The Breast 24 (2015) 321–330

Contents lists available at ScienceDirect

The Breast

journal homepage: www.elsevier.com/brst

Review

New approaches for improving outcomes in breast cancer in Europe

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ARTICLE INFO

Article history:
Received 9 October 2014
Received in revised form
18 February 2015
Accepted 6 March 2015
Available online 31 March 2015

Keywords:
Breast cancer
Pathogenesis
Classification
Treatment
Biomarker
Resistance

ABSTRACT

Considerable progress has been made in breast cancer treatment in Europe over the past three decades, yet survival rates for metastatic disease remain poor, underlining the need for further advances. While the use of predictive biomarkers for response to systemic therapy could improve drug development efficiency, progress in identifying such markers has been slow. The currently inadequate classification of breast cancer subtypes is a further challenge. Improved understanding of the molecular pathology of the disease has led to the identification of new targets for drug treatment, and evolving classifications should reflect these developments. Further ongoing challenges include difficulties in finding optimal combinations and sequences of systemic therapies, circumventing multidrug resistance and intra-tumor heterogeneity, problems associated with fragmentation in clinical trials and translational research efforts. Adoption of some of the strategies identified in this article may lead to further improvements in outcomes for patients with the disease.

Introduction

Breast cancer is the most common cancer in women worldwide, being responsible for over 500,000 deaths in 2004 [1]. The disease places a considerable burden on patients and healthcare systems, accounting for 10% of overall cancer costs in the European Union [2]. Nevertheless, progress has been made in the treatment of breast cancer in the Western world over the past three decades, with age-standardized, 5-year relative survival rates in Europe increasing from 73% to 83% between 1992 and 2008 [3]. Despite these advances, 5-year relative survival rates for metastatic disease remain poor [4], though the modest improvements in prognosis observed with the advent of modern systemic treatments suggest that more progress could be made as a result of new therapeutic approaches [5–7]. Nonetheless, survival rates for the disease in Europe still lag behind those observed in the United States [8], underlining the need for further advances across the region.

One of the problems facing the medical treatment of breast cancer in Europe is the high cost in the current economic climate. While the use of predictive biomarkers for response to systemic therapy could improve treatment efficacy and reduce costs, progress in identifying such markers has been slow. Additional challenges include the difficulty in finding optimal combinations, sequencing of chemotherapy and biologic therapy, circumventing multidrug resistance and intra-tumor heterogeneity, along with problems associated with disconnects between clinical trials and translational research efforts.

Breast cancer subtypes: evolving definitions and clinical relevance

Invasive breast carcinoma has traditionally been classified according to histomorphologic features into several variants, the

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http://dx.doi.org/10.1016/j.breast.2015.03.001

most common of which are the ductal and lobular types (reflecting ductal carcinoma in situ [DCIS] and lobular in situ neoplasia, respectively) [9]. Such histologic classifications are currently used in clinical practice along with determination of TNM stage (Tumor size, Nodal involvement, presence of Metastases) to make predictions of disease prognosis [10,11], though they have limited usefulness when selecting the best systemic therapies. More recently, it has become clear that breast tumors are highly heterogeneous in their molecular composition [12], with different subtypes varying in their characteristics and natural history [13–16]. Measurement of these molecular subtypes, which includes determination of estrogen receptor (ER), progesterone receptor (PgR) and human epidermal growth factor receptor 2 (HER2) status, and sometimes also the proliferation marker Ki-67, is important since such factors can assist in the estimation of both disease recurrence risk and response to therapy [9]. Receptor status also has an impact on survival, with triple (ER/PgR/HER2) negative breast cancer (TNBC) being associated with the poorest outcome (Table 1) [17].

Breast cancer subtypes can be defined by the use of gene expression microarrays such as Affymetrix GeneChip (Affymetrix, Santa Clara, USA) [18,19] or Illumina (Illumina Inc., San Diego, USA) [20], or by clinical use of gene expression to direct therapeutic decisions: Mammaprint [21], Oncotype DX (Genomic Health Inc., USA) [22], or PAM50 [23]. However, such methods are not commonly undertaken at present due to cost and limited availability, so a simplified approximation to this classification using clinicopathologic determination of ER, PgR and HER2 is often used to help guide treatment selection in clinical practice [24,25]. Such clinicopathologic criteria involve the use of immunohistochemistry (IHC) to assess receptor status and score protein levels semi-quantitatively, while *HER2/neu* gene amplification is evaluated by fluorescent in situ hybridization (FISH). However, use of IHC is not without problems, with quality assurance for interpretation of the results and quantification being particularly challenging [26]. Indeed, such issues have led to sizeable discrepancies between centers in the results of receptor measurements according to central pathology review. As assessment by IHC is now the predominant determinant of treatment for breast cancer, accurate determination of receptor status is crucial since false negatives/positives have an impact on disease management [26]. Consequently, the precise cut-off points for receptor measurement in clinical trials should be considered with care, with protocols following guidelines for the determination of ER, PgR and HER2 [27,28]. Measurement of Ki-67 may be used in order to differentiate between luminal A and B breast cancer and to identify candidates for chemotherapy. Use of Ki-67 measurement remains controversial due to the wide variations in analytical methods employed and lack of quality control; however, such variability is expected to decrease following recent recommendations on pre-analytical and analytical assessment of this marker [29]. Epidermal growth factor receptor (EGFR), cytokeratin (CK) 5/6 expression and other markers can also be measured in order to determine basal subtype in patients with TNBC [25].

