Title Page

Title: Optimising Endocrine Therapy in Postmenopausal Women with Advanced Breast Cancer.

Author: Thomas Ho Lai Yau and Kwok-Leung Cheung
Email address: kl.cheung@nottingham.ac.uk
School of Medicine, University of Nottingham

Keywords: Breast cancer, Hormone-receptor positive, Advanced, Postmenopausal, Endocrine Therapy, Combination, Sequential.
Word count: 4832 words

Abstract

Hormone receptor-positive breast cancer is commonly treated with endocrine therapy; however, overtime cancer cells can develop endocrine resistance. This review aims to document combination therapy and sequential therapy in the use of endocrine agents and targeted agents. By conducting two systematic searches using 4 databases: Cochrane Library, MEDLINE, EMBASE, and Web of Science. A total of 26 studies that covered combination therapy were obtained and included for the review. 14 were phase III documenting combinations of mechanistic target of rapamycin (mTOR), phosphoinositide-3-kinase (PI3K), vascular endothelial growth factor receptor (VEGFR), human epidermal growth factor receptor 2 (HER2), and cyclin dependent kinase 4/6 (CDK4/6) inhibitors. The remaining studies were of phase II nature that reported combinations involving inhibitors in mTOR, endothelial growth factor receptor (EGFR), CDK4/6, and tyrosine kinase inhibitor (TKI). Interesting findings in inhibitor combinations involving; CDK4/6, mTOR and PI3K

suggest clinical activity that can overcome endocrine resistance. On the other hand, there were 0 studies that covered sequential therapy. Overall findings showed that combination therapy improved treatment efficacy over monotherapy in postmenopausal patients with hormone-receptor positive advanced breast cancer. Inevitably, the benefits are accompanied with increased toxicity. To optimise endocrine therapy, further research into combinations and effective patient selection will need to be defined. Additionally, this review warrants future studies to explore sequential therapy.

Introduction

Endocrine therapy (ET) is often used as first line treatment in patients with hormone receptor positive (HR+) breast cancer and preferred to chemotherapy when there are no signs of visceral crisis (Reinert and Barrios 2015). In terms of efficacy, ET improves progression-free-survival (PFS), time to progression (TTP), objective response rate (ORR) and clinical benefit response (CBR), while possessing a favourable toxicity profile when compared to chemotherapy. Although, the therapeutic action of ET is slower than chemotherapy, the duration of response in ET is more sustainable with longer-term survival benefits (Cheung 2007). Recent data from the FALCON trial observed significant improvements in not just PFS and TTP but also overall survival (OS) for postmenopausal patients with endocrine naïve, HR+ locally advanced/metastatic breast cancer (LABC/MBC) when treated with fulvestrant 500mg, as opposed to anastrozole 1mg (Robertson, et al. 2016). All HR+ breast cancer can be represented with the presence of oestrogen receptor (ER) and/or progesterone receptor (PR) (Cheung 2007). The Americain Soceity of Clinical Oncology/College of American Pathologists recommended HR+ tumours be defined as having at least 1% of tumour nuclei stained positively for either ER or PR on

immunohistochemistry (Hammond, et al. 2010). Unfortunately, patients with ER+ breast cancer are susceptible to risks of progressive disease (PD) or develop endocrine resistance (Dixon 2014). As a result, investigations in modalities of ET agents have been thorough and produced a wide-range of ET options for patients to use.

A greater understanding in cancer biology has shown that ESR1 mutation is associated with mechanisms of endocrine resistance, especially to tamoxifen and fulvestrant (Jeselsohn, et al. 2015). About 15-20% of ER+ LABC/MBC were shown to have ESR1 mutation, with increased frequencies detected in patients with multiple ET exposure. Research into biochemical pathways associated with proliferation has identified that cross-talk between signalling pathways can activate ERs, despite conventional ER pathways being blocked or inactivated (Dixon 2014; Pietras 2006). For instance, cross-talk between ER and specific pathways such as the phosphoinositide 3-kinase /v-akt murine thymoma viral oncogene /mammalian target of rapamycin (PI3K/AKT/mTOR) can result in continued proliferation of the cancer cells and hence develop resistance to ET (Dixon 2014). Targeted therapy agents (TA) are designed to interfere with specific targets that are involved with growth. Often TA act on specific molecular targets to achieve blockade of cell proliferation and potential cross-talks between the ER mediated pathway and other signalling pathways. Most TA are categorised by their molecular target (see Table 1). Accordingly, the concomitant use of TA with other cancer therapeutics can potentially further increase treatment efficacy and overcome endocrine resistance (Pietras 2006). However, combination therapy is prone to a greater toxicity profile when compared to monotherapy. Hence, an alternative would be the sequential application of ET and TA, which is expected to lessen the toxicity profile of these regimen. In sequential therapy, the patient will be exposed to only one toxicity profile at once rather than two during combination therapy. From figure 1, it was of interest if

sequential application of an ET agent (blue) and TA (red) will produce similar efficacy when compared to combination therapy (green). Another interesting comparison of these treatments would be to compare the results of different sequencing pattern in sequential ET (in this case treatment B and C). Henceforth, this was the definition of combination therapy and sequential therapy in this review.

