Advances in the treatment of oestrogen receptor positive advanced breast cancer

Nicholas Turner\textsuperscript{1}, Patrick Neven\textsuperscript{2}, Sibylle Loibl\textsuperscript{3}, Fabrice Andre\textsuperscript{4}

1 Royal Marsden Hospital and Institute of Cancer Research, Fulham Road, London, UK
2 Multidisciplinary Breast Centre & Gyn Oncol, University Hospitals Leuven, Department of Oncology, B-3000 Leuven, Belgium
3 German Breast Group, c/o GBG Forschungs GmbH Martin-Behaim-Str. 12, 63263 Neu-Iserburg, Germany and Sana Klinikum Offenbach, Germany
4. INSERM U981, Gustave Roussy Cancer Center, Université Paris Sud, Villejuif, France
Abstract

Oestrogen receptor (ER) positive breast cancer is the most common subtype of breast cancer. Endocrine therapies that target the dependence of this subtype on ER have substantial activity, yet resistance to therapy is inevitable in advanced cancer. Major progress has been made in identifying the drivers of ER positive breast cancer and the mechanisms of resistance to endocrine therapy. This has translated into major advances in the treatment of advanced breast cancer, with a number of targeted therapies that enhance the efficacy of endocrine therapy. Substantial improvements in progression free survival have been demonstrated with mTOR inhibitor and CDK4/6 inhibitors. A new wave of targeted therapies is being developed, including PI3K, AKT, HER inhibitors and new generation of ER degraders. Substantial challenges remain in patient selection, selection of the most appropriate order of therapies, and whether there is cross-resistance between therapies.

Introduction

Luminal oestrogen receptor (ER) positive HER2 negative breast cancer encompasses 70% of all breast cancers. Endocrine therapies form the mainstay of treatment for these cancers that are characterised by expression of the oestrogen receptor but lack of HER2 amplification, with adjuvant endocrine therapy reducing the relative risk of recurrence by approximately 40%\textsuperscript{1}. However many patients relapse despite endocrine therapy, and although endocrine therapy is often initially successful in the treatment of metastatic breast cancer resistance inevitably develops.

Our understanding of the mechanisms of resistance to endocrine therapy has evolved over the last two decades. Initial laboratory research identified upregulation of receptor tyrosine kinase signalling as a common mechanism of endocrine
resistance\textsuperscript{2}, although it was not possible to translate this scientific understanding to improve outcome in the clinic\textsuperscript{3,4}. Substantial progress has been made after these initial disappointments, resulting from major advances in our understanding of the biology of this subtype of breast cancer. First, molecular mechanisms of endocrine resistance have been discovered. Mutations in the ER gene (\textit{ESR1}) are selected as a driver of resistance to endocrine therapy in 15 to 30\% of the patients\textsuperscript{5-7}. Activation of mTOR signalling is likely involved in resistance\textsuperscript{8}, although the prevalence of this mechanism has not been established. Second, the mechanisms of oncogenesis of ER positive breast cancer have more clearly been established. Cyclin dependent kinases CDK4/6 have been identified a key driver of proliferation in this subtype of breast cancer\textsuperscript{9}, and the somatic genetic events of luminal breast cancer have been described\textsuperscript{10}, identifying a large number of potential new therapeutic targets, including \textit{PIK3CA} (30\%), \textit{AKT1} (4\%), \textit{ERBB2} (2\%) mutations and \textit{FGFR1} amplification (10\%).

Most of the data on molecular epidemiology come from research on primary tumors, and we are lacking large studies of molecular analysis on recurrent metastatic breast cancer.

Here we discuss recent efforts to augment the efficacy of endocrine therapy in advanced breast cancer, and treat endocrine therapy resistance advanced breast cancer with targeted therapies.

**Progress in endocrine therapy**

\textit{Clinical data}

The standard endocrine therapies for advanced breast cancer have remained largely unchanged for the last two decades including tamoxifen, used particularly in pre-menopausal women, and aromatase inhibitors (AIs) for post-menopausal women that inhibit the aromatase enzyme to ablate oestrogen production. For pre-menopausal women ovarian suppression is frequently given alongside other therapies, and absolutely necessary for AIs. Recent interest has focused on selective oestrogen
receptor degradors (SERDs), with fulvestrant-500mg likely the most effective endocrine therapy after progression on initial therapy\(^\text{11}\). In a phase II study fulvestrant was also found to be superior to aromatase inhibitors in treatment naïve advanced breast cancer\(^\text{12,13}\). Results from a phase III study (FALCON, NCT01602380) confirmed these findings and showed that fulvestrant 500 mg improves PFS in treatment-naïve metastatic breast cancers\(^\text{14}\). Fulvestrant has formed the backbone for the development of many new therapies in advanced breast cancer.