Although breast cancer subtypes defined by clinicopathologic criteria are similar to intrinsic subtypes identified by gene expression profiling and represent a useful surrogate definition, they are not identical. Furthermore, this classification of breast cancer subtypes remains suboptimal as a means of directing therapeutic decisions since substantial heterogeneity exists within each molecular subtype, leading to considerable variability in response to therapy. However, it is hoped that molecular subtyping using gene expression profiling will become routine practice after 2015, should the large trials TAILORx (**T**rial **A**ssigning **I**ndividua**L**ized **O**ptions for Treatment [**Rx**]) and MINDACT (**M**icroarray **I**n **N**ode negative **D**isease may **A**void **C**hemo**T**herapy) release 'positive' results, namely that low proliferative luminal cancers can be safely treated with endocrine therapy only. Gene expression profiling has already been endorsed by the latest St Gallen International Breast Cancer Consensus Conference (2013) for making adjuvant therapy decisions [30].

Current treatment options and unmet needs in breast cancer

Multidisciplinary team (MDT) meetings, involving oncologists, surgeons, radiologists, nurses and pathologists, are considered ideal for the management of early breast cancer so that diagnostic and treatment aspects of patient care can be discussed. Indeed, regular MDT meetings are common in Europe, particularly for complex cases, with treatment recommendations being based on national or European guidelines [25,31].

Treatment for breast cancer is dependent on disease stage, histologic and molecular subtypes and menopausal status. Further aspects influencing treatment choice for early breast cancer include balancing the risk of relapse with the benefit of intervention and patient factors such as the impact of treatment on fertility. Surgery (mastectomy or breast-conserving surgery with or without lymph node dissection) and radiotherapy play an important role in early breast cancer: systemic therapy may be used for almost all women and is the predominant treatment for those with advanced disease [25,32]. Tamoxifen, with or without ovarian function suppression, is recommended for premenopausal women with hormone-sensitive (ER+) disease and an aromatase inhibitor (AI) is the preferred option for postmenopausal women. AI therapy can be induced upfront or sequentially by switching from tamoxifen to AI and vice versa

Table 1
Breast cancer receptor subtypes and associated 5-year survival rates. Reproduced with permission from Onitilo et al. 2009 [17].

Characteristic	Overall survival, % (95% CI)	Disease-free survival, % (95% CI)
Subtype		
ER/PgR+, HER2− (luminal A)	90.3% (87.6–92.5)	86.8% (83.8–89.4)
ER/PgR+, HER2+ (luminal B)	88.7% (79.2–94.1)	83.2% (74.0–89.6)
ER/PgR−, HER2+	78.8% (66.0–87.7)	66.0% (53.9–76.3)
ER/PgR−, HER2−	79.0% (70.8–85.3)	73.5% (65.0–80.5)
ER/PgR status		
ER/PgR+	90.1% (87.5–92.2)	86.4% (83.6–88.8)
ER/PgR−	79.0% (72.4–84.4)	70.8% (63.9–76.8)
HER2 status		
Positive	84.6% (77.3–89.9)	75.9% (68.6–81.9)
Negative	88.5% (85.9–90.6)	84.7% (81.9–87.2)
Overall	87.8% (85.4–89.9)	83.1% (80.5–85.5)

CI, confidence interval; ER, estrogen receptor; HER2, human epidermal growth factor receptor; PgR, progesterone receptor.