An ever-growing arsenal of anticancer agents requires knowledge in optimal application for clinicians and patients to make informed decisions regarding therapeutic strategies. The aim was to assimilate methodologies and conclusions of randomised control trials (RCTs) investigating the benefits/limitations of combination and sequential therapy of ET/TA.

Methods

This systematic review was conducted by electronic searches to include relevant phase II/III RCTs that have reviewed the application of ET and TA in combination therapy or sequential therapy. Relevant literatures were screened for their title, followed by evaluation of abstracts befitting the selection criteria. Lastly, availability of full articles and abstracts in eligible literature were reviewed. Two separate searches were performed in parallel to accommodate the aims of the review.

A comprehensive search was performed with multiple databases: Medline, EMBASE, Cochrane Library and Web of Science. Both searches included ‘endocrine therapy’, ‘hormone’, ‘advanced breast cancer’, ‘metastatic’ and ‘postmenopausal’. Additional search terms: ‘combination’, ‘plus’, ‘add’ and ‘together’ were incorporated into the search for combination therapy. Whereas, search terms: ‘sequential’, ‘switch’, ‘concurrent’, and ‘concomitant’ were included for the sequential therapy search. Cross-referencing of relevant literature was also conducted to expand the literature search. Conference abstracts

were also considered for screening, to include on-going studies for review. The search was limited to English language and RCTs that investigated combinations or sequential applications of ET and TA in postmenopausal patients with HR+ advanced/metastatic breast cancer in phase II/III. The search was carried out from 1998 onwards, because trastuzumab was approved by the Food and Drug Administration on this year (Roche and Ingle 1999). The Critical Appraisal Skill Programme (CASP) RCT checklist was used for critical appraisal of founded studies.

Inclusion criteria

- ET combination with TA
- Sequential use of ET with TA
- Primary interest of ET agents includes:
    - Selective Oestrogen Receptor Modulators (SERMs): tamoxifen
    - Steroidal third-generation Aromatase Inhibitors (AIs): exemestane
    - Non-steroidal third-generation AIs: anastrozole or letrozole
    - Selective Oestrogen Receptor Downregulators (SERDs): fulvestrant
- Study title must be a RCT that report any of the following molecular TA with ET:
    - HER2 inhibitors
    - mTOR inhibitors
    - CDK4/6 inhibitors
    - VEGFR inhibitors
    - EGFR inhibitors
    - PI3K inhibitors
    - TKIs

- Study must offer full text or abstract that provide details in:
    - Background/Introduction
    - Methods
    - Results
    - Discussion/Conclusion
- HR+ breast cancer may include:
    - ER+, PR+, HER2+
    - ER+, PR+, HER2-
    - ER+, PR-, HER2-
    - ER+, PR-, HER2+
    - ER-, PR+, HER2+
    - ER-, PR+, HER2-
- Study must recruit postmenopausal patients or in addition to premenopausal patients
- Prior chemotherapy was acceptable in abstract screening of RCTs

Exclusion criteria

- Keywords “chemotherapy” or “radiotherapy” stated in title or in combination with ET
- Combination of ET agents (SERDs, AIs, SERMs)
- “Premenopausal” or “Early breast cancer” stated in title
- Study solely on premenopausal patients
- Non-human studies
- Neo-adjuvant studies

Primary outcome

The primary objective was to evaluate the effectiveness of combination therapy and sequential therapy in optimising ET. The optimisation of ET will be measured by observed improvements in PFS, ORR, TTP, CBR and overall survival (OS). Remarks of overcoming endocrine resistant will also be considered.

Secondary outcome

The benefits and limitations of combination therapy and sequential therapy were evaluated. Parameters included: quality of life (QoL), toxicity and cost-effectiveness will also be considered.

It was hypothesised that combination therapy was a more suitable option to optimising ET when compared to sequential therapy in terms of improving treatment efficacy and overcoming endocrine resistance.

Results

Combination therapy search

From Figure 2, an initial detection of **2866** articles from the 4 databases. A final total of **26** studies was achieved, after removal of duplicates, title and abstract screening according to the inclusion and exclusion criteria stated in methods.