Mutations in \(ESR1\) are very rare in primary breast cancer, but sequencing studies of pre-treated advanced breast cancer identified \(ESR1\) mutations in 15-40% of advanced ER positive breast cancer\(^\text{6,15}\). The majority of mutations cluster in three amino acids in the ligand binding domain of ER, where they result in ligand independent activation of the ER\(^\text{15}\). \(ESR1\) mutations are a mechanism of acquired resistance to aromatase inhibitors, occurring in particular when AIs are used to treated advanced breast cancer\(^\text{16}\). Cancers with \(ESR1\) mutation may be targetable by SERDs that degrade the oestrogen receptor\(^\text{6}\). A new generation of potent oral SERDs are in development. In a phase I study, two out of nine tumours with \(ESR1\) mutations had objective response to the oral SERD GDC-0810\(^\text{17}\).

**Discussion / perspectives**

Although there is hope that the new generation of potent oral SERDs will improve outcome, the next generation of clinical trials will determine whether they will improve outcome as compared to fulvestrant and/or AI, in cancer with and without \(ESR1\) mutations. The oral SERDs also have potential to improve outcome in earlier settings in endocrine naïve populations, although the safety profile of new SERD is currently not well described and should define the future development in first line and adjuvant setting.
CDK4/6 inhibition

Scientific basis

Dysregulation of the cell cycle leads to uninhibited cell proliferation as one of the hallmarks of cancer. The cyclin D1-CDK4/6-retinoblastoma pathway is critical for cell proliferation and its dysregulation is frequent in breast cancer biology\textsuperscript{18,19}. CDK4 pathway is reported in Figure 1a. CDK4 and CDK6 are activated early in the cell cycle by cyclin D1 (CCND1), and other D-type cyclins, to facilitate cell cycle progression through the G1 restriction point\textsuperscript{20}. Activated cyclin-CDK complexes phosphorylate and inactivate the tumor suppressor retinoblastoma 1 (RB1, also known as Rb), activating the transcription of factors involved in S-phase entry. The cyclin-dependent kinase inhibitor 2A gene (CDKN2A) encodes p16 an inhibitory protein which blocks the formation of the cyclin D-CDK4/6 complexes, resulting in cell cycle inhibition\textsuperscript{18,19}.

Cyclin D1 is frequently overexpressed in ER positive luminal breast cancer\textsuperscript{19,21}, in part through \textit{CCND1} amplification\textsuperscript{10}, activating CDK4/6 in ER-positive breast cancer. Subsequent cell culture experiments demonstrated that inhibition of CDK 4/6 attenuates cell proliferation of luminal ER positive cell lines\textsuperscript{22,23}. CDK4/6 was also shown to continue to promote the proliferation of breast cancer cells with \textit{in vitro} derived resistance to endocrine therapy\textsuperscript{24}. These data provided a strong rational to investigate the effects of CDK4/6 inhibition in breast cancer.

Clinical evidence with palbociclib

Endocrine sensitive cancers

The PALOMA 1/TRIO 18 was a phase II randomised open label study conducted in first line ER+/HER2- advanced breast cancer, with patients randomised to receive the aromatase inhibitor letrozole or letrozole in combination with the CDK 4/6
inhibitor palbociclib.\textsuperscript{25} The study recruited two cohorts; the 1\textsuperscript{st} was unselected, and the second included only patients whose tumours had $CCND1$ amplification and/or loss of p16 (CDKN2A). In combined analysis of both studies, progression free survival was improved by 10 months (HR $0.488$, 95% CI $0.319–0.748$; one-sided $p=0.0004$) (Table 1). The PALOMA1 study led to accelerated approval of palbociclib in the US, but not in other territories. These results were confirmed in the phase III PALOMA2 trial also with a 10 month improvement in PFS, $24.8$ (22.1-NR) months for the palbociclib plus letrozole group and $14.5$ (12.9-17.1) months for the letrozole plus placebo (HR $0.58$, 95%CI: $0.46$-$0.72$, $P<0.0001$).\textsuperscript{26}