[33]. Extended AI therapy with the addition of 5 years of letrozole therapy after 5 years of tamoxifen has also been shown to prolong disease-free survival in postmenopausal women with early breast cancer [34], with the greatest benefit seen in those who were premenopausal at the time of diagnosis but postmenopausal following completion of tamoxifen [30,35]. Nevertheless, the adverse effects of AIs must be considered carefully, particularly in those with pre-existing ischemic cardiac disease [30,36,37]. Neoadjuvant endocrine therapy with an AI or tamoxifen may facilitate tumor shrinkage to allow breast conservation in postmenopausal women with highly endocrine-responsive disease, and 1-year adjuvant treatment with an anti-HER2 agent (e.g. trastuzumab) is advocated for patients with HER2+ disease in addition to chemotherapy [25]. The patient groups that benefit from chemotherapy are not well defined and biomarkers for response are lacking. Nevertheless, indications for treatment include breast cancer of high histologic grade, high levels of proliferation (determined by Ki-67 measurement), low hormone receptor status, HER2+ status and the presence of TNBC. Anthracyclines and taxanes are the preferred chemotherapeutic agents, though no single standard of care exists [25]. Patients with luminal A breast cancer do not respond well to chemotherapy; consequently, such treatment has lost popularity for this subtype but remains in use in the presence of a high tumor burden (e.g. at least four positive nodes). While patients with luminal B breast cancer can respond to chemotherapy, the optimal treatment is uncertain and improved ways of identifying those who will benefit are required. No single treatment algorithm can be defined for patients with advanced disease due primarily to the lack of biologically driven, predictive markers of treatment activity. Consequently, treatment selection must be made according to individual circumstances, taking into account a number of factors including response to previous treatments, disease-free interval, tumor burden, biomarker expression, presence/absence of symptoms, clinical trial availability and patient preference. Therapeutic goals for those with advanced disease include disease control, preservation or improvement in quality of life and prolongation of survival, with symptom palliation and end of life care advocated for those with end-stage disease [38].

Despite the considerable progress made in the treatment of breast cancer in recent years, challenges still remain. In particular, for patients with early disease, there is a significant need for new targeted agents along with novel ways of identifying those who will benefit from them, especially for individuals with luminal B breast cancer and TNBC. Indeed, management of patients with these tumor subtypes is one of the major issues in breast cancer and improved understanding of drivers and poor prognostic groups are needed. Unmet needs in HER2+ breast cancer include treatment of trastuzumab-resistant disease and prediction of the subpopulation that will benefit from costly dual targeting interventions, such as the combination of trastuzumab and pertuzumab.

Challenges in advanced breast cancer include the need for biologically driven criteria to guide treatment choice, identification of the optimal combinations or sequences of targeted agents and integration of new agents into current regimens. In addition, the optimal duration of anti-HER2 therapy for patients with HER2+ breast cancer remains under active investigation in the adjuvant setting. Further research is also needed into the appropriate means of monitoring disease progression, though incorporation of imaging into clinical trials and the development of circulating markers (including circulating tumor DNA [ctDNA]) may help in this regard. The cost of long-term treatment for patients with advanced breast cancer is also a pressing concern for the future, since welcome recent advances in therapy mean that such patients may receive treatment for a considerable length of time.

Challenges in new drug development in breast cancer and the importance of biomarkers

The development of new drugs for breast cancer is hindered by high cost, low likelihood of success due to high attrition rates and poor understanding of the subpopulations that benefit from their use [39]. Despite almost 100,000 patients being enrolled in clinical trials with taxane-based regimens, progress in the knowledge of which patients benefit from such treatment has been minimal and no biomarkers for response or resistance have been validated. Biomarkers for targeted therapy are similarly lacking and available data are conflicting. Of particular concern is the fact that no single marker, beyond HER2, has been able so far to robustly identify patients unlikely to benefit from anti-HER2 therapies.

There is a need for novel trial designs to be adopted in order to hasten drug development for breast cancer in patient subgroups likely to derive substantial benefits. One such approach is the preoperative 'window of opportunity' trial [40] in which patients receive the investigational drug before surgery, with biopsies taken before the drug is given and prior to surgery. Such trials recruit patients faster than neoadjuvant trials, involve less than 150 individuals and can be used to assess whether a targeted agent affects the putative target in humans. However, recruitment into such trials can be an issue since there may be little direct benefit for the patients enrolled; patients may also be deterred by additional biopsies or the potential risk of troublesome side-effects. Neoadjuvant trials are also useful to establish a proof of concept for new drugs, and can help inform the decision as to whether to pursue development in the adjuvant setting; safety should be a primary consideration for these trials as the patient population is potentially curable, with particular care being required for anti-vascular endothelial growth factor drugs to avoid wound healing issues after surgery. The post-neoadjuvant setting is also attractive; here, one would randomize patients with residual disease after neoadjuvant chemotherapy to receive the investigational agent in addition to standard of care, namely endocrine treatment (for patients with luminal B breast cancer), or trastuzumab (for those with HER2+ breast cancer) or placebo for individuals with TNBC (as there is no clear standard of care in this setting) versus standard of care alone. These trials do not need to be large, since they focus on 'high-risk' populations.

