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REVIEW ARTICLE

Advances in systemic therapy for metastatic breast cancer: future perspectives

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Abstract Breast cancer (BC) is the most common cancer in women worldwide. One in eight women will develop the disease in her lifetime. Notwithstanding the incredible progress made in this field, BC still represents the second most common cause of cancer-related death in women. Targeted drugs have revolutionised breast cancer treatment and improved the prognosis as well as the life expectancy of millions of women. However, the phenomenon of primary and secondary pharmacological resistance is becoming increasingly evident, limiting the efficacy of these agents and calling for a better in-depth knowledge and understanding of the biology as well as the biochemical crosstalk underlying the disease. The advent of laboratory technologies in the clinical setting such as the routine use of next generation sequencing has allowed identification of new genetic alterations as well as providing a precise picture of the molecular landscapes of each tumour. Consequently, new specific therapeutic approaches are becoming available to minimise or delay the occurrence of resistance. In this review, we analyse the latest research and news from the clinical development side for each BC subtype.

Keywords Metastatic BC · BC · Systemic · Cancer

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Background

Breast cancer (BC) still remains the most common type of cancer occurring in women, second only to lung cancer as cause of mortality. Compared to the 1990s, the death rate of BC has slowly declined in 10 years, even though this cancer still remains frequent within the population [1]. Metastatic BC (mBC) is treatable but still virtually an incurable disease, with the main goals of care being finalised to the optimisation of length and quality of life. The European biannual Advanced Breast Cancer Conference (ABC) provides evidence-based international and multidisciplinary guidelines for the diagnosis and treatment of MBC confirming how systemic treatment should be tailored to the molecular characteristic of each individual case (Fig. 1).

ER/PR+ve metastatic breast cancer

Around 70% of breast tumours express the oestrogen receptor (alpha) and/or the progesterone receptor [3, 4]. These tumours are generally characterised by a favourable prognosis. In fact, since the advent of tamoxifen, anti-oestrogen therapies have completely modified the natural history of the disease, becoming the backbone of hormone-positive BC treatment. However, aside from the cases of de novo resistance displayed by some of these tumours, eventually the majority of ER+ve metastatic breast cancers develop resistance to targeted anti-oestrogen treatment [5, 6]. Several mechanisms underlying the occurrence of pharmacological resistance to targeted therapies have been identified. Accumulation of new genetic mutations (i.e. ESR1) and/or constitutive activation of other signalling pathways represent some of the strategies that tumour cells

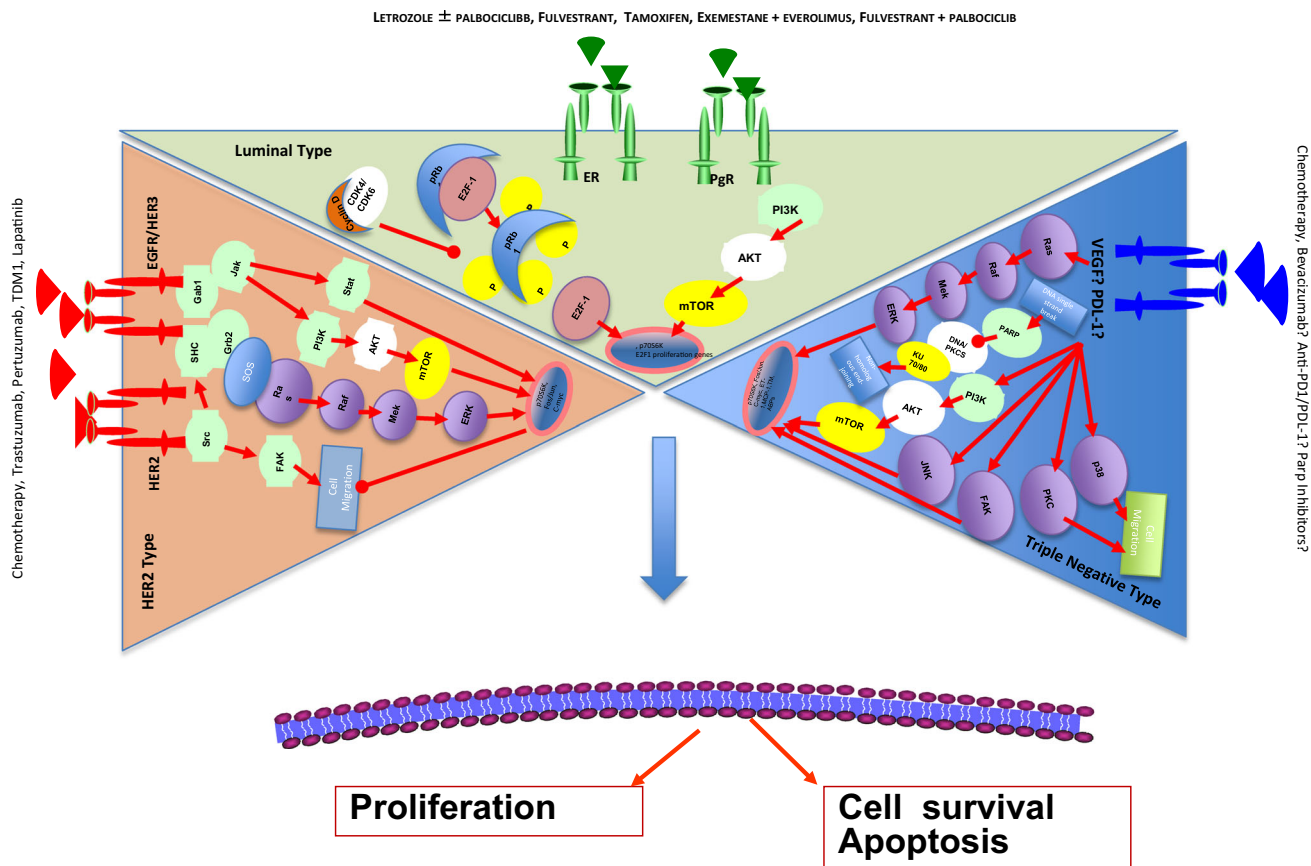## Signal Trasduction and Treatments in Breast Cancer Subtypes