\textit{Endocrine pre-treated}

The PALOMA-3 phase III trial investigated the efficacy of palbociclib in patients with advanced breast cancer that had progressed on prior endocrine therapy, either on or within 12 months of adjuvant therapy, or on therapy for advanced breast cancer.\textsuperscript{9} Post and pre/perimenopausal women were included, and randomised to receive fulvestrant with palbociclib or placebo. Progression free survival was $9.2$ months ($7.5$-NE) in the fulvestrant plus palbociclib group and $3.8$ (3.5-5.5) months in the fulvestrant plus placebo group (HR $0.42$, 95% CI $0.32$-$0.56$; $p<0.001$]. Efficacy was not different in pre- and post-menopausal patients.\textsuperscript{9} The PALOMA2 and 3 studies are anticipated to lead to regulatory approval in many territories.

\textit{Clinical evidence with ribociclib and abemaciclib}

Two other CDK4/6 inhibitors (abemaciclib, ribociclib) have reported data from early phase studies, and have recruited Phase 3 clinical trials. In patients with HR+/Her2- mBC, abemaciclib single agent was associated with $19.7\%$ (13.3%-27.5%) objective response rate in a phase II study of 132 patients.\textsuperscript{27} A phase I\textsuperscript{b} trial in 47 patients testing ribociclib combined with letrozole was reported 79% of the treatment-naïve patients presented a clinical benefit (objective response or stable disease $>24$
weeks). The MONALESSA2 phase III trial testing ribociclib in endocrine sensitive cancers, the same population as PALOMA2, was recently stopped at interim analysis because the study met its primary endpoint (NCT01958021).

**Safety**

Asymptomatic neutropenia is the main adverse effect of palbociclib and ribociclib. In PALOMA3, 62% of patients presented a grade III/IV neutropenia. This high rate of neutropenia was not complicated by infections, with no increase in febrile neutropenia. Neutropenia is caused by cell cycle arrest induced by palbociclib, and potentially does not result in neutrophil dysfunction induced by chemotherapy. In contrast, abemaciclib causes less neutropenia, and more diarrhea and fatigue.

There is no robust published data to explain the different safety profile between palbociclib/ribociclib and abemaciclib.

**Predictive biomarkers for the efficacy of CDK4 inhibitors**

Several studies have tried to define the molecular alterations associated with higher sensitivity to CDK4/6 inhibitors. In PALOMA1, neither CCND1 amplification, nor p16 loss were associated with efficacy, and in PALOMA3, PIK3CA and ESR1 mutations were not predictive for the efficacy of palbociclib. Finally, in a window of opportunity trial (POP trial), neither PIK3CA mutations, CCND1 amplification, Rb expression, p16 expression were predictive. Interestingly, in two trials, early changes in pRb were predictive for the efficacy of CDK4 inhibitors.

**Perspectives**

CDK4/6 inhibitors in combination with endocrine therapy are the new standard of care for advanced HR+/Her2- BC. Indirect comparison between trials suggests that for patients who present with endocrine sensitive advanced cancer, initial treatment offers greater PFS benefit than later treatment.
months in PALOMA3). There are no biomarkers to identify which patients derive larger benefit from CDK4/6 inhibition. The next advance in the field will come from the understanding of resistance mechanisms. One preclinical study suggested that PI3K activation could be an early mechanism of adaptation, and that cyclin E1 amplifications and RB1 loss could mediate acquired resistance to CDK4 inhibitors. Multiple ongoing studies are assessing CDK4/6 inhibitors in the adjuvant setting as the next step of drug development (PENELOPE-B NCT01864746, PALLAS NCT02513394).

mTOR inhibition

*Scientific basis*

mTOR (mammalian Target Of Rapamycin) is a serine threonine kinase involved in mRNA translation, metabolism, resistance to autophagy, functioning in two distinct protein complexes TORC1 and TORC2. Downstream targets of mTOR in TORC1 include S6K and 4EBP1, with S6K mediating phosphorylation of estrogen receptor. At least two pathways regulate mTOR activation in breast cancer, namely PI3K/AKT and LKB1/AMPK/TSC pathways. PI3K/AKT / mTOR pathway is reported in Figure 1b.