At present, registration authorities favor demonstration of an overall survival (OS) advantage in clinical trials. However, OS can be a challenging endpoint, particularly in studies employing crossover from standard therapy to the new therapy (which reflects clinical practice). Indeed, use of OS as a mandatory endpoint for advanced breast cancer registration trials may lead to a lack of new first- or second-line treatment options for patients with metastatic disease; therefore, alternative endpoints are needed. While some trials employ quality of life as a surrogate endpoint [41], the questionnaires used may not accurately reflect patients' daily life. One endpoint that has utility as a surrogate for long-term outcome, particularly for neoadjuvant trials in patients with ER− breast cancer, is pathologic complete response (pCR) [42,43]. Indeed, the US Food and Drug Administration has stated that pCR may be used as a surrogate endpoint to support accelerated approval for neoadjuvant treatment of early breast cancer, and its use is also under discussion by the European Medicines Agency [44,45]. Ki-67 drop under neoadjuvant endocrine therapy is a possible surrogate endpoint for longer disease-free survival for individuals with ER+ tumors.

Future efforts in drug development in breast cancer are likely to focus on implementing molecular screening and next-generation sequencing (NGS) into drug development [46]. The knowledge

base provided by recent NGS studies is unprecedented, and it will take many years before the therapeutic hypotheses developed from this vast data repository will be addressed. Nonetheless, a new treatment paradigm is evolving whereby deep genomic analysis will drive treatment decisions based on a pharmacopoeia of cell type and pathway-matched therapies. However, funding for pivotal phase III clinical trials for novel agents targeting rare mutations is an important concern since such agents may only benefit small numbers of patients, presenting a considerable economic challenge for drug development. Over the past few decades, National Cancer Institute (NCI) Clinical Trials Co-operative Groups, charities and government-initiated research councils have played a key role in the funding and organization of clinical trials in oncology. However, in the current economic climate, government and public funding for such trials has progressively decreased. Furthermore, recruitment of patients with specific molecular aberrations into trials will be challenging given the relatively low frequency of the wide range of alterations. Indeed, trials involving patients with rare mutations may be more suited to international collaboration due to the numbers of patients required for screening. As a result, future trials are likely to require interactions between co-operative groups, academia, industry and networks of institutions involved in molecular screening (e.g. the Breast International Group [BIG] program) [47] to increase the likelihood of success.

In the last two decades, most of the academic research efforts have focused on early-stage disease. However, recently there is growing interest in metastatic disease with the hope of eradicating existing metastases [48], preventing tumor-cell dissemination [49,50] or reducing the ability of disseminated cells from adapting to the novel microenvironment at distant sites [51,52]. Additional changes to drug development in the future may involve strategies to target multiple signaling pathways in order to minimize resistance and improve efficacy, and the development of pharmacodynamic endpoints (e.g. imaging, ctDNA, circulating tumor cells [CTCs]) to assess the activity of targeted agents and monitor disease progression [53,54]. CTC detection has already been shown to be an early marker for disease progression with prognostic relevance after the start of treatment for metastatic disease [55–57]. Further ongoing trials (e.g. SWOG 0500 and NCT01185509) should clarify the role of CTCs in the management of patients with breast cancer and confirm its utility for both monitoring the efficacy of treatment and as a predictive tool. ctDNA, analyzed by multiplex tumor mutation sequencing, has also been shown to be a non-invasive means of monitoring disease progression, with higher concentrations being correlated with worse OS [58,59]. Assessment of such circulating blood biomarkers holds great promise for the future, though their routine incorporation into clinical trials will require the development of robust standardized assays.

Biomarkers of response or resistance are also required to enhance treatment efficacy and reduce costs; their identification is likely to play a major role in any further advances in breast cancer management. Since the impact of different mutation drivers may vary according to stage, biomarker research must investigate mutations in both the primary tumor and metastases. Care is also needed to ensure that biomarker trial designs are appropriate and have sufficient power; with the increasing fragmentation of breast cancer into small molecular subsets, there is a need for collaboration within large networks.

Resistance to targeted agents and the challenge of intra-tumor heterogeneity

Inter- and intra-tumor heterogeneity in breast cancer and other solid tumors has significant implications for both therapeutics and biomarker discovery, presenting a significant barrier to improving survival outcomes [60]. Discordance in ER, PgR and HER2 receptor status between primary and metastatic breast cancer has been well reported [61,62], yet therapeutic decisions for patients with metastatic disease are frequently based on the features of the primary tumor. Biopsies of breast cancer metastases can help to individualize therapy based on the profile of the metastatic disease; however, clinical and genomic heterogeneity also exists across metastases and tumors continually evolve over time [63]. Molecular screening programs such as that undertaken by the BIG aim to perform large-scale screening and sequencing of metastatic breast cancer patients with an effort at understanding better the clonal evolution of the disease. The results of these platforms will assist in determining the optimal treatment for patients with metastatic disease in the future. Until this time, biopsy of the first metastasis to confirm metastatic disease and hormone status prior to treatment selection is recommended by most guidelines [31].