Fig. 1 Signal trasduction and treatments in breast cancer subtypes. Treatments change in accordance to the molecular characteristics of the tumour. In patients that are HR-positive and HER2-negative, the endocrine therapy should be the first-line of treatment. Chemotherapy could always be added during the course of the disease. *ER* oestrogen receptor, *PR* progesterone receptor, *TDM-1* trastuzumab emtansine. *Conventionally, there is always a combination of endocrine therapy with ovarian suppression in pre-menopausal women. Ovarian suppression is recommended in pre-menopausal women in order to reduce the formation of the breast cancer-feeding hormones [2]

employ to override oestrogen receptor inhibition [7]. In particular, phosphatidylinositol 3-kinase (PI3K)—mammalian target of rapamycin (mTOR) signalling pathway activation—has been shown to be associated with resistance to endocrine treatment [8–12].

Oestrogen receptor interaction with the ligand induces a biochemical cascade of events culminating, amongst others, with AKT phosphorylation and mTOR activation [13], which translates into cellular proliferation and metabolic signals, and ultimately survival advantage. Constitutive activation of the PI3K signal downstream, mainly mediated by activating mutations of the catalytic subunit of the kinase [13], AKT kinase mutations or PTEN tumour suppressor deletions [14] overcomes oestrogen receptor-targeted inhibition [15]. Moreover, PI3K activation has been shown to cause a decrease in ER levels and therefore a lesser degree of response to anti-oestrogen therapies [16]. It becomes clear how targeting the PI3 kinase pathway has

the potential to restore sensitivity to ER inhibition and many studies are investigating this possibility using PI3K and mTOR inhibitors in combination with endocrine therapies.

The BOLERO-2 study evaluated the mTOR-specific inhibitor everolimus (Afinitor, Novartis) in combination with exemestane (Aromasin, Pfizer) in comparison with exemestane monotherapy in patients with metastatic breast cancer, previously treated with a non-steroidal aromatase inhibitor [17, 18]. The study enrolled 724 patients and showed a significant improvement with the combination regime in the progression-free survival (PFS) (10.6 versus 4.1 months) leading to the regulatory approval of the combination for mBC treatment. Very interestingly, in a quest for biomarkers of response to targeted therapies, a parallel translational study run during the BOLERO-2 found that the presence of multiple signalling pathways aberrations is associated with lack of response to

everolimus [19]. The data may suggest a multiple targeted combinatorial approach should be reserved to these patients.

Everolimus is undergoing further studies to assess its anti-tumour activity in combination with endocrine therapies in adjuvant and neoadjuvant settings (BOLERO-4, NCT01698918; BOLERO-6, NCT01783444; NCT02291913; NCT02742051 and NCT01805271). Also, a new oral dual mTORC1/2 inhibitor, TAK228, is currently being evaluated in the neoadjuvant setting in combination with tamoxifen (NCT02988986) and letrozole (NCT02619669) in women with ER-positive, PR-positive high-risk early breast cancer.

Other PI3K signalling inhibitors have shown significant anti-tumour activity in preclinical studies and have therefore reached clinical development. AZD5363 a specific AKT inhibitor is being tested in monotherapy (NCT0277569 and NCT01226316) as well as in combination with fulvestrant (Faslodex, AstraZeneca) in metastatic BC previously progressed on aromatase inhibitors (NCT01992952). New PI3K-targeted therapies BYL719 (alpelisib) (specifically designed to target PI3KCA mutations) (NCT01219699 and NCT02437318) and taselisib (NCT02340221) are also being evaluated in the same combinatorial regime with fulvestrant, as well as with letrozole (NCT01923168, NCT01791478, NCT02273973). Buparlisib is also being evaluated in two Phase III trials in combination with fulvestrant in patients previously treated with AIs (BELLE-2, NCT01610284) and after resistance to mTOR inhibitors developed (BELLE-3, NCT01633060). Of note, the BELLE-4 trial evaluated buparlisib in combination with paclitaxel in advanced and metastatic BC with or without PI3K mutation (NCT01572727) and was terminated due to the absence of activity.

The FERGI study is a Phase II trial comparing fulvestrant versus fulvestrant plus pan-PI3K inhibitor pictilisib (GDC-0941) in post-menopausal patients with ER-positive metastatic BC [20, 21]. Results showed no improvement in the median progression-free survival (PFS) with the combination. Also, the study failed to show any correlation between the PI3K mutational status and the combination efficacy [20]. These data are in contrast with the report from the BELLE-2 and BELLE-3 with buparlisib; the mutation status of PI3KCa was predictive of response/outcome in those patients receiving the PI3Kca inhibitor [22]. Taken altogether, these data suggest more translational studies are required in these settings to understand the clinical role of the PI3Kca status with regard to the PI3KCa or/and mTOR inhibitors in mBC.

About the importance of the PI3KCa status, it will also be interesting to evaluate whether specific PI3K isoforms inhibitors in development are more effective than the pan inhibitors [23].

Moreover, inhibition of PI3K or mTOR signalling seems to induce pharmacologic resistance via activation of parallel pathways. Therefore, inhibition of AKT kinase or PI3K upstream of mTOR or combined inhibition of PI3K and mTOR together seems to be the preferred strategies [24]. In fact, dual inhibition of PI3K and mTOR is currently being assessed in a Phase II trial looking at the combination of BYL719 and everolimus plus exemestane (NCT02077933).

Of note, the association of metformin to the above-mentioned combinations (NCT01589367 and NCT01042379) appears to add to the anti-tumour activity, perhaps decreasing the insulin receptor (IR) expression as well as AKT phosphorylation [25] and avoiding the occurrence of hyperglycaemia (the major drug-related toxicity associated with PI3K inhibitors, due to mTOR activation and inhibitory feedback to IGF) [26–28] and insulin resistance.