From Table 2, there are 9 studies addressing ET/mTOR, 3 ET and CDK4/6, 1 study addressing ET/PI3K, 3 studies addressing ET/HER2, 2 studies addressing ET/VEGFR, 5 studies addressing ET/EGFR, and 3 studies addressing ET/TKI combinations. 2 studies had CBR as their primary endpoint and the rest of the studies had PFS.

ET combinations with mTOR inhibitors (phase III/II)

The combination of exemestane and everolimus was well documented in the international, phase 3, multicentre, randomised, double-blind, placebo-controlled trial: BOLERO-2 (Baselga, et al. 2012; Burris, et al. 2013a; Burris, et al. 2013b; Piccart, et al. 2012; Yardley, et al. 2013). The targeted population consisted of postmenopausal women with HR+, HER2- locally ABC or MBC whom experienced PD from letrozole or anastrozole. Eligible patients were randomised in a blind manner at a 2:1 ratio for the experimental arm (25mg/day exemestane and 10mg/day oral everolimus) or matching placebo. The investigation in BOLERO-2 showed significant improvements in PFS and other efficacy parameters (see Table 8). These improvements in efficacy were also maintained in patients with visceral disease, elderly and of Asian ethnicity. Thus, the everolimus/exemestane combination represents an improvement in managing a wider population of postmenopausal women with HR+, HER2- ABC. Furthermore, BOLERO-2 is the only study that reported QoL. Burris et al. reported similar baseline global health status score in treatment and placebo regimen (64.7 *vs* 65.3) (Burris et al. 2013b). The similar outcome of QoL further supports the use of everolimus with ET.

Despite BOLERO-2 advocated the benefits of using mTOR inhibitor, contrasting finding in PFS was observed in the HORIZON study (Wolff, et al. 2013). This study involved investigation in the use of letrozole in combination with the oral mTOR inhibitor temsirolimus. This combination failed to improve PFS (8.9 vs 9.0 months), ORR (27% vs 27%) and OS. Moreover, a raised toxicity profile in the combination arm resulted in more grade 3/4 AEs (37% vs 24%). However, it was speculated that the contrasting findings in both trials were due to key differences in eligible patient characteristics (Wolff et al. 2013). For instance, HORIZON excluded patients with prior AI exposure within 12 months, whereas eligible patients in

BOLERO-2 required progression from a non-steroidal AI during or within 12 months. This speculation highlights the significance of patient selection to determining the success of the treatment regimen. Interestingly, it was noted in the HORIZON study observed an improved PFS (9.0 vs 5.6 months) limited to patients aged ≤65 treated with the combination letrozole/temsirolimus rather than in patients aged ≥65 (8.5 vs 10.1 months). This finding suggests that temsirolimus activity may favour the younger population over the older population (Wolff et al. 2013). Again, this proposal accentuates the importance of patient selection for treatment success.

From the open-labelled RCT (TAMRAD) that investigated the tamoxifen/everolimus combination. An interesting finding in CBR suggested possible reversal of ET resistance and subsequent improvements. Overall CBR at 6 months was 61% vs 42%. Moreover, improvements in CBR were consistent in patients with secondary resistance (74% vs 48%) and in patients with primary resistance (46% vs 38%). Similar findings in TTP (14.8 vs 5.5 months) was more prominent in patients with secondary resistance as oppose to those with primary resistance (5.4 vs 3.8 months) (Bachelot, et al. 2012). Therefore, this combination may benefit patients with AI-resistance MBC. However, this trial was relatively small with a total of 111 patients and may be prone to bias. Small imbalances between groups' performance status were notable (Bachelot et al. 2012). Hence this study was confirmed only for hypothesis generating and warrant further study into this area (Bachelot et al. 2012).

ET combinations with CDK4/6 inhibitor (phase III/II)

Positive results were observed when novel CDK4/6 inhibitor palbociclib was added to ET. From table 3, PALOMA-2 (letrozole/palbociclib) and PALOMA-3 (fulvestrant/palbociclib)

have shown improvements in efficacy parameters. In both PALOMA-2 and PALOMA-3, significant improvements in PFS, ORR and CBR were reported. In terms of toxicity, neutropenia (79.5% vs 6.3%) was evident when palbociclib was added. Nonetheless, PALOMA-2 confirmed the significant clinical benefits and safety of using palbociclib/letrozole to treat postmenopausal patients whom had no prior systemic therapy for their ER+, HER2- ABC (Finn, et al. 2016a; Finn, et al. 2016b).