Resistance to systemic therapies is an additional obstacle [64–66]. Many patients with metastatic breast cancer present with intrinsic endocrine resistance, and all patients develop acquired resistance to multiple agents over time [67]. Evidence is emerging that low-frequency subclones may determine outcome, raising challenges to biomarker approaches in oncology. For example, low-frequency presence (~1%) of the EGFR T790M mutation, which confers resistance to EGFR tyrosine kinase inhibitors, leads to poorer progression-free survival (PFS) outcomes following EGFR targeted therapy in non-small cell lung cancer. However, current biomarkers are often not directed at the detection of such low-frequency genetic events, underlining the need for improved methods of detection that will better predict outcome following therapy. Genetic events present early in the evolution of the tumor (e.g. those found in the trunk of the tumor's phylogenetic tree) may present optimal biomarkers and therapeutic targets [68]. A further issue is the fact that several signaling pathways have been associated with the development of endocrine resistance, including the PI3K/AKT/mTOR (phosphatidylinositol 3-kinase/serine/threonine kinase/mammalian target of rapamycin), CDK 4–6 (cyclin-dependent kinase 4–6)/cyclin D1 E2F and AP-1 pathways [69–71], complicating the development of new agents.

A number of strategies may be exploited in the future to either manage or impede resistance to targeted therapy as described below. For example, cross-talk exists between ER and growth factor receptor signaling, with hyperactivation of the PI3K pathway (a frequently mutated pathway in breast cancer) being shown to promote resistance to endocrine therapy (Fig. 1) [70]. The BOLERO-2 trial has demonstrated the effectiveness of targeting this pathway, given the 6-month gain in PFS seen when an mTOR inhibitor (everolimus) was added to exemestane in patients in whom a non-steroidal AI had failed [72]. Moreover, a number of PI3K inhibitors are currently in development, and the possibility of combining these agents with endocrine therapy is being investigated as a therapeutic strategy for patients developing resistance to hormonal agents.

Another promising therapeutic strategy for luminal breast cancer treatment is inhibition of CDK 4–6, as shown in several preclinical studies [71,73] and recent data from a phase II trial of the CDK 4–6 inhibitor palbociclib (PD-0332991) used in combination with letrozole as first-line treatment [74]. Results of preclinical studies also suggest that resistance to endocrine treatment is associated with oxidative stress and elevated AP-1 activity, and that AP-1 levels are higher in tamoxifen-resistant tumors [75,76]. Although further validation is needed, this indicates that blockade of AP-1 function may well reverse tamoxifen resistance. A further approach that may affect the development of resistance is sequencing or intermittent use of endocrine therapy. This strategy has shown promise in a xenograft study employing intermittent

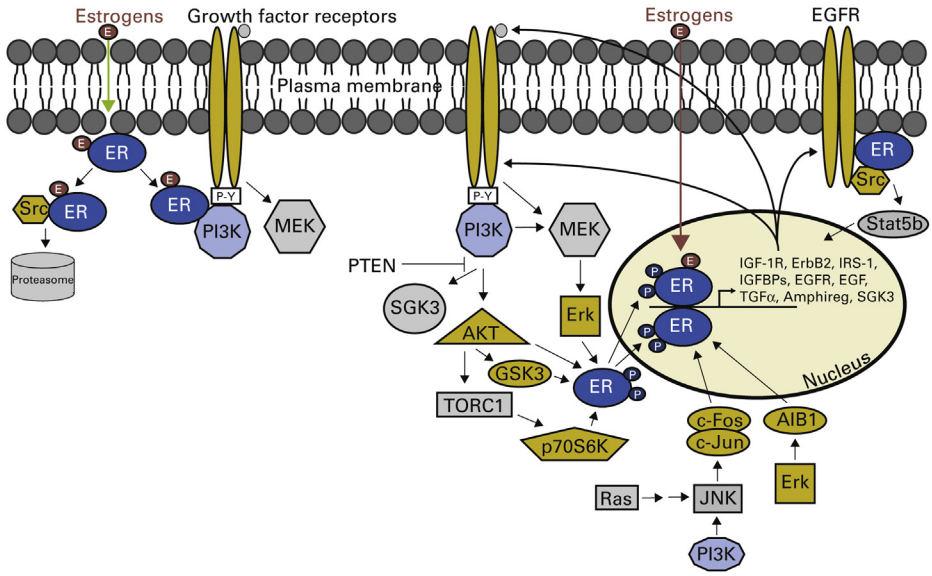

Fig. 1. Cross-talk between estrogen receptor (ER) and growth factor receptor signaling pathways. Reproduced with permission from Miller et al., 2011 [70]. Receptor tyrosine kinases (RTKs) and G-protein-coupled receptors activate phosphatidylinositol 3-kinase (PI3K; blue) and MAPK/Erk (MEK) signaling pathways. These signal transducers then phosphorylate ER (green arrow) and/or coactivators and corepressors to modulate ER transcriptional activity not necessarily dependent on ER ligands. In turn, ER transcribes genes encoding components of growth factor signaling pathways, thus completing the signaling cycle of RTKs to ER to RTKs. ER also complexes with RTKs and Src to rapidly induce nongenomic signaling. ER-interacting proteins are shown in color. AIB1, amplified in breast cancer 1; EGFR, epidermal growth factor receptor; GSK-3, glycogen synthase kinase-3; IGF-1R, insulin-like growth factor-1 receptor; IGFBP, insulin-like growth factor binding protein; IRS-1, insulin receptor substrate 1; JNK, c-Jun N-terminal kinase; MAP, mitogen-activated protein kinases; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; RTK, receptor tyrosine kinase; SGK3, serum/glucocorticoid-regulated kinase-3; Stat5b, signal transducer and activator of transcription 5b; TGFα, transforming growth factor alpha; TORC1, target of rapamycin complex 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