Disruption of the cyclin D/CDK/pRB pathway occurs in 50–70% of breast cancers [3]. Cyclin D1 amplification is the most frequent alteration and is found in around 70% of BCs [29], in particular in luminal B and HER2-positive BCs [3]. A growing body of evidence suggests the existence of a crosstalk between oestrogen receptor and the cyclin D1/CDK4-6/RB pathway, via downstream signals such as PI3K and mTORC or the convergence of other growth factors intracellular signals [30]. This crosstalk overrides the inhibition of the oestrogen receptor upstream, therefore being responsible for resistance to anti-oestrogen therapies [31] as well as to PI3K-targeted therapies. Also, retinoblastoma tumour suppressor gene-negative breast tumours have shown resistance to tamoxifen in xenografts models and in the clinic, further confirming the convergence of the oestrogen and cyclin D/CDK4/6/RB pathways [32, 33].

Palbociclib (Ibrance, Pfizer Inc.) as a single agent showed minimal activity in a Phase II study in patients with RB-positive breast cancer, with 7% partial responses and 14% rate of stable disease lasting for more than 6 months [34]. Although "partial", these results indicated the potential for synergistic anti-tumour activity in combination with other targeted drugs and the available data pointed towards a combination with hormone therapies. PALOMA-1, a randomised Phase I/II trial, investigated the combination of palbociclib and letrozole in comparison with letrozole alone, for the treatment of advanced breast cancers in post-menopausal women [35]. The following Phase 2 study showed superiority of the combination of palbociclib and letrozole in comparison with letrozole with a marked increase in the progression-free survival from 7.5 to 26.1 months (HR 0.37, $p < 0.001$) [36]. The combination was very well tolerated with neutropenia, leukopenia, anaemia and fatigue being the main toxicities detected. These outstanding results gained palbociclib the designation of "breakthrough therapy" and subsequent approval by the Food and Drug Administration (FDA) as new first-line therapy for patients with advanced or metastatic ER-positive, HER2-negative tumours. Further results

for the overall survival analysis will be gained from an ongoing randomised Phase III study (PALOMA-2, NCT01942135). Palbociclib was also granted FDA approval in combination with fulvestrant, as first-line treatment of metastatic ER-positive, HER2-negative breast tumours which have progressed after prior endocrine therapy, irrespective of menopausal status, on the basis of the results obtained in the PALOMA-3 study (palbociclib combined with fulvestrant in hormone receptor-positive HER2-negative metastatic breast cancer after endocrine failure [37]).

The other CDK4/6 inhibitors have also yielded extremely clinically relevant results. Ribociclib (LEE011, Novartis) was granted "breakthrough therapy designation" by the FDA in view of the results of the Phase 3 MON-ALEESA-2 trial. In this study, the combination of ribociclib and letrozole significantly improved progression-free survival (PFS) from 14.7 months in the placebo group to 19.3 months to "not reached" in the ribociclib group (HR 0.59; $p = 0.002$) in first-line treatment of post-menopausal women with ER-positive/HER2-negative advanced breast cancer [38]. At 18 months, PFS rate in the experimental arm was 63% (95% CI, 54.6–70.3) versus 42.2% in the letrozole arm (95% CI, 34.8–49.5). Other ribociclib-containing combinations are currently being evaluated [39]. Abemaciclib (LY283519, Eli Lilly), another CDK4/6 inhibitor in clinical development, was also granted "breakthrough therapy designation" as a single agent for the treatment of patients with hormone receptor-positive advanced or metastatic breast cancer on the basis of the MONARCH 1 clinical trial results. [40] The drug is currently being evaluated in two Phase 3 trials in comparison with fulvestrant (MONARCH 2) and with a non-steroidal aromatase inhibitor (MONARCH 3) in post-menopausal patients with advanced or metastatic ER-positive/HER2-negative breast cancer [40, 41].

Several trials are investigating multiple combinations of cell cycle inhibitors, PI3K inhibitors and endocrine therapies (NCT02088684 and NCT01872260) [42, 43].

ESR1 mutation in ER+/PR+ metastatic breast cancer and new target tehrapies

The occurrence of new somatic mutations is one of the well-recognised mechanisms of acquired resistance to endocrine therapies in metastatic BC. Recently, two different research groups independently reported relatively high prevalence of previously described mutations of the oestrogen receptor 1 (ESR1) in patients with metastatic BC [42, 43]. Single nucleotide mutations in the ligand-binding domain of the ESR1 gene give rise to constitutively active mutated variants of the receptor, which retain ligand-independent activity

[44, 45]. Toy et al. [43] identified ESR1 mutations in 9/36 (25%) metastatic tissue samples from women with ER+ metastatic BC previously treated with multiple endocrine therapies regimes. In the same fashion, further studies found ESR1 mutations in 55 and 38 and 12% of metastatic biopsies from patients with ER+ metastatic BC pre-treated with multiple endocrine therapies [42, 46, 47]. Whilst the first reports linked the occurrence of ESR1 mutations to previous aromatase inhibitors containing regimens, further studies showed the mutations in treatment-naïve and tamoxifen-treated patients [46, 48, 49]. With the advent of "liquid biopsies", it has become much easier and less invasive for the patient to collect information on the genetic status of the metastases using circulating tumour cells (CTCs) and free circulating DNA (cfDNA). In fact, both of these parameters are thought to provide reliable "indirect pictures" of metastatic disease [50]. Therefore, larger cohorts of patients have been investigated and the ESR1 mutation impact on treatment and prognostic significance looked at [51].