Several studies have shown that mTOR signaling can mediate resistance to endocrine therapy; Cancer cell lines derived to be resistant to oestrogen deprivation in vitro have increased mTOR activity; AKT1 activation mediates resistance to endocrine therapy in endocrine sensitive cell lines, and biomarker studies on breast cancers have shown that mTOR activation, assessed by the level of phosphorylation of 4EBP1, can be acquired during resistance to endocrine therapy; Preclinical studies demonstrated synergy between mTOR inhibitors and endocrine therapy.
Two classes of mTOR inhibitors are being developed for the treatment of cancer. Rapalogs are rapamycin-derivatives, including everolimus and temsirolimus, that allosterically inhibit mTOR in the TORC1 complex and have been extensively investigated in breast cancer. ATP-competitive inhibitors inhibit mTOR in both TORC1/2 complexes, are theoretically more bioactive, although no comparison with rapalogs have yet been reported.

Early drug development evaluated different doses, schedules and combinations of rapalogs\textsuperscript{40-43}. From these studies it became clear that a small subset of ER+ advanced BC have objective tumour response or shrinkage when treated with rapalogs as a single agent\textsuperscript{40,41}. Prolonged disease control, often without substantial tumour response, was obtained when rapalogs were combined with endocrine therapy\textsuperscript{42}. Dose and schedule were highly important for efficacy\textsuperscript{40}. These trials lead to further evaluation of rapalog in combination with endocrine therapy in randomised trials.

Two randomized trials have evaluated temsirolimus. A phase II randomized trial (n=90) reported that adding temsirolimus (10 mg daily or 30 mg intermittent) to letrozole improved the rates of disease control at one year (69%, 62%, 48% respectively)\textsuperscript{44}. A subsequent phase III randomized trial (n=1112) evaluated temsirolimus (30 mg daily, 5 days every two weeks) in combination with letrozole in patients with endocrine sensitive advanced breast cancer, not previously treated with an AI\textsuperscript{45}. Despite pre-clinical evidence, there was no improvement in PFS with the addition of temsirolimus (HR, 0.90; 95% CI, 0.76 to 1.07; \( P = .25 \)). Several hypotheses have been put forward to explain this failure, including that dosage/schedule of temsirolimus was likely sub-optimal\textsuperscript{46}. 
Three randomized trials have evaluated the efficacy of everolimus in combination with endocrine therapy. These trials are summarized in the Table 2. A phase II randomized trial (n= 270) reported that adding everolimus to letrozole improved the clinical response rates (68% vs 59%, p=0.06, prespecified significance <0.1, 1ry endpoint) in women with primary breast cancer in the neoadjuvant setting. A phase II randomized trial (n=111) performed in patients with advanced BC resistant to AIs reported that adding everolimus to tamoxifen improved time to progression (TTP) from 4.5 months to 8.6 months (hazard ratio [HR], 0.54; 95% CI, 0.36 to 0.81), and overall survival (HR, 0.45; 95% CI, 0.24 to 0.81). Finally, a phase III registration trial (BOLERO-2) evaluated the efficacy of everolimus in combination with exemestane, in post-menopausal patients with advanced BC and prior progression on an non-steroidal AI, demonstrating improved median PFS to 6.9 from 2.8 months (HR: 0.43 (0.35-0.54), p<0.001). The study was not powered to assess overall survival, and which was not improved by everolimus (30 versus 26 months, HR=0.89, 95%CI: 0.73-1.10, p=0.14). Ongoing trials are testing everolimus in premenopausal women (NCT02313051), or in comparison with capecitabine chemotherapy (NCT01783444).

Results of BOLERO2 trial led to drug approval in many territories for post-menopausal women with advanced breast cancer whose disease has progressed on a prior aromatase inhibitor, and endorsement by many guidelines (NCCN, ABC).

Safety
Toxicity management is an issue with everolimus. Mucositis is observed in 67% patients (grade 3 severe in 8%) and usually occur within the first two months of therapy. There is also an increase in fatigue, rash, diarrhea, anorexia, dyslipidaemia, bone marrow suppression and infections. Non-infectious pneumonitis is observed in 20% (grade 3 severe in 4%) with an incidence constant over the time, and patients presenting with chest symptoms should be carefully evaluated to assess for drug
related pneumonitis, bacterial pneumonia, and for pneumocystis infection. There is no predictive factor to anticipate side effects and dose interruption / reduction is the best management. Recently, some investigators have reported efficacy of mouth washes to prevent mucositis. This could improve compliance, and allow maintenance of high dose therapy.

