]. BRCA-ness of BRCA wildtype tumours can for example result from CpG island hypermethylation of the BRCA promoter, leading to transcriptional silencing of the BRCA gene [48]. Another possibility for BRCA proficient tumours to gain properties of BRCA mutated cancers is the down-regulation of cyclin-dependent kinase 1 (CDK1) [49]. CDK1 phosphorylates BRCA, which is essential for the formation of BRCA foci in the vicinity of double strand breaks.

In triple negative metastatic breast cancer, a phase II trial of gemcitabine and carboplatin with or without the PARP inhibitor *iniparib* has shown an impressive increase of the overall survival from 7.7 to 12.3 months [50]. Unfortunately, the results of the following phase III study were sobering. No OS benefit could be demonstrated [51]. It is possible that the inconsistency of these results is due to the heterogeneity of the triple negative population; in particular, BRCA status or markers of BRCA-ness were not assessed in the trial, making it impossible to tell whether basal-like breast cancer or BRCA deficient tumours were more likely to respond to iniparib therapy. Additionally, iniparib might be a rather weak PARP inhibitor when compared to other compounds in development (see also [52]). Interestingly, a phase II trial with the oral PARP inhibitor olaparib exclusively in BRCA mutated breast cancer patients showed a response rate of 41% in a heavily pretreated population [53]. BRCA mutated cancers might therefore represent the better target for PARP inhibition than triple negative breast cancer. Although the development of PARP inhibitors was slowed down by the negative phase III trial described above, the chances are that they will ultimately find their way into the clinic.

In this situation, efforts to identify predictive biomarkers for PARP inhibition therapy are mandatory. Larger trials assessing the relevance of BRCA mutation as a marker are needed. PARP expression predicts response to neoadjuvant therapy with anthracyclines or taxanes, but we do not know whether it determines response to PARP inhibitors or not [54]. Different other single gene markers have been tested, but none is robust enough for clinical use. In addition, there is still no standardised way to assess BRCA-ness, thus precluding the use of BRCA-ness as a predictive marker at this time [55].

However, nature has been very inventive when developing mechanisms of resistance against anticancer drugs. This is also true for PARP inhibitors. A particularly artful way of evading tumour therapy has recently been elucidated: secondary somatic BRCA mutations may restore BRCA functionality in carcinomas from women with germline mutations of BRCA1/2. These secondary mutations possibly predict resistance to platin derivatives and PARP inhibitors [56].

Anti-angiogenic therapy

Angiogenesis, the sprouting of new vessels from existing ones, and vasculogenesis, the *de novo* formation of vessels, are rate-limiting steps in the progression of malignant tumours. Thus, blocking vessel formation may lead to tumour starving and regression.

Anti-VEGF-A

The first anti-angiogenic drug approved by the FDA was bevacizumab, a monoclonal antibody that binds the vascular endothelial growth factor A (VEGF-A). In the neoadjuvant setting, two phase III studies have shown an increase of pathological complete response in women with localised ErbB2-negative breast cancer [57, 58]. Three large phase III trials have investigated bevacizumab in the first line therapy of metastasised breast cancer: in the pivotal ECOG E2100 trial, paclitaxel plus bevacizumab prolonged the PFS and increased the objective response rate [59, 60]. The AVADO trial demonstrated similar though less impressive improvements when combining docetaxel with bevacizumab [61]. Finally, the RIBBON-1 study showed an increase in PFS from 5.7 to 8.6 months in the cohort with capecitabine and bevacizumab, and an increase from 8 to 9.2 months in the taxane/anthracyclin and bevacizumab group [62]. However, all three trials failed to demonstrate an OS benefit. Further information comes from the Athena registry, whose OS data are now mature. Athena is a one-arm outcome study. Patients with ErbB2 negative metastatic breast cancer received a taxane or another non-anthracycline chemotherapy in combination with bevacizumab. If chemotherapy was discontinued for toxicity or patient/physician choice (but not for progress), bevacizumab could be continued as monotherapy. The median OS was 30.0 months in patients who continued bevacizumab after discontinuation of chemotherapy and 18.4 months in patients who discontinued bevacizumab before or at the same time as stopping chemotherapy [63]. A subgroup analysis indicated that triple negative breast cancer might benefit most from anti-VEGF-A therapy. However, since this is not a randomised and controlled trial, there may be important confounding factors, which significantly interfere with the results. Thus, there is still no proof that bevacizumab increases OS in patients with advanced breast cancer. Whether OS and/or PFS are useful endpoints in clinical trials with targeted therapies in mammary tumours is discussed controversially. It is debatable whether OS is a fair benchmark for the activity of new drugs, when today's patients suffering from metastasised breast cancer receive an average of 3–6 palliative chemotherapy lines without any evidence of OS improvement for later-line chemotherapies. On the other hand, PFS may not be an important endpoint unless side-effects and quality of life are taken into consideration as well. These complex considerations together with other aspects including economic and public health issues form the basis of an ethical discourse which will not only be of importance for regulatory health authorities but for society as well.