Cell-free DNA in the blood of women enrolled in the BOLERO-2 trial showed a 29% prevalence of ESR1 mutations in this patient cohort, with 6% of the patients carrying double mutations. When the authors looked at progression-free survival (PFS) in the exemestane arm of the trial, patients with ESR1 mutation (D538G) showed a decreased PFS in comparison with the wild-type receptor patients (2.7 vs. 3.9 months, respectively). In the everolimus plus exemestane arm, the PFS increased consistently irrespective of the mutational status of the receptor [51]. When the overall survival (OS) was examined, patients with ESR1 mutations did worse than wild-type patients (median OS 22 vs. 32 months, respectively) and it seems that the type of genetic mutation influenced the OS figures.

In another study (PALOMA-3), patients with advanced or metastatic BC were randomised to receive fulvestrant alone or in combination with palbociclib [52]. In this trial, analysis of the ESR1 status revealed a prevalence of 25% mutations. The benefit of the combination therapy in terms of PFS was seen in the ESR1-mutated population as well as in the wild-type population (with an increase in the PFS from 3.6–9.4 in patients treated with the combination regime).

The intrinsic characteristics of the mutations imply that only drugs that interact directly with the oestrogen receptor, such as SERMs and SERDs, are able to retain their activity against the mutated cells, whereas aromatase inhibitors are not effective in these patients. In fact, as demonstrated in the FERGI study, fulvestrant is active in the patients who carry the ESR1 mutation (37% of the total number of patients in this cohort), with a similar PFS in wild-type and mutated patients in both arms of the trial irrespective of the presence of the PI3K inhibitor [53].

Table 1 Ongoing clinical trials for ER+/PR+ metastatic BC

Trial name	Phase	Administered drug(s)	Status notes	ID
MONALEESA-2	3	Letrozole ± ribociclib	Not recruiting	NCT01958021
MONALEESA-3	3	Fulvestrant ± ribociclib	Recruiting	NCT02422615
MONALEESA-7	3	Tamoxifen or non-steroidal aromatase inhibitor + goserelin ± ribociclib	Recruiting	NCT02278120
MONARCH-2	3	Fulvestrant ± abemaciclib	Not recruiting	NCT02107703
MONARCH-3	3	Non-steroidal aromatase inhibitor ± abemaciclib	Not recruiting	NCT02246621
PENELOPE-B	3	Placebo versus palbociclib	Recruiting	NCT01864746
PALOMA-2	3	Letrozole ± palbociclib	Not recruiting	NCT01740427
PALOMA-3	3	Fulvestrant ± palbociclib + goserelin in pre-menopausal pts	Not recruiting	NCT01942135
PALOMA-4	3	Letrozole ± palbociclib	Recruiting	NCT02297438
PEARL	3	Exemestane + palbociclib versus capecitabine (prior non-steroidal aromatase inhibitor)	Recruiting	NCT02028507
NeoPAL	2	Chemotherapy versus letrozole + palbociclib	Recruiting	NCT02400567
PALLAS	3	Endocrine therapy (5 years) versus palbociclib (2 years)	Recruiting	NCT02513394
CLEE011X2106[175]	1b	Ribociclib + exemestane + everolimus	Recruiting	NCT01857193
CLEE011X2107[176]	1b/2	Letrozole + ribociclib + alpesilib	Recruiting	NCT01872260
JPBH [177]	1b	Abemaciclib + other anti-cancer therapies	Recruiting	NCT02057133
BELLE-2	3	Fulvestrant ± BKM120 (AIs refractory pts)	Not recruiting	NCT01610284
BELLE-3	3	Fulvestrant ± BKM120 (Pts previously on mTOR inhibitors)	Not recruiting	NCT01633060
SOLAR-1	3	Fulvestrant ± alpelisib (AIs refractory pts)	Recruiting	NCT02437318
GO29642	1b/2	GDC-0810 ± LHRH agonist ± palbociclib		NCT01823835
RAD1901-005	1	RAD1901	Recruiting	NCT02338349
D6090C00001	1	AZD9496	Not recruiting	NCT02248090
CLSZ102X2101	1/1b	LSZ102 ± LEE011 (ribociclib) or BYL719	Recruiting	NCT02734615

New SERDs are in different stages of clinical development and eagerly waited for. The GDC-810 is a new orally available selective oestrogen receptor degrader currently being evaluated in patients with metastatic breast cancer with or without ESR1 mutations (NCT01823835), after the encouraging results showed in a Phase I trial [54]. Other SERDs in clinical development are Rad-1901, NCT02338349; AZD-9456, NCT02248090; and LCZ-102, NCT02734615.

It is noteworthy that these new drugs are orally available. Their better bioavailability characteristics will likely overcome the limits of fulvestrant and perhaps favour a more extensive use of this very effective family of oestrogen receptor inhibitors.

The main ongoing clinical trials studying safety and anti-tumour activity of experimental compounds or combinations of targeted drugs in ER/PR+ve metastatic BC are detailed in Table 1.

HER2-positive metastatic breast cancer

Human epidermal growth factor receptor 2 gene amplification and/or protein overexpression are found in 15–20% primary breast tumours [55–57] and are associated with aggressive biological behaviour and poor prognosis [55]. Notably, in inflammatory BC, Zell et al. [58] reported up to 40% HER2 protein overexpression. Other epithelial-derived tumours aside from breast cancer have been shown to carry protein overexpression or gene amplification of HER2 (also reviewed by Yan et al. 2014).

HER2-positive BC effectively illustrates the concept of "oncogene addiction": tumour cells of this subtype depend on activation and downstream signalling of the main "driver", HER2, for proliferation and survival. Inhibition of the signalling cascade induces cell cycle arrest, apoptosis and tumour shrinkage in vivo. This phenomenon, together with the transmembrane position and the tyrosine kinase activity of the receptor, made HER2 an extremely attractive therapeutic target. Trastuzumab (Herceptin, Genentech) was the first monoclonal antibody specifically directed against the juxtamembrane portion of the HER2 receptor [59]. Its advent, as well as profoundly changing the approach to cancer therapy, has greatly improved the prognosis of HER2-positive BC [60], not only in the early stages, but also in patients with advanced or metastatic disease [61]. The combination of trastuzumab with chemotherapy in the adjuvant and neoadjuvant settings represents now the standard treatment for this BC subtype,

after several studies demonstrated the significant superiority of the combination over chemotherapy alone [60, 62, 63]. Notwithstanding the incredible results achieved with trastuzumab, the phenomenon of primary and secondary pharmacological resistance has become increasingly frequent, ultimately limiting treatment outcome [64].