**Predictive biomarkers for efficacy**

Given the adverse effect profile of everolimus, predicting who benefits is of high importance although identification of a biomarker has so far been elusive. No specific somatic genetic events have been validated to predict for sensitivity to mTOR inhibition. In a retrospective analysis from BOLERO2, *PIK3CA* mutations do not associate with mTOR activation and do not predict the efficacy of everolimus$^{52}$, and not other genetic events including *CCND1* amplification, *FGFR1* amplification, *ESR1* D538G mutations were predictive for the sensitivity to everolimus$^{5,52}$ Using the same material, it was reported that high level of genomic instability$^{52}$ and ESR1 Y537S$^5$ mutations could be associated with lower benefit, but these data were generated in the context of multi hypothesis testing on small number of patients, and therefore need further validation. Several case reports have suggested that rare mTOR mutations$^{53}$, *TSC1/2* mutations$^{54}$ or *AKT1* mutations$^{55}$ could be associated with objective response to everolimus. Assessment of phosphorylated 4EBP1 could be associated with sensitivity to everolimus$^{56}$, and this hypothesis is now being tested prospectively in SAFIRTOR trial (NCT02444390). Assessment of phosphoproteins by immunohistochemistry is challenging, and potentially gene expression signatures may be an alternative option to assess mTOR activation$^{57}$.

A vast majority of the patients will develop resistance to rapalogs, and the mechanisms of resistance are well described in pre-clinical research. Inhibiton of mTOR relieves feedback loops that result in activation of PI3 kinase signalling$^{58}$. 
Several strategies are being developed to overcome it, including combination with IGF1R inhibitors, combination with PI3K inhibitors (NCT02077933), although these combinations are challenged by toxicity.

**Perspectives**

Although everolimus is approved in patients who are resistant to non-steroidal aromatase inhibitors, the impact on public health in the clinic is still not yet defined, and there is a need for more research on how to safely administer the drug. As previously mentioned, the major research question is the identification of predictive biomarkers, especially biomarkers that could identify patients who benefit from mTOR inhibitors over CDK4/6 inhibitors. Everolimus is currently being evaluated in the adjuvant setting in patients with high risk of relapse (NCT01805271). Rapalogs do not fully suppress mTOR activity, they do not fully attenuate cap-mediate mRNA translation, and ATP-competitive mTOR inhibitors could overcome this issue.

**PI3 kinase inhibition**

**Scientific basis**

Phosphoinositides (PI) account for 10-15% of membrane phospholipids. Since the late 80s, phosphorylation of these PI lipids has been recognised as a key signal transduction mechanism of oncogenic receptor tyrosine kinases. The PI3Ks are heterodimers of a 110 kDa catalytic subunit, encoded by one of four genes \( \text{PIK3CA} \) (\( \alpha \)), \( \text{PIK3CB} \) (\( \beta \)), \( \text{PIK3CD} \) (\( \delta \)), \( \text{PIK3CG} \) (\( \gamma \)), and a 85 kDa regulatory subunit, encoded by one of three genes \( \text{PIK3R1-3} \). PI3Ks phosphorylate PIs to activate several protein kinases in the PI3K/AKT/mTOR pathway, regulating processes like metabolism, proliferation, migration, survival and angiogenesis. Negative regulation of this pathway is conferred by PTEN (phosphatase and tensin homolog) an important tumor suppressor.
Due to the crucial roles of PI3K, it is not surprising that activating alterations in this signalling pathway are one of the most frequent genetic events in cancer and a major focus for drug development. *PIK3CA* is frequently mutated in breast cancer, with 40% ER+ breast cancers presenting a activating mutation\textsuperscript{10}. This gene is mutated less frequently in ER negative breast cancer except for androgen receptor positive triple negative breast cancers\textsuperscript{69}. Mutations in *PIK3CA* lead to increased downstream signalling and oncogenesis\textsuperscript{70, 71, 72}, in parts regulating ER transcription and expression\textsuperscript{73}. Combined inhibition of PI3K with endocrine therapy is synergistic and may help overcome resistance to endocrine agents\textsuperscript{74}.