letrozole [77], and is being investigated in an extended adjuvant phase III trial of continuous versus intermittent letrozole in patients with ER+/node+ breast cancer who are disease free after 5 years of adjuvant endocrine therapy.

New therapeutic targets for drug treatment in breast cancer

Agents directed at intra-tumoral targets

A number of new potential intra-tumoral targets have been identified in breast cancer, including tyrosine kinases, fibroblast growth factor (FGF) signaling, insulin-like growth factor, hepatocyte growth factor and c-MET (Table 2) [78]. Amplification of one of these targets, FGF receptor 1, is seen in around 10% of breast cancers, with increased expression correlating with poor prognosis and conferring tamoxifen resistance [79]. Expression of c-MET also correlates with poor prognosis and is seen in all breast cancer subtypes [80]. Further novel targets include second messengers such as PI3 kinase mutation, phosphatase and tensin homolog (PTEN) loss, rare mutations including AKT, PTEN and Janus kinase (JAK), DNA repair alterations and modulation of the p53 pathway. Interruption of DNA repair has been investigated as a strategy for enhancing the activity of chemotherapy in a number of cancers, with efforts focusing on the use of poly(ADP-ribose) polymerase (PARP) inhibitors in DNA repair-deficient cancer [81]. In breast cancer, PARP inhibitors are being investigated as a monotherapy for the treatment of patients with BRCA1/BRCA2 mutations [82] and those with TNBC. While monotherapy with PARP inhibitors is generally well tolerated, a potential but as yet unknown risk of mutation induction and second cancer induction must be considered with chronic inhibition of PARP, given its roles in DNA repair.

Although identification of new intra-tumoral targets is an area of active research in breast cancer, drug development requires improved understanding of tumor biology and biomarkers of outcome, using preclinical studies such as those employing mouse xenografts to identify the molecular profiles likely to respond [83]. Patient-derived breast tumor xenografts (PDXs) are also likely to play a key role in improving our understanding of the complexity and molecular heterogeneity of breast cancer since they retain the morphology, heterogeneity and molecular profiles of the original tumor [84]. Although not all intrinsic breast cancer subtypes are currently represented in PDX models, their use holds particular promise for screening therapeutic agents prior to clinical trials, which is likely to greatly assist the development of future personalized cancer therapy.

There are also challenges associated with the integration of novel agents into current breast cancer management, including optimal scheduling and combination with cytotoxics, though pharmacodynamic parameters can be helpful in this regard. In particular, studies suggest that the optimal biologic dose of the agent should be identified rather than the maximum tolerated dose [85]. Continuous low-dose administration of chemotherapy may also be more effective than intermittent high doses [86], the concept of cyclotherapy has been proposed [87] and the combination of new targeted agents with metronomic chemotherapy also merits exploration.

Agents targeting the tumor stromal compartment

Immunotherapy is thought to be the future direction for biologic therapy in breast cancer as a strategy to target diversity, and novel therapies are expected to control tumor growth without the side effects of traditional treatments. Current research efforts are focusing on a number of immune targets that could be exploited in breast cancer, using antibodies or vaccines to augment the innate immune response against cancer cells. For example, antibody therapy can be used to target the priming or effector phase responses to the antigen of the tumor, through blockade of proteins

Table 2
Selected new targets for drug treatment in breast cancer and novel agents in development.