Most of the molecular changes investigated to explain de novo and/or acquired resistance to HER2-targeted therapies are to be attributed to the phenomenon of the "adaptive response": when the primary oncogenic driver is inhibited, tumour cells are able to survive by activating other "secondary" signalling pathways, ultimately overcoming their "oncogenic dependency" on the main mutation.

Several signalling pathways (either downstream of or "parallel" to HER2) are activated in response to anti-HER2 therapies and therefore responsible for pharmacologic resistance. Phosphoinositide 3-Kinase (PI3K-Akt), EGFR, IGFR, mTOR and MAPK/ERK pathways are the most commonly involved [65–69].

Loss of PTEN or activation of mTOR is able to overcome HER2 signal blockade [69, 70]. Other molecular aberrations involved with trastuzumab resistance include gene mutations and expression of a truncated form of the HER2 protein [71].

More recently, after several reports highlighted the presence of cell cycle molecular aberrations in HER2-positive BC, as well as pointing out the requirement for cyclin D1 and CDK4/6 in HER2 models of tumourigenesis [3, 72, 73], Goel et al. [74] provided stronger evidence of the involvement of the cell cycle in pharmacologic resistance to targeted anti-HER2 therapies. Hyper-activation of the cyclin D1/CDK4/6/pRB pathway has been linked to acquired resistance to anti-HER2 targeted therapies. A small group of tumour cells were shown to be able to survive HER2 targeted inhibition: nuclear overexpression of cyclin D1, the common molecular feature amongst these cells, represents direct indication of cell cycle activation [74].

These data altogether provide enough evidence to highlight the need for new more effective anti-HER2 therapies, specifically in a combinatorial approach, to block the crosstalk between pathways, to achieve simultaneous inhibition of multiple signalling pathways or to obtain complete HER2 signal blockade (using more than one targeted therapy when the mechanisms of action of each compound differ), therefore preventing pharmacologic resistance [75–77].

There is increasing evidence that some of these approaches are paying off.

So far, four anti-HER2 targeted therapies have become available: trastuzumab, pertuzumab, lapatinib and ado-trastuzumab emtansine. Review of each of these agents is beyond the scope of this article. More in-depth information can be found elsewhere [78–80].

Lapatinib (Tykerb, GlaxoSmithKline) is a small molecule with tyrosine kinase activity, which therefore displays totally different characteristics in terms of mechanism of action, safety and toxicity profile from those of trastuzumab. In particular, lapatinib is effective in patients whose tumours express the truncated HER2 protein [81] or lack the tumour suppressor PTEN [82]. Instead, both these aberrations induce resistance to trastuzumab. This is just one example reinforcing the concept of a dual blockade of the epidermal growth factor receptor 2. In fact, two recent trials NeoALTTO and NeoSphere have confirmed superior efficacy of the dual blockade/combination of anti-HER2 agents in comparison with single therapy with each agent alone in early BC [83, 84]. Both studies showed much higher rates of pathologic complete remission when the patients received either trastuzumab plus lapatinib or trastuzumab plus pertuzumab, plus chemotherapy, rather than anti-HER2 monotherapy plus chemotherapy. Lapatinib is currently approved in combination with letrozole or capecitabine for HER2-positive metastatic BC resistant to trastuzumab, when hormonal therapy is indicated.

Pertuzumab (Perjeta, Genentech) inhibits the dimerisation of HER2 and therefore signalling through the receptor. The CLEOPATRA study evaluated the combination of trastuzumab plus chemotherapy plus or minus pertuzumab as first-line therapy for patients with metastatic breast cancer (CLEOPATRA, NCT00567190) [85]. The study showed significant PFS (6.3 months difference) and OS (15.7 months difference) improvements in patients treated with the combination of pertuzumab, trastuzumab and docetaxel versus patients randomised to receive only trastuzumab plus chemotherapy. These data contributed to the FDA approval of pertuzumab in combination with trastuzumab plus chemotherapy (docetaxel) in patients with HER2-positive metastatic BC. In 2013, the antibody was also approved for the neoadjuvant treatment of HER2-positive BC [86].

Pertuzumab is currently being tested in combination with other approved or experimental drugs in the neoadjuvant setting in patients with BC (I-SPY 2 trial, NCT01042379).

Neratinib and afatinib are the second-generation small molecule inhibitors of HER1, HER2 and HER4 (only neratinib) [78, 87]. These agents are currently being evaluated in the clinic in monotherapy or in combination with chemotherapy as well as with other targeted drugs, in the adjuvant and neoadjuvant settings (NCT00915018, NCT00878709, NCT01271725, NCT01441596, NCT01125566) [88, 89]. A Phase II randomised trial of neratinib monotherapy versus the combination of lapatinib plus capecitabine (approved by the FDA) in HER2-positive advanced BC showed significant activity of the second-generation HER2 inhibitor (neratinib) as single-agent

therapy, but failed to demonstrate the superiority or inferiority of this molecule to lapatinib plus capecitabine [90].

The LUX-Breast 1 trial (NCT01125566) also compared trastuzumab plus vinorelbine to afatinib plus vinorelbine in patients with metastatic HER2 BC previously treated with at least one trastuzumab-based regime, but failed to show the superiority of the afatinib/vinorelbine combination.

Tucatinib (ONT-380) is also being evaluated in combination with other HER2 inhibitors in Phase I clinical trials (NCT01921335, NCT0198501 and NCT02025192).