From PALOMA-3, patients with HR+, HER2- MBC were randomised in a double-blind manner to fulvestrant (500mg, intramuscular injections on days 1 and 15 of cycle one and then on day 1 of each 28-day cycle) and palbociclib or placebo (125mg/day oral for 3 weeks, followed by 1 week off in a 28-day cycle). Although, this trial recruited both pre- and postmenopausal women, premenopausal women were treated with goserelin (LHRH agonist) to induce postmenopausal status. Significant improvements in PFS (9.5 vs 4.6 months), ORR (66% vs 15%) and CBR (67% vs 40%) were observed. The benefits of palbociclib/fulvestrant in PFS compared to fulvestrant/placebo were consistent irrespective of the degree of HR expression, PIK3CA mutation, ET resistance and ethnicity. These findings propose the possibility of re-sensitising endocrine sensitivity in ET resistant tumours by targeting of CDK4/6. Common toxicities include: neutropenia, leukopenia, fatigue and anaemia were observed in ET/palbociclib arms. These haematological changes should be considered during patient selection for this therapeutic strategy. Endocrine monotherapy had limited efficacy in patients with PD from prior ET, proposing a need for further investigations into the effective use of combination regimens to overcome this problem (Cristofanilli, et al. 2016).

ET combinations with PI3K inhibitors (phase III)

BELLE-2 was a randomised, double-blinded, placebo-controlled phase III trial that investigated the addition of buparlisib to fulvestrant. Overall promising results were observed; with PFS, ORR and CBR all being improved in the experimental arm. The toxicity profile of the addition of buparlisib seems to be associated with liver function; with increase in alanine aminotransferase (26% vs 1% and aspartate aminotransferase (18% vs 3%). Hence, the use of buparlisib in patients with poor liver function should be cautioned. Interestingly, Baselga J et al. reported that buparlisib significantly improved median PFS, ORR and CBR in patients with PIK3CA mutant ctDNA but the same activity was not observed in patients without the mutation. Furthermore, patients characterised with PIK3 mutated tumours are associated with endocrine-resistant HR+, HER2- ABC (Baselga, et al. 2016). This proposes the possibility that the targeting of PI3K pathway may be an area to explore for overcoming endocrine resistance.

ET combinations with HER2 inhibitors (phase III)

Positive results of adding HER2 inhibitor to ET was shown in the TAnDEM study (anastrozole/trastuzumab) and in a phase III study that investigated letrozole in combination with lapatinib (Burstein, et al. 2014; Johnston, et al. 2009; Kaufman, et al. 2009). PFS and CBR were greatly enhanced, with a doubling of PFS was seen in both studies (see Table 3). However, the increase in PFS did not correlate with OS. More AEs were reported in the combination arm in both studies. Moreover, an increase in cardiac events (14 vs 2) was observed in anastrozole/trastuzumab when compared to anastrozole alone. Johnston et al. also discussed the problem of ET resistance in HR+, HER2+ breast cancer and concluded that the addition of lapatinib did not delay disease progression with letrozole in

endocrine-sensitive tumours. In general, the studies concur that addition of HER2 inhibitors to ET in HR+, HER2+ breast cancer can prolong chemoprevention and increase ET efficacy. CALGB 40302 was a randomised, double-blinded, placebo-controlled phase III study that investigated the fulvestrant/lapatinib combination. Conversely, there was a lack of improvement in clinical outcomes. Though, it was noted that PFS was improved in patients with HER2+ tumours (5.9 vs 3.3 months) as oppose to HER2- tumours (4.1 vs 3.8 months) when lapatinib was added. However, this study had a small number of HER2+ cases (18%) with the majority being HER2- tumours (81%). Hence, this could be a limitation of the study that patient recruitment could have been amended to include more HER2+ cases to maximise activity of the HER2 inhibitor. Although the experimental regimen was generally tolerable, there were more AEs and treatment discontinuation caused from the raised toxicity. Overall, CALGB 40302 concluded that lapatinib did not significantly improve clinical benefits when added to fulvestrant (Burstein et al. 2014).

ET combinations with VEGFR inhibitor (phase III)

From table 3, the CALGB 40503 (letrozole with bevacizumab) and LEA study (letrozole/fulvestrant with bevacizumab), reported of contrasting findings in PFS. According to the CALGB 40503 study, the addition of bevacizumab to letrozole improved PFS (20.2 vs 15.6 months) when compared to the placebo arm. Moreover, ORR (69% vs 49%) and CBR (80% vs 62%) exhibited similar improvements from the addition of bevacizumab. However, the significant improvement in PFS, ORR and CBR did not correlate with OS (47.2 vs 43.9 months) (Dickler, et al. 2016). Similar improvements in PFS (19.3 vs 14.4 months), ORR (41% vs 22%) and CBR (77% vs 66%) were observed in the LEA study. However, the difference in PFS was not statistically significant: the hazard ratio of the combination arm vs ET alone was

0.83 (p=0.126) (Martin, et al. 2015). Unsurprisingly, bevacizumab combinations were associated with increased AEs; mainly hypertension and proteinuria. The LEA study reported of deaths in the bevacizumab arm that seem to be associated with conditions that may have been worsened from the hypertensive side-effects (Martin et al. 2015). As a result, patients with hypertensive conditions should avoid the use of bevacizumab.

One of the limitations of the LEA study was the lack of comparison of letrozole and fulvestrant when in combination with bevacizumab. All the data assimilated was grouped together either as ET/bevacizumab and ET alone. Further sub-groups within ET/bevacizumab to compare letrozole/bevacizumab and fulvestrant/bevacizumab would have provided more information on optimal application of bevacizumab to ET.

ET combinations with EGFR inhibitor (phase II)

Marked advantage in PFS was reported when gefitinib was added to anastrozole in comparison to placebo (see Table 4) (Cristofanilli, et al. 2010; Valero, et al. 2009). Improvement in PFS was also observed in the study of tamoxifen in combination with gefitinib. For this trial, patients were split into two groups: stratum 1 (PD after tamoxifen) and 2 (PD during/after AI). PFS was only improved in stratum 1 (10.9 vs 8.8 months), but not in stratum 2 (5.7 vs 7.0 months). The significant improvement of PFS in stratum 1 suggests possible endocrine re-sensitisation when gefitinib was added to an ET (tamoxifen, in this case) that was previously used (Osborne, et al. 2011). A sub-analysis of PFS in patients with prior ET therapies (11.2 vs 7.1 months) and ET naïve (20.2 vs 8.4 months) was observed using gefitnib/anastrozle vs placebo arm (Cristofanilli et al. 2010). These findings suggest a potential role of overcoming ET resistance from using gefitinib. On the other hand, Tryfonidis et al. argued that the toxicity profile (mainly skin and gastrointestinal related) of

gefitinib resulted in premature therapy interruption in 33% of patients. Additionally, the PFS rate at 1 year was only 35% for combination arm and 32% for placebo arm (Tryfonidis, et al. 2016). Hence, the use of gefitinib was not supported in a risk/benefit point of view. Carlson et al. echoed similar opinion in further trials of combinations of gefitnib with anastrozole/fulvestrant, despite modest findings in anti-tumour activities (Carlson, et al. 2012). Overall PFS comparison seemed similar (5.3 vs 5.2 months in anastrozole and fulvestrant arms respectively) but in patients who had prior chemotherapy, a significant deterioration in PFS was seen in the fulvestrant/gefitinib arm (2.6 months) (Tryfonidis et al. 2016). Although it was unexplained why these changed were observed, it can be inferred that prior treatment can have an impact on future treatments.

ET combinations with TKI (phase II)

The general consensus toward TKI/ET combinations seem negative. Johnston et al. reported a 3 arms trial of anastrozole (1mg/day) in combination with AZD8931 at 20mg (twice daily), 40mg (twice daily) or placebo. Although PFS (13.8 vs 14.9 vs 10.9 months) was increased, it was statistically insignificant (see Table 4) (Johnston, et al. 2016). This therapeutic strategy does not seem to enhance ET responsiveness and was generally associated with a greater toxicity profile when compared to ET alone. Wright et al. reported that the addition of dasatinib to fulvestrant did not improve PFS (6.0 months vs 5.3 months), CBR and OS. In fact, CBR (28.0% vs 32.7%) and OS (17.0 vs 21.7 months) seemed to worsen with dasatinib/fulvestrant when compared to placebo (Wright, et al. 2011). This may suggest that a worse safety profile and patient tolerability could potentially influence the patient's QoL and ultimately OS. Finally, in the fulvestrant/dovitinib study, an improvement in PFS (10.9 vs 5.5 months) was observed. Though only limited to patients with FGF pathway-amplified

breast cancer in fulvestrant/dovitinib vs placebo arm respectively. Contrastingly, patients without FGF-pathway-amplification gained no effect from the addition of dovitinib (5.5 vs 5.5 months), other than the increased toxicity associated in combination therapy (Musolino, et al. 2017). This discovery highlights the importance of patient selection by identifying cancer biology to maximise treatment prognosis.

Sequential therapy search

From figure 3, an initial detection of **901** articles. A final total of **0** studies was identified, after removal of duplicates, title and abstract screening according to the inclusion and exclusion criteria stated in methods. Therefore, the search for relevant literature in the sequential application of ET and TA was unsuccessful.