**PI3 kinase inhibitors**

A number of different PI3K inhibitors are in clinical development, which can be classified into dual pan-PI3K-mTOR inhibitors, pan-PI3K inhibitors and isoform-specific inhibitors designed to be selective to one or more of the 4 isoforms of the p110 catalytic subunit. The majority of non-selective pan-PI3K-mTOR inhibitors have ceased development due to toxicity, and are not discussed.

**Pan-PI3K inhibitors**

The pan-isoform PI3K inhibitor buparlisib (BKM120) has been studied in combination with chemotherapy or endocrine therapy\textsuperscript{75}. The phase III BELLE2 study randomised 1147 post-menopausal women with advanced breast cancer that had previously progressed on an AI, between buparlisib plus fulvestrant and placebo plus fulvestrant. The addition of buparlisib significantly improved PFS (6.9 versus 5 months, HR 0.78, P<0.001)\textsuperscript{76}. The most common toxicities noted in BELLE2 included hyperglycemia, rash, fatigue, elevated transaminase, stomatitis, and gastrointestinal
side effects like nausea, vomiting and diarrhea. Mood disorders like anxiety, irritability, and depression are frequent as buparlisib crosses the blood-brain barrier. Overall, results of BELLE2 did not support the use of buparlisib in patients with HR+ mBC due to the small magnitude of benefit and the toxicity observed.

A number of other trials have been negative with pan-PI3 kinase inhibitors. Pictilisib (GDC-0941) did not improve PFS in a phase II study which randomized 168 postmenopausal women with AI-resistant advanced ER+ breast cancer to fulvestrant with or without pictilisib81.

**Isoform-specific PI3K inhibitors**

PI3K isoform-specific inhibitors have been developed that selectively inhibit the PI3K p110 isoforms82. By inhibiting more selectively the driver oncogene, toxicities may be reduced resulting in more potent inhibition of the targeted oncogene. There is substantial interest in alpha-selective PI3K inhibitors in cancers with PIK3CA mutations, and two drugs are in later stage development. In a phase I trial combining fulvestrant and taselisib (GDC-0032), six out of twelve patients with PIK3CA mutation presented an objective response83. The SANDPIPER phase III trial of taselisib with fulvestrant is currently recruiting (NCT02340221). In a study of 50 patients with PIK3CA-mutant metastatic breast cancer, alpelisib (BYL179) combined with fulvestrant was associated with 24% objective response84. This combination is under investigation in the SOLAR phase III trial (NCT02437318).

**Biomarkers**

Activating mutations in PIK3CA are one of the most common actionable genetic event in ER positive breast cancer10, and increasing data suggests that PI3 kinase inhibitors are most effective in PIK3CA mutant cancers. An exploratory analysis of the phase III BELLE-2 study reported that patients harbouring PIK3CA mutations in plasma DNA, benefited the most from addition of buparlisib (PIK3CA mutant, PFS
HR 0.56 (95%CI: 0.39-0.80). Phase I/II studies also suggest that PIK3CA mutations could be a strong predictor of sensitivity to alpha-selective PI3K inhibitors, with statistically significant longer PFS reported for patients with PIK3CA mutations. There have been several mechanisms shown to explain resistance to PI3K inhibitors that re-activate down-stream pathways, including activation of mTOR and CDK4/6 signalling, MYC amplification, PTEN loss, and expression of ribosomal S6 kinases.