Molecular target	Agents in development	Company	Development phase
Intra-tumoral targeted agents			
Transmembrane tyrosine kinases			
IGF	Cixutumumab	ImClone Systems Inc.	I/II
	MEDI-573	MedImmune	I/II
	BMS-754807	Bristol-Myers Squibb	I
HGF	Ficlatuzumab	AVEO	II
	Rilotumumab	Amgen	II
	TAK-701	Millennium Pharmaceuticals	I
cMET	Onartuzumab (OA5D5)	Genentech	II
	LY-2875358	Eli Lilly	II
	Tivantinib (ARQ-197)	Daiichi Sankyo	II/III
	Foretinib (XL-880)	Elexis	II
Secondary messengers			
P13K/AKT/mTOR	XL147	Exelixis/Sanofi-Aventis	I
	BYL719	Novartis	I
	BKM120	Novartis	I
	GDC-0032	Genentech	I
DNA repair alterations			
PARP	Rucaparib	Clovis	II
	Olaparib	AstraZeneca	II
	Veliparib	Abbott	II
	MK-4827	Merck	I
	CEP-9722	Cephalon	I
	BMN673	Biomarin	I
	E7016	Eisai	I
Others			
AR	Enzalutamide	Medivation/Astellas	II
	Abiraterone	Janssen	II
Hsp90	NVP-AUY922	Novartis	II
	Ganestespib	Synta	III
CDK	PD0332991	Pfizer	II
Extracellular compartment targeted agents			
Angiogenic vascular cells			
VEGF	Tivozanib	AVEO/Astellas	I/II
	Sunitinib	Pfizer	III
	Sorafenib	Bayer	III
	Ramucirumab	Eli Lilly/Imclone	III
Infiltrating immune cells			
PD-1/PD-L1	MDX-1105-01	Bristol-Myers Squibb	I
	AMP-514	MedImmune	I
	MPDL3280A	Genentech	I
CTL	Ipilimumab (MDX-010)	Yervoy	II
TAM	PLX3397	Plexxikon	I/II
Cancer-associated fibroblastic cells			
MMP	Tanomastat (BAY 12-9566)	Bayer	I
TRAIL	Tigatuzumab	Daiichi Sankyo	I
	PRO95780	Genentech	I

AR, androgen receptor; CDK, cyclin-dependent kinase; CTL, cytotoxic T-lymphocytes; HGF, hepatocyte growth factor; Hsp90, heat shock protein 90; IGF, insulin-like growth factor; MMP, matrix metalloproteinase; PARP, poly(ADP-ribose) polymerase; P13K/AKT/mTOR, phosphatidylinositol 3-kinase/serine/threonine kinase/mammalian target of rapamycin; TAM, tumor-associated macrophages; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; VEGF, vascular endothelial growth factor.

involved in T-cell regulation (cytotoxic T-lymphocyte antigen 4 [CTLA-4] and programmed death 1 [PD-1], respectively) [88,89]. Additional novel agents in development that target the non-malignant tumor stroma include trabectedin, which inhibits macrophage differentiation, receptor activator of nuclear factor κ B (RANK) ligand inhibitors (e.g. denosumab) and drugs targeting the tumor necrosis factor-related apoptosis inducing ligand (TRAIL) pathway (e.g. mapatumumab, dulanermin) [90–93]. Matrix metalloproteinase (MMP)-targeted agents are also under investigation (e.g. marimastat, prinomastat) since MMPs are up-regulated in malignant tissues [94,95]; however, it should be noted that all MMP-targeted agents produced to date have failed in clinical development [96].

The development of immunotherapeutic agents should focus on the use of immunomodulators, alone or in combination with low-dose cytotoxics, to decrease the immunosuppression induced by T-cell effector up-regulation. TNBC and HER2+ breast cancer may be useful settings for the initial study of immunotherapy, as these tumor subtypes have shown higher levels of immune infiltration compared with luminal BC [97–99]. Combination of an anti-HER2 drug with the novel immunotherapy agent should be considered in this population. It should be noted, however, that TNBCs are highly heterogeneous and may be classified into seven different subtypes by gene expression microarray [100]. Differential responses have already been reported among these molecular subtypes [101], and it may be anticipated that their responses to immune stimulation could also differ.

Breast cancer medicine is increasingly moving towards a new era of personalized therapeutics, with strategies targeting cancer cell intrinsic and extrinsic pathways as well as mediators of the tumor microenvironment. The importance of the microenvironment and its potential in cancer therapy is just being established. Among modalities that target the microenvironment, the harnessing of immune responses has long been pursued, though efforts must avoid immune over-activation. Nevertheless, blockade of

immune cell-intrinsic checkpoints, such as CTLA-4 or the PD-1 receptor, has provided the first evidence of activity of an immune-modulation approach in the treatment of a solid tumor and further advances in this field are expected.

Summary

The advent of targeted therapy has led to considerable progress in the treatment of breast cancer in recent years, though challenges remain, including the currently inadequate classification of breast cancer subtypes. Improved understanding of the molecular pathology of breast cancer has resulted in the identification of new targets for drug treatment and evolving classifications should reflect these developments. Further ongoing issues include resistance to systemic therapy, the high cost of drug treatment and the slow progress made in reducing rates of metastatic disease. However, Table 3 provides a detailed vision for improvement, suggesting a number of strategies designed to address these problems. Adoption of some of these approaches may be expected to lead to further improvements in both treatment and outcomes for patients with breast cancer.