Discussion

This review aimed to explore options for the optimisation of ET with TA by methods of combination therapy or sequential therapy. From assimilating relevant studies, it was clear that combination therapy is investigated more thoroughly than sequential therapy. The identification of benefits and limitations in both combination and sequential therapy was not met due to the absence of literature available in sequential therapy. The result of 0 articles warrants the need of future investigation in this area.

It was hypothesised that combination therapy would be the better option in optimising ET. Most combinations of ET and TA have yielded extremely promising results, notably in enhancing treatment efficacy (PFS, ORR and CBR). The classes of TA reviewed in this systematic review included: mTOR inhibitors, EGFR inhibitors, TKI, CDK4/6 inhibitors, VEGFR inhibitors, PI3K inhibitor, and HER2 inhibitors. Most treatment combinations were effective

in treating patients with HR+, HER2- ABC/MBC. Evidently, the best combination arms included CDK4/6 inhibitor, PI3K inhibitor and mTOR inhibitors in treating this population. These combinations seem to optimise ET by producing significant improvements in PFS, CBR and ORR, regardless of patients' treatment history and overcoming endocrine resistant. The additional benefits from combination therapy were associated with an increase in toxicity. This was a common trend in all included studies. Consequently, combination therapy may prove difficult in patients whom do not tolerate these regimens, for instance in the elderly population.

All studies documented the toxicity profile of the combination against the comparison arm. However, it was unknown how these toxicities may have impacted the patient being treated. Most studies had stated that one of the main reasons for patient discontinuation was related to treatment toxicity. Data in these areas should identify treatment tolerability, patients' QoL and financial feasibility for sustainable treatment. Therefore, clinicians will be provided with a better understanding on the ideal application of ET and TA.

Throughout the review, it was evident that some combinations (TKI, EGFR and VEGFR) failed to produce any benefits over ET alone. Differences in study design seemed to be the most likely explanation for contrasting findings in RCTs with similar experimental arms. Most RCTs used methods such as: double-blinding, placebo-controlled, and 2-arm trial. Although some RCTs deviated from this and employed an open-label approach and the absent of placebo. Hence those RCTs may be of lower power than those that used the double-blinding and placebo-control methods to minimise chances of bias.

Patient selection

It was implied that the importance of patient selection seemed to influence treatment prognosis. From assimilating relevant study findings, this review suggests that patient selection can be categorised into 3 main areas: patient characteristics, cancer biology and pharmacology.

Patient Characteristics:

Patient characteristics such as age have shown to influence drug efficacy. In the HORIZON study, temsirolimus produced PFS benefits in younger patients as opposed to older patients (Wolff et al. 2013). Thus, the use of SERMs and SERDs in combination to temsirolimus may exhibit greater benefit in selected younger patients than using AIs which are restricted to the postmenopausal population. However, it should be reminded that not all postmenopausal patients are of the older population. Younger patients can obtain the postmenopausal status via oophorectomy or the use of a luteinising hormone releasing hormone agonist. Another aspect to consider in older patients would be treatment tolerability. From the LEA study, details of patients' deaths were reported in the bevacizumab arm (n=8) (Martin et al. 2015). Some deaths were associated with conditions that may have been exacerbated from the hypertensive side effects. Further inspection, revealed that the patient age ranged from 53-82 years old and 5 out of 8 patients had hypertension as baseline co-morbidity (Martin et al. 2015). Therefore, specific co-morbidities in individual patients should be considered when selecting regimens. As evidently different classes of TA are associated with specific toxicities: palbociclib (neutropenia), bevacizumab (hypertension), trastuzumab (cardiac events), and EGFR inhibitors (skin and gastrointestinal).

Cancer Biology:

The identification of specific targets can broaden the options for therapeutic strategies. For instance, the use of dovitinib (TKI that inhibits FGF pathways) in combination with fulvestrant was shown to significantly improve PFS in patients with FGF pathway-amplified breast cancer (10.9 vs 5.5 months) when compared to the placebo arm. Whereas, patients without FGF pathway amplification did not benefit from the dovitinib/fulvestrant combination (5.5 vs 5.5 months) (Musolino et al. 2017). Burstein et al. also reported greater improvement in PFS and ORR, when the HER2 inhibitor lapatinib was added to fulvestrant in patients with HER2+ status than in those with HER2- (Burstein et al. 2014). These findings support the importance of patient selection, by identifying cancer biology to maximise treatment success.

Pharmacology:

Pharmacology was another factor that should be considered during patient selection for suitable therapeutic strategy. It was clear from the findings in this systematic review, that prior therapy can influence treatment prognosis. This was evident in studies of ET/EGFR combinations, whereby prior ET or chemotherapy had caused dramatic changes in treatment outcome. In the phase II study that investigated the anastrozle/gefitinib combination, Cristofanilli et al. reported an exploratory post hoc subset analysis of patients with endocrine naïve and prior ET. An all-round improvement in PFS was observed in both subset. But, the data seem to suggest superior benefits in PFS for patients with endocrine naïve (20.2 months) in contrast to patients who had prior ET (11.2 months) (Cristofanilli et al. 2016). From these findings, it was confirmed that endocrine monotherapy had limited efficacy in patients with PD from prior ET, proposing a need for further investigations into

the effective selection of combination regimens to overcome this problem. Furthermore, this proposes that the use of combination therapy in a first line setting may benefit those with naïve treatment. Although, some combinations (CDK4/6, PI3K, EGFR, and mTOR) have shown activity to overcome ET resistance in patients with prior ET exposure. Yet it was unspecified if the number of prior therapies may further diminish the outcome in combination therapy. Hence this may be another area to be for future investigations.

Overcoming resistant:

One of the criteria for optimising ET in this review was to overcome ET resistance. This question was met in findings from phase III PALOMA-3 and BELLE-2 studies suggesting that targeting CDK4/6 and PI3K hold the most promise. This was supported by in vitro evidence suggesting cancer cells that have developed ET resistance remain dependent on cyclin D1 and CDK4 for proliferation. Similarly, pre-clinical evidence has identified a potential cause of endocrine resistance via cross-talk between ER and PI3K pathways (Milani, et al. 2014). Additional findings from phase II ET combinations with gefitinib and everolimus suggested signs of delaying ET resistance or re-sensitising tumours with ET resistance promise (Bachelot et al. 2012; Tryfonidis et al. 2016). This prompts further research into overcoming ET resistance by targeting these pathways.

Sequential application

There was evidently a lack of knowledge about the sequential application of ET and TA. This review has identified areas that combination therapy has failed to impress and a new approach in optimal application of specific target agents was needed. For instance, the activity of gefitinib with ET has suggested effects of delaying ET resistance. But in a

combination setting, the regimen seemed to only increase toxicity while retaining similar efficacy seen in endocrine monotherapy (Tryfonidis et al. 2016). Hence the sequential application of these agents could be a feasible alternative. A predicted decrease in toxicity would provide a more tolerable profile for patients. This will be important for management of the elderly population where tolerability may be an issue. Classes of TA such as TKIs, VEGFR inhibitors and HER2 inhibitors when in combination created unfavourable tolerability in patients. Therefore, those classes of agents may benefit from this sequential approach.

Limitations

The term "targeted agents" was narrowly defined to fit the feasibility of generating this systematic review. Several agents were excluded from this review included: proteasome inhibitors and farnesyltransferase inhibitors. Moreover, combination therapy was strictly defined to only include 2 agent combinations and excluding studies that have explored the feasibility of more than 2 agent combinations such as triple combinations. Thus, this review does not reflect the true potential depth of combination therapy and diversity of TA available for optimising ET.

The method in selecting papers was rigorously determined by the presence of specific keywords. Studies that were excluded solely based on title alone, may have contained relevant information in the abstract or within the full text. Thus, there was the possibility that relevant studies were missed.

Furthermore, many trial status were "on-going" or "results pending", this resulted in a narrow range of agents being incorporated into this review. This was especially evident in the attempt of including novel agents that targeted the PI3K pathway. Consequently, the protocol was amended to allow inclusion of abstracts to generate a wider pool of agents

and subsequent findings. However, limited information was provided in the abstracts when compared to full text. This was evident during analysis of study design and results.

Conclusion

Combination of ET and TA have proven to be effective at improving treatment efficacy over monotherapy in postmenopausal patients with HR+ ABC/MBC. However, not all combinations are adding benefit to ET and some are only increasing the toxicity profile. Indisputably, tolerability of toxicity in combination therapy of the elderly population possess an issue in patient management. As a result, this may be an opportunity for sequential therapy of ET and TA to be explored in this specific population.

Declaration of interest

Thomas Ho Lai Yau has declared no conflict of interest.

Declaration of conflict of interest for Kwok-Leung Cheung:

- Research Funding - AstraZeneca
- Consulting or Advisory Role - Genomic Health
- Travel, Accommodation, Expenses - Genomic Health

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgment

Not applicable.

Reference

Bachelot T, Bourgier C, Cropet C, Ray-Coquard I, Ferrero JM, Freyer G, Abadie-Lacourtoisie S, Eymard JC, Debled M, Spaeth D, et al. 2012 Randomized Phase II Trial of Everolimus in Combination With Tamoxifen in Patients With Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Metastatic Breast Cancer With Prior Exposure to Aromatase Inhibitors: A GINECO Study. *Journal of Clinical Oncology* **30** 2718-2724.