Importantly, anti-VEGF-A therapies such as bevacizumab have side-effects, the most important being hypertension, proteinuria, bleeding and thromboembolic events. Some

concerns have also been raised that bevacizumab might increase the rate of heart failure in breast cancer patients [64]. However, major toxicity of bevacizumab was a rare event in the Athena registry [63]. Elderly patients had the same rate of side-effects except for hypertension, which was more common than in younger patients [65]. There is some evidence that polymorphisms of VEGF-A may influence response and toxicity of anti-VEGF-A therapy [66]. However, these results need to be confirmed in larger prospective clinical trials.

VEGFR inhibitors

Instead of blocking the ligand, VEGF signalling can be suppressed by VEGFR kinase inhibitors. A randomised phase II trial compared paclitaxel and bevacizumab with paclitaxel and motesanib, a VEGFR1, -2, -3, PDGFR and cKit inhibitor [67]. The clinical benefit rate was the same for the motesanib and the bevacizumab cohort, but more side-effects occurred in the motesanib group. An open-label, multicentre phase III trial compared paclitaxel plus sunitinib versus paclitaxel plus bevacizumab [68]. This trial was terminated early by the independent data monitoring board during an interim analysis. At data cut off, the objective response rate was the same for both treatment arms but the duration of response was longer in the bevacizumab arm. This is but one of 4 phase III trials that failed to provide clinical evidence of activity of sunitinib. Together with several other negative trials of VEGFR inhibitors (reviewed by [69]) there is limited hope that VEGFR kinase inhibition will provide a significant benefit in breast cancer therapy.

However, anti-angiogenic compounds directed against other targets, such as for example PlGF or angiopoietins, have been successfully tested in preclinical models of breast cancer, suggesting that further trials with new anti-angiogenic compounds will follow. One of the main obstacles for anti-angiogenic therapy remains the lack of reliable biomarkers. Despite significant research efforts to identify cell populations or serum proteins predictive of response to anti-angiogenic treatment, such markers have yet to be found.

Conclusions and outlook

The era of targeted therapies has brought rapid progress to the treatment of breast cancer. Targeted therapies increase cure rates in localised and prolong survival in metastasised breast cancer. The list of targets for drug treatment has dramatically increased with a deeper understanding of the molecular pathology of breast cancer. However, there are some concerns that need to be addressed in the future. First, a reproducible sub-classification of breast cancer needs to be developed. Terms such as basal-like, triple negative and BRCA-ness have to be better defined and accommodated in such a classification. Ideally, a new classification should mirror targets for drug treatment rather than mere prognosis. Second, every targeted therapy is in need of an adequate biomarker. Although the use of biomarkers will reduce the number of patients who potentially can be treated with a compound, the clinical benefit and cost-effectiveness is ex-

pected to be higher in selected populations. Third, specific weaknesses of targeted therapies should be addressed. For example, therapeutic efficacy is a main concern with antibodies while specificity is major issue with kinase inhibitors. Finally, the rapidly growing body of new drugs will force us to rethink the way we do clinical trials in breast cancer research. Performing large phase III trials with every single one of these new compounds will not be possible. Selection of promising agents earlier in development will have to be more rigorous. However, development of new targeted therapies will undoubtedly continue at a quick pace and hopefully further increase both the life span and the quality of life of breast cancer patients.

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