Other trials are underway to study the anti-tumour effect of these new agents, as well as to find markers of response to treatment, in breast cancer (NCT01670877 and NCT01042379) and other solid tumours overexpressing HERI or HER2 (mainly lung and gastric cancer) (NCT01522768). An area of great interest and possible application for these second-generation inhibitors, as well as for lapatinib, are the treatment of central nervous system metastatic disease (NCT02650752 and NCT01921335) [91]. The incidence of brain metastases in patients with advanced HER2-positive breast cancer is in fact relatively high, and successful treatment of distant disease in this area would tremendously improve the prognosis for this patient cohort [92, 93].

T-DM1 (Kadcyla, Genentech), a conjugate drug coupling trastuzumab with a cytotoxic anti-microtubule drug called maytansinoid, has shown good results in the early phases of clinical development [94, 95] and was approved by the FDA in February 2013 for treatment of HER2-positive metastatic BC previously treated with trastuzumab and taxanes, after the encouraging results of the EMILIA trial [96]. This agent is currently being tested in a Phase Ib/II trial in combination with the anti-CDK4/6 agent ribociclib compared to trastuzumab plus ribociclib in women with metastatic HER2-positive BC (NCT02657343). New antibody–drug coupled compounds are being evaluated in the clinic: MM-302 uses nanoparticle technology to deliver

anthracyclines to HER2-positive tumours cells. The HERMIONE trial looked at the combination of MM-302 with trastuzumab in comparison with chemotherapy plus trastuzumab in patients with advanced/metastatic HER2-positive breast cancer never treated with anthracyclines, who progressed under trastuzumab or TDM-1 therapy (NCT02213744) [97]. The study was prematurely terminated as the experimental combination failed to show any benefit.

Data from BOLERO-3 [98] support the combinatorial approach with anti-mTOR and trastuzumab for women with trastuzumab-resistant, HER2-positive advanced BC.

Other PI3K inhibitors are being evaluated in the clinic for this type of metastatic BC: the PANTHER study, a Phase Ib/II single-arm trial, is currently evaluating the safety, pharmacokinetic profile as well as the anti-tumour activity of copanlisib (PI3Kinase inhibitor) in combination with trastuzumab in recurrent or metastatic HER2-positive BC previously pre-treated with anti-HER2 targeted therapies (NCT02705859).

The first clinical studies testing the combination of trastuzumab and palbociclib, in advanced or metastatic cancers, as well as other combinations of CDK4/6 inhibitors with targeted anti-cancer therapies are also underway (Table 2). The NA-PHER2 trial (NCT02530424) investigates the combination of trastuzumab, pertuzumab, palbociclib and fulvestrant for neoadjuvant treatment of ER-positive, HER2-positive invasive breast cancer. The PATRICIA study (NCT02448420) is a Phase 2 trial designed to compare the combination of palbociclib and trastuzumab plus or minus letrozole for the treatment of HER2-positive, ER+ or ER−, locally advanced or metastatic breast cancer in post-menopausal women previously treated with chemotherapy and trastuzumab.

Lastly, immunotherapies are currently being tested in this molecular subtype of cancer (Table 2): atezolizumab is being evaluated in combination with trastuzumab

Table 2 Ongoing clinical trials for HER2-positive metastatic BC

Trial name	Phase	Administered drug(s)	Status notes	ID
CLEE011XUS20T	1b/2	Ribociclib + trastuzumab or TDM1	Recruiting	NCT02657343
monarcHER 2	2	Abemaciclib + other anti-cancer therapies	Recruiting	NCT02675231
NA-PHER2	2	Trastuzumab + pertuzumab + fulvestrant + palbociclib (neoadjuvant)	Not recruiting	NCT02530424
PATRICIA	2	Trastuzumab + palbociclib ± letrozole	Recruiting	NCT02448420
ARRY-380	1	ONT-380 + trastuzumab	Not recruiting	NCT01921335
LUX-Breast 2	2	Afatinib ± vinorelbine or paclitaxel	Not recruiting	NCT01271725
201209135	2	Neratinib ± fulvestrant in mutated HER2 BC	Recruiting	NCT01670877
Panther	1b/2	Copanlisib + trastuzumab	Recruiting	NCT02705859
GO29831	1b	Atezolizumab + trastuzumab and pertuzumab or TDM-1	Recruiting	NCT02605915
PANACEA	1b/2	Pembrolizumab + trastuzumab in trastuzumab-resistant HER2 BC	Recruiting	NCT02129556

emtansine (T-DM1) or with trastuzumab plus pertuzumab in patients with HER2-positive BC (NCT02605915), whilst PANACEA is a Phase Ib/II trial investigating the efficacy of pembrolizumab in combination with trastuzumab in women with HER2-positive, metastatic breast cancer, who progressed whilst on trastuzumab (NCT02129556).

As it becomes clear from the type and number of ongoing studies, the current issues regarding the therapeutic approach to HER2-positive breast cancer are being addressed mainly by designing multiple combinations of agents that target different features of transformed cells at the same time. This strategy weakens the very ability of cancer to "adapt" to targeted therapies, depriving cells of the biochemical signals necessary for survival and proliferation. However, more clinical trials are needed to identify reliable biomarkers with the aim to define and target subgroups of patients that are most likely to benefit from specific combinations of therapies. Such achievement will really start the era of "personalised" medicine.

New therapeutical strategies in triple-negative metastatic breast cancer

Historically the idea of immunotherapy against cancer goes back to the early 1910s when William Coley proved that injection of *Streptococcus* and *Serratia* into tumours (specifically round-cell sarcoma), caused them to shrink [99]. The bacteria evoked an immune response that ultimately led to shrinkage of the cancer. Today various immunotherapies have been developed based on four different strategies: (1) non-specific immune stimulation, (2) adaptive cell transfer, (3) vaccination and (4) immune checkpoint blockade. The latter represents the most promising approach, in particular after the outstanding results obtained in melanoma and NSCLC [100, 101].