Baselga J, Campone M, Piccart M, Burris IHA, Rugo HS, Sahmoud T, Noguchi S, Gnant M, Pritchard KI, Lebrun F, et al. 2012 Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. *New England Journal of Medicine* **366** 520-529.

Baselga J, Im SA, Iwata H, Clemons M, Ito Y, Awada A, Chia S, Jagiello-Gruszfeld A, Pistilli B, Tseng LM, et al. 2016 PIK3CA status in circulating tumor DNA (ctDNA) predicts efficacy of buparlisib (BUP) plus fulvestrant (FULV) in postmenopausal women with endocrine-resistant HR+/HER2-advanced breast cancer (BC): First results from the randomized, phase III BELLE-2 trial. *Cancer Research. Conference: 38th Annual CTRC AACR San Antonio Breast Cancer Symposium. San Antonio, TX United States. Conference Start* **76**.

Burris H, Gnant M, Hortobagyi G, Hart L, Yardley DA, Eakle J, Provencher L, Brechenmacher T, Saletan S, Taran T, et al. 2013a Characterization of response to everolimus (EVE) in BOLERO-2: A phase 3 trial of EVE plus exemestane (EXE) in postmenopausal women with HR+, HER2-advanced breast cancer. *Cancer Research. Conference: 36th Annual CTRC AACR San Antonio Breast Cancer Symposium. San Antonio, TX United States. Conference Start* **73**.

Burris HA, Lebrun F, Rugo HS, Beck JT, Piccart M, Neven P, Baselga J, Petrakova K, Hortobagyi GN, Komorowski A, et al. 2013b Health-related quality of life of patients with advanced breast cancer treated with everolimus plus exemestane versus placebo plus exemestane in the phase 3, randomized, controlled, BOLERO-2 trial. *Cancer* **119** 1908-1915.

Burstein HJ, Cirrincione CT, Barry WT, Chew HK, Tolaney SM, Lake DE, Ma C, Blackwell KL, Winer EP & Hudis CA 2014 Endocrine therapy with or without inhibition of epidermal growth factor receptor and human epidermal growth factor receptor 2: A randomized, double-blind, placebo-controlled phase III trial of fulvestrant with or without lapatinib for postmenopausal women with hormone receptor-positive advanced breast cancer - CALGB 40302 (alliance). *Journal of Clinical Oncology* **32** 3959-3966.

Carlson RW, O'Eill A, Vidaurre T, Gomez HL, Badve SS & Sledge GW 2012 A Randomized Trial of Combination Anastrozole plus Gefitinib and of Combination Fulvestrant plus Gefitinib in the Treatment of Postmenopausal Women with Hormone Receptor Positive Metastatic Breast Cancer.

Cheung KL 2007 Endocrine therapy for breast cancer: an overview **16** 327-343.

Cristofanilli M, Turner NC, Bondarenko I, Ro J, Im S-A, Masuda N, Colleoni M, DeMichele A, Loi S, Verma S, et al. 2016 Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phase 3 randomised controlled trial. *The Lancet Oncology* **17** 425-439.

Cristofanilli M, Valero V, Mangalik A, Royce M, Rabinowitz I, Arena FP, Kroener JF, Curcio E, Watkins C, Bacus S, et al. 2010 Phase II, randomized trial to compare anastrozole combined

with gefitinib or placebo in postmenopausal women with hormone receptor-positive metastatic breast cancer. *Clinical Cancer Research* **16** 1904-1914.
Dickler MN, Barry WT, Cirrincione CT, Ellis MJ, Moynahan ME, Innocenti F, Hurria A, Rugo HS, Lake DE, Hahn O, et al. 2016 Phase III trial evaluating letrozole as first-line endocrine therapy with or without bevacizumab for the treatment of postmenopausal women with hormone receptor-positive advanced-stage breast cancer: CALGB 40503 (Alliance). *Journal of Clinical Oncology* **34** 2602-2609.
Dixon JM 2014 Endocrine Resistance in Breast Cancer. *New Journal of Science* **2014** 27.
Finn RS, Martin M, Rugo HS, Jones S, Im SA, Gelmon K, Harbeck N, Lipatov ON, Walshe JM, Moulder S, et al. 2016a Palbociclib and Letrozole in Advanced Breast Cancer. *New England Journal of Medicine* **375** 1925-1936.
Finn RS, Martin M, Rugo HS, Jones SE, Im SA, Gelmon KA, Harbeck N, Lipatov ON, Walshe JM, Moulder SL, et al. 