**Exploratory approaches**

**Rare genomic segments**

Large collaborative efforts have now defined the somatic genetic landscape of breast cancer. These efforts have identified great diversity in the genetic events, mutations and copy number changes. Many mutations occur in a small percentage of breast cancers, yet for many of these genetic events there is now evidence that matching targeted therapies have efficacy. Targetable genomic alterations and their matched therapies are reported in Figure 2. For example mutations in AKT1 occur in 4% of luminal breast cancer and treatment with AKT inhibitor (AZD5363) led to three objective responses out of 18 cancers with AKT1 mutations, with some tumor shrinkage observed in 14 out of 18 patients. Further studies will evaluate the combination with endocrine therapy. ERBB2 (HER2) mutations occur in 2% of luminal breast cancer, at the same rate in primary and advanced cancer, and have been associated with high sensitivity to neratinib in preclinical studies. In a phase II trial, five out of 14 patients with ERBB2 activating mutations presented a clinical benefit (OR or SD>24 weeks) to neratinib. Lobular carcinoma may have a higher rate of ERBB2 mutations. BRCA1 and BRCA2 germline mutations occur in around 4% of luminal BC. This genomic alteration is associated with high sensitivity to PARP inhibitors. Finally, FGFR1 amplifications occur in around 10% of luminal BC. This
genomic alteration has been associated with sensitivity to lucitanib, a multikinase inhibitor, in a phase I trial\textsuperscript{95}. The clinical translational challenge for these therapies is identifying patients eligible for the trials, considering the low prevalence of the alterations. Large scale tumour targeted sequencing is one approach, but increasingly in advanced cancer screening with circulating tumour DNA is seen as the answer to screening. Tumours release DNA into the blood stream, and highly sensitive assays can analysis tumour mutations and provide a current assessment of the tumour genetics and tackle changes that have occurred through prior treatment\textsuperscript{96}.

\textit{HDAC inhibition}

Efficacy has potentially been seen with the HDAC inhibitor entinostat in a phase II study that randomised patients with advanced breast cancer between exemestane plus entinostat and exemestane with median PFS improved to 4.3 months from 2.3 months (HR 0.73, one-sided P = .055, predetermined P<0.1)\textsuperscript{93}. Patients with biomarker of elevated protein acetylation in peripheral blood mononuclear cells possibly associated with benefit from etinostat\textsuperscript{97}. A phase III study is currently recruiting.

\textbf{Perspectives}

The major advances in the treatment of ER positive advanced breast cancer have led to a set of new challenges and emerging clinical questions.

\textit{Identifying long term responders with endocrine therapy alone}

A subset of patients treated with endocrine therapy alone have long durable disease control. Identifying these patients prior to treatment, would better define who should receive CDK4/6 inhibitors and who could be treated with endocrine therapy alone. Gene expression assays, developed to determine prognosis in early breast cancer,
may also assist in predicting outcome on endocrine therapy for advanced cancer\textsuperscript{58}, but further research is required.

\textit{Defining natural history of genomic segments}

As previously mentioned, ER+ advanced BC includes large number of molecular segments, each of them a target for new drug development. With the exception of \textit{ESR1} mutations, it is currently unclear whether genomic alterations are be associated with specific phenotypes, whether response to standard treatments such as endocrine therapy varies between segments, and the prognosis associated with individual segments. A greater understanding of the diversity of ER positive breast cancer could allow better selection of which patients need a combination therapies,

\textit{Offering optimal molecular portraits to all patients}

ER and HER2 assessed by immunohistochemistry, together with assessment of germline \textit{BRCA1/2} mutations in selected cases, are currently the gold standard for patients with advanced BC. Considering that cancer can evolve over the time, it is recommended to re-assess molecular markers at the time of recurrence\textsuperscript{50}. As discussed previously, \textit{ESR1}, \textit{PIK3CA}, \textit{AKT1}, \textit{ERBB2} mutations have been associated with responses to targeted therapies and sequencing diagnostics are likely to be implemented in the near future to identify these mutations. Assays for circulating tumour DNA will also likely enter clinical practice, with the potential to provide more accurate real-time assessment of tumour genetics and for disease monitoring. Several studies have shown that ctDNA have good analytical validity to assess \textit{ESR1} and \textit{AKT1} mutations, and beyond offering a non-invasive approach could save cost compared to tissue biopsies. Clinical studies are beginning to approach very rare genomic events, that occur in <1% of breast cancer overall, to establish the potential to target these rare genetic events (SAFIR02, NCT02299999).
Until these trials are achieved, there is no evidence that large panel of genes should be used for treatment decision.99

Defining which treatment and when
The availability of multiple targeted treatments currently licensed, or with impending licensing, presents in a clinical challenge; Which treatments should be used and in which order? Development of predictive biomarkers for CDK4/6 and mTOR inhibitors could help solving this issue, and in many health care settings the cost of individual approaches will affect availability. The current clinical consensus has CDK4/6 inhibitors as the initial targeted therapy combination, based on the high efficacy and relative good tolerability of CDK4/6 inhibitors, reinforced by the strong biological rational (Figure 3). This presents a relatively simple choice for patients who relapse on adjuvant endocrine therapy, with a CDK4/6 inhibitor in combination with fulvestrant. For patients who present with advanced disease without prior treatment, or who relapse late after stopping adjuvant endocrine therapy, the duration of disease control on front line endocrine therapy alone is over a year (Figure 4), extending into many years of disease control in some women. A research priority is to identify who in this setting needs upfront CDK4/6 inhibitor, and who can be treated with endocrine therapy alone avoiding increased toxicity and expense.