Breast cancer tissue immune profiling exposed how T-lymphocytes represent the main population of immune cells found in the context of the tumour (70–80%), with the rest of the immune cells composed of B-lymphocytes, macrophages, antigen-presenting cells (APC) and natural killer cells (NKC) [102, 103]. A portion of TNBC has been proved to be highly immunogenic, showing relatively high percentage of tumour infiltrating lymphocytes (TILs) [104], higher levels of B7 gene family member type I [105] programmed cell death 1 ligand 1 (PD–L1) protein [106, 107] and mRNA [108, 109] expression levels in comparison with other breast cancer subtypes. It has been shown that there is a strong and consistent correlation between the presence of immune markers and/or TILs and the likelihood of achieving a pCR after neoadjuvant chemotherapy in TNBC [110]. High expression of tumour markers and high TIL density are associated with benefit from chemotherapy in TNBC [111–114]. These data suggest that chemotherapy regimens elicit better results in the presence of a proficient immune system, as initially proposed by preclinical data [115, 116]. Moreover, many research groups have found a strong association between high levels of immune markers or TILs and low risk of relapse and/or death of TNBC patients early treated with systemic chemotherapy [104, 108, 117–126]. These data altogether provide a strong rationale for testing immunotherapies in a highly immunogenic cohort of TNBC.

Many immunotherapy-based studies on breast cancer [126–130] suggest an effective engagement of the immune system, although insufficient to entirely eradicate the tumour, can help reduce the risk of metastasis or maintain tumour dormancy [131].

A new class of immunotherapies targeting the so-called immune checkpoints (mainly PD-1 and its ligand, PDL-1 and CTLA-4) has shown promising results.

Two Phase I trials evaluated immune checkpoint inhibitors in advanced-stage TNBC [132, 133]. In one of them (KEYNOTE-012), anti-PD-1 pembrolizumab induced a response in 18.5% of ER-positive breast cancer patients enrolled in the study [132]. Expression of PD-L1 measured by immunohistochemistry (IHC) was used as criteria for selecting patients for these two trials [132, 133]. However, the validity of PD-L1 as a selection marker remains still controversial from data coming from studies on other solid tumours, where substantial survival benefit was shown when anti-PD-L1 antibodies were used in PD-L1-negative patients [134, 135]. Table 1 summarises key ongoing clinical trials evaluating immune checkpoint inhibitors in combination with chemotherapy in patients with metastatic TNBC. In the Phase III trial NeoTRIPaPDL1 (NCT02620280), patients with metastatic TNBC will be randomly assigned to receive nab-paclitaxel and carboplatin with or without PD-L1 inhibitor (atezolizumab). In another Phase II trial (NCT02530489), atezolizumab will be evaluated in combination with nab-paclitaxel. Also, the safety and efficacy of anti-PD-L1 inhibitor durvalumab will be tested in combination with nab-paclitaxel followed by dose-dense chemotherapy containing cyclophosphamide and doxorubicin in a Phase I/II trial (NCT02489448). Overall, the monoclonal antibodies anti-PD-1 pembrolizumab (Keytruda), nivolumab (Opdivo), PDR001 and immunotherapy drugs, the anti-CTLA4 tremelimumab and anti-PD-L1 atezolizumab (MPDL3280a), MEDI4736 and durvalumab have been tested in patients with metastatic TNBC in the ongoing clinical trials in Table 3.

Other noteworthy molecular targets under clinical investigation for metastatic TNBC are PARP inhibitors, exploited for their "synthetic lethality" [136] PI3K inhibitors [137], histone deacetylase (HDAC) inhibitors

Table 3 Key ongoing immunotherapy clinical trials for metastatic TNBC

Study	Phase and design	Drug	Primary outcome
Metastatic TNBC			
Single-agent pembrolizumab (MK-3475) was compared to the single-agent chemotherapy for metastatic TNBC NCT02555657	Phase III Randomised Open label	Anti-PD1 (MK-3475, pembrolizumab) comparison with chemotherapy	PFS, OS
Single-agent pembrolizumab (MK-3475) for TNBC NCT02447003	Phase II Single arm Open label	Anti-PD1 (MK-3475, pembrolizumab monotherapy	ORR safety
Atezolizumab (MPDL3280A) in combination with nab-paclitaxel compared with placebo with nab-paclitaxel for metastatic TNBC NCT02425891	Phase III Randomised Double blind	Anti-PDL1 (MPDL3280A, atezolizumab) with nab-paclitaxel compared with placebo and nab-paclitaxel	PFS
Tremelimumab in patients with advanced triple-negative breast cancer (and other solid tumours) NCT02527434	Phase II Single arm Open label	Anti-CTLA4 (tremelimumab) monotherapy with the option for the paitents to be followed by MEDI4736 alone or in combination with tremelimumab after progressive disease	ORR
Anti-PD1 (PDR001) administered to metastatic TNBC NCT02404441	Phase I/II Single arm Open label	Anti-PD1 (PDR001) monotherapy	DLT Safety ORR
Anti-PDL1 (MPDL380A, atezolizumab) treatment in patients with locally advanced or metastatic non-small cell lung, bladder and TNBC after investigational imaging NCT02478099	Phase II Single arm Open label	Anti-PDL1 (MPDL380A, atezolizumab) monotherapy	ORR Efficacy
Neoadjuvant study with anti-PDL1 (MPDL380A, atezolizumab) for metastatic TNBC undergoing treatment with nab-paclitaxel and carboplatin NCT02620280	Phase III Randomised Open label	Carboplatin, nab-paclitaxel, anti-PDL1 (MPDL3280A, atezolizumab), anthracycline versus carboplatin, nab-paclitaxel and anthracycline	Event-free survival
Metastatic TNBC Anti-PDL1 (MPDL380A, atezolizumab) with nab-paclitaxel compared to placebo with nab-paclitaxel NCT02425891	Phase III Randomised Double blind	Anti-PDL1 (MPDL3280A, atezolizumab) with nab-paclitaxel compared to placebo with nab-paclitaxel	PFS and OS
Metastatic TNBC treated with Entinostat with atezolizumab NCT02708680	Phase I/II Randomised Double blind (subject, caregiver, investigator, outcomes assessor)	Anti-PDL1 (MPDL3280A, atezolizumab) with entinostat	DLT, MTD and PFS
Metastatic TNBC treated with eribulin mesylate and anti-PD1 (MK-3475, pembrolizumab) NCT02513472	Phase I/II Single arm Open label	Eribulin mesylate and anti-PD1 (MK-3475, pembrolizumab)	DLT, Safety ORR
Metastatic TNBC treated with cyclophosphamide with anti-PD1 (MK-3475, pembrolizumab) NCT02768701	Phase II Single arm Open Label	Cyclophosphamide with anti-PD1 (MK-3475, pembrolizumab)	PFS
Treatment of advanced solid tumours, including metastatic TNBC, with TAK-659 with anti-PD1 nivolumab (Opdivo). NCT02834247	Phase 1 Non-Randomised Open label	TAK-659 with anti-PD1 nivolumab (Opdivo)	MTD ORR
Metastatic TNBC treated with niraparib with anti-PD1 (MK-3475, pembrolizumab) NCT02655822	Phase I/II Single-group assignment Open label	Niraparib with anti-PD1 (MK-3475, pembrolizumab)	DLT ORR