Conflict of interest statement

All authors received honoraria from Astellas Pharma EMEA for their participation in the meeting that led to the development of this manuscript. Angelo Di Leo has received honoraria for participation in advisory boards and as a speaker at sponsored symposia from AstraZeneca, Novartis, Pfizer and Roche. Luca Malorni has received research funding from Pfizer and provides consultation for AstraZeneca. Christos Sotiriou has provided consultation for Roche, Novartis and Merck, and has several patents on gene expression signatures associated with prognosis and response to therapies in breast cancer. Alastair Thompson has provided consultation for Roche and Astellas. Andrew Tutt has received honoraria for participation in advisory boards from AstraZeneca, Pfizer, Roche, Clovis, Sanofi-Aventis and Biomarin, and discovery payments under a reward to inventor's scheme from the Institute of Cancer Research

Table 3
Challenges in breast cancer and proposals for addressing them.

Challenge	Proposal for addressing
Treatment selection relies on accurate determination of hormone and HER2 receptor status, yet measurement varies considerably between centers	• Standardization of antibodies and IHC assays (including cut-off points) across clinical trials to avoid false positives/negatives
No single treatment algorithm can be defined for patients with advanced disease as many factors have an impact on treatment decisions	• Treatment selection based on individual circumstances, including both patient and tumor factors • Factors to be taken into account when selecting treatment include response to prior treatments, clinical trial availability, tumor burden, biomarker expression, presence/absence of symptoms, comorbidities and patient preference • Clinical trial efficiency and availability for patients in the future may be determined by a molecular triage by biopsy and next-generation sequencing or molecular imaging of sites of recurrent disease
Dual targeting interventions for patients with HER2+ breast cancer are expensive and ways of identifying the subpopulation that will benefit from such treatment are needed	• Use of imaging before and after anti-HER2 therapy in the neoadjuvant setting to identify appropriate patients with truly HER2-addicted tumors
Although advances have been made in the treatment of early breast cancer, little progress has been seen in reducing rates of metastatic disease	• Improved means of monitoring disease progression, including incorporation of imaging into clinical trials • Development of markers to identify disease progression (e.g. ctDNA in plasma and CTCs)
Development of new drugs for breast cancer is limited by high cost, poor success rates and lack of understanding of the subpopulations that would benefit	• Identification of predictive biomarkers for response/resistance to systemic therapy to improve treatment efficiency and reduce costs • Novel trial designs to accelerate a biomarker-driven drug development process: ◦ Pre-operative 'window of opportunity' trials for early assessment of the ability of a targeted agent to affect the putative target ◦ Neoadjuvant trials to establish a proof of concept for new drugs • Integration of NGS and molecular screening into drug development and collaboration between pharmaceutical companies and molecular screening networks to increase the likelihood of success • Targeting multiple signaling pathways to minimize resistance and improve efficacy • Development of pharmacodynamic endpoints (e.g. imaging, ctDNA, CTCs) to assess the activity of targeted agents and monitor disease progression in 'real time' • Use of preclinical studies (e.g. mouse xenografts) to identify the molecular profiles likely to respond
Use of OS as a mandatory endpoint for advanced breast cancer registration trials may limit new first- or second-line options for patients with metastatic disease	• Use of alternative endpoints (e.g. pCR) for trials in patients with ER− breast cancer or Ki-67 reduction for patients with ER+ tumors
Many patients with metastatic breast cancer present with intrinsic endocrine resistance and all patients develop acquired resistance to multiple agents over time	• Strategies to manage or impede resistance to targeted therapy, including: ◦ Sequencing or intermittent use of endocrine therapy ◦ Blockade of AP-1 function (may reverse tamoxifen resistance) ◦ Use of PI3K/mTOR inhibitors in combination with endocrine therapy in patients resistant to anti-estrogens
Integration of novel agents into current management is challenging, and optimal scheduling and combination with cytotoxics has not been determined	• Identification of the optimal biologic dose of agents rather than the MTD • Studies to investigate the effectiveness of continuous low-dose administration, cyclotherapy and combination of new targeted agents with metronomic chemotherapy

ctDNA, circulating tumor DNA; CTCs, circulating tumor cells; ER, estrogen receptor; HER2, human epidermal growth factor 2; IHC, immunohistochemistry; MTD, maximum tolerated dose; NGS, next-generation sequencing; OS, overall survival; pCR, pathologic complete response; P13K/mTOR, phosphatidylinositol 3-kinase/mammalian target of rapamycin.

with regard to PARP inhibitor therapies. Martine Piccart is a member of the Board of PharmaMar and provides consultation for Sanofi-Aventis, Amgen, Roche-Genentech, Bayer, AstraZeneca, Verastem, MSD, Synthon, Invivis, Astellas and Pfizer. All other authors report no additional conflicts of interest.

Role of the funding source

This manuscript and the original meeting that led to its development were supported by an educational grant from Astellas Pharma EMEA.

Acknowledgments

Highfield Communication Consultancy, Oxford, UK (funded by Astellas Pharma EMEA) provided editorial assistance in the preparation of the manuscript.

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