Preclinical data suggests substantial efficacy for combinations of CDK4/6 inhibitors with mTOR inhibitors, and with PI3 kinase inhibitors, in part by circumventing the activation of compensatory pathways that allow cancers to become resistant to either CDK4/6 inhibitors or PI3 kinase inhibitors. A number of early stage clinical trials are examining the safety and tolerability of triplet therapies with endocrine therapies, CDK4/6 inhibitors and PI3 kinase/mTOR inhibitors. Further clinical research will identify whether such combinations are tolerable, and whether drugs should be used
in triplet combination up front, or used sequentially after disease progression on initial therapy.

**Understanding resistance**

The next wave of new compounds will likely be developed by the discovery of resistance mechanisms to CDK4/6, mTOR and PI3K inhibitors. Inhibitors of CDK2 and AKT inhibitors could be of particular interest in patients who develop acquired resistance to CDK4/6 and PI3K inhibitors respectively, given the involvement of Cyclin E activation and PTEN loss in resistance to these compounds.

**Conclusion**

The treatment of ER+ advanced breast cancer has dramatically improved in the last 20 years through stepwise evolution. Recent studies suggest that overall survival range between 4 and 5 years for patients with ER+/Her2- metastatic breast cancers (figure 4). The first wave of improvement resulted from the development of new endocrine therapies, including aromatase inhibitors and first generation ER-degraders (fulvestrant). Second wave of improvement came through the development of targeted therapies that target kinases important to ER breast cancer biology, with two drugs are now approved that target mTOR and CDK4/6 pathways. The anticipated third wave of advances will include therapies targeted at oncogenic genomic alterations. It is expected that at least five drug families will show outcome improvement in same number of genomic segments (*PIK3CA*, *AKT1*, *ERBB2*, *ESR1*, *BRCA1/2* mutations). Potentially, a fourth wave may be the enhancement of the immune system to treat ER positive breast cancer. How these drugs will be sequenced and/or combined will be addressed in the next generation of clinical trials.

**References**

**14 FALCON results, ESMO 2016**

26. Finn R, Martin M, Rugo H et al. PALOMA-2: Primary results from a phase III trial of palbociclib (P) with letrozole (L) compared with letrozole alone in postmenopausal women with ER+/HER2− advanced breast cancer (ABC). *J Clin Oncol* 2016; 34: Abst 507
29 Ribociclib: reference ESMO, to be filled in sept, during the proof


List of Figures:

**Figure 1a CDK4 pathway**
Figure 1b PI3K / AKT / mTOR pathway

Tyrosine kinase

PIP2

PTEN

PI3K

PIP3

AKT

TSC2

TSC1

TORC1

mTOR

S6K1

4E-BP2

eIF-4E

Figure 2: Targetable genomic alterations in ER+/Her2- metastatic breast cancers
Figure 3: Current treatment landscape and research questions in post-menopausal women with ER+/Her2- metastatic breast cancers

- Backbone therapy: nsAI, fulvestrant, exemestane, chemotherapy
- Targeted therapies approved in all comers: CDK4 inh, mTOR inh
- Targeted therapies matched to genomic alterations (under investigation):
  - α-spe PI3K inh
  - ER-degraders, neratinib, PARP inh, FGFR inh, AKT inh
- New combinations:
  - Endocrine therapy: CDK4 inh, mTOR inh, α-spe PI3K inh

Questions:
- Predictive biomarkers?
- Optimal sequence?
- Mechanisms of Zty resistance?
- Positioning?
- Safety?
- Efficacy vs sequential approach?

Figure 4: Recent improvements in first line therapy for ER+/Her2- metastatic breast cancer patients

- TTP$^{12}$: Anastrozole 13 mos, Fulvestrant 23 mos
- PFS$^{26}$: Letrozole 14 mos, Letrozole + palbociclib 24 mos
- OS:
  - Fulvestrant 1st line: 48 mos
  - Anastrozole + Fulvestrant 1st line: 55 mos

Confirmatory trial: FALCON