Table 3 continued

Study	Phase and design	Drug	Primary outcome
Metastatic TNBC treated with anti-PD1 (MK-3475, pembrolizumab)in combination with chemotherapy (carboplatin and gemcitabine) NCT02755272	Phase II Randomised Open label	Anti-PD1 (MK-3475, pembrolizumab)with carboplatin and gemcitabine	ORR Safety
Metastatic TNBC treated with anti-PD1 (MK-3475, pembrolizumab)with radiotherapy NCT02730130	Phase II Single arm Open label	Anti-PD1 (MK-3475, pembrolizumab)with radiotherapy	ORR
Metastatic TNBC treated with anti-PD1 (MPDL3280A, atezolizumab) and nab-paclitaxel in combination before surgery and atezolizumab alone after surgery NCT02530489	Phase II Single arm Open label	Anti PD1 (MPDL3280A, atezolizumab) with nab-paclitaxel before or after surgery	pCR
Metastatic TNBC treated with anti-PD-L1 with MEDI4736 with nab-paclitaxel and dose-dense doxorubicin/cyclophosphamide (ddAC) chemotherapy NCT02489448	Phase I–II Single arm Open label	Anti-PD-L1 with MEDI4736 with weekly nab-paclitaxel and dose-dense doxorubicin/cyclophosphamide (ddAC) chemotherapy	pCR
Investigation of tolerability and anti-tumour activity of anti-PD1 (MK-3475, pembrolizumab) in participants with advanced triple-negative breast cancer (TNBC) (Cohort A), advanced head and neck cancer (Cohorts B and B2), advanced urothelial cancer (Cohort C), or advanced gastric cancer (Cohort D) NCT01848834	Phase Ib Non-Randomised Open label	Anti-PD1 (MK-3475, pembrolizumab)	Number of participants experiencing adverse events Number of participants discontinuing from study treatment due to adverse events Overall Response Evaluation Criteria in Solid Tumours version Overall RECIST 1.1
Anti-PDL1 atezolizumab (MPDL3280A) administered as single agent by intravenous (IV) infusion to patients with locally advanced or metastatic solid malignancies including metastatic TNBC. NCT01375842	Phase 1 Single-group assignment Open label	Anti-PDL1 atezolizumab (MPDL3280A).	DLTs

DLT dose-limiting toxicity, *Nab* albumin-bound nanoparticle, *ORR* objective response rate, *OS* overall survival, *PFS* progression-free survival, *TNBC* triple-negative breast cancer, *CTLA4* cytotoxic T-lymphocyte-associated protein 4, *PD1* programmed cell death 1, *PDL1* programmed death ligand 1, *DLT* dose-limiting toxicity, *pCR* pathologic complete response

[138, 139], MEK inhibitors [140], heat shock protein 90 (HSP90) inhibitors [141, 142], EGFR inhibitors [143], FGFR inhibitors [144, 145] and angiogenic pathway inhibitors [143–145]. There are several PARP inhibitors currently being tested in clinical trials, such as veliparib (Phase III study; NCT02163694), talazoparib (Phase III study; NCT01945775), niraparib (Phase III study; NCT01905592) and rucaparib (Phase I/II study; NCT01074970) to treat patients with MBC, including TNBC; buparlisib a pan-PI3K inhibitor (buparlisib, BKM120, Novartis) is being evaluated in combination with olaparib to treat patients with solid tumours that include met TNBC (Phase I study; NCT01623349); lastly, HDAC inhibitor KHK2375 is being tested either as a monotherapy (Phase I study; NCT02623751) or in combination with cisplatin, in the treatment of patients with met TNBC (Phase I/II study; NCT02393794).

Conclusions

In this review, we have highlighted the importance of integrating biological and clinical data for the clinical development of new drugs in metastatic breast cancer. The new drugs, recently approved in the different settings of mBC, provide concrete clinical opportunities to induce "chronicity" of disease with a respect to the patient's quality of life. Careful selection of patients and growing opportunities for enrolment in clinical trials represents a great chance to expand treatment options whilst deepening our understanding of cancer biology. Indeed, a molecular stratification of breast cancer patients would be the key for future research in the field helping into the identification of the proper drug for any single patient. In this scenario, various ongoing Phase III clinical trials are testing the efficacy of new molecules according to the hypothesised timing of the single BC subset

driver, such as ER or HER2+ or immune phenotype. Novel molecular targets are also emerging in subtypes of breast cancer traditionally lacking actionable mutations. Furthermore, biomarker studies in the metastatic setting should be conducted in the upcoming future and new tools for molecular diagnosis (such as CNV or protein gene expression profile) should become more readily available to move to the concept of precision medicine.

Compliance with ethical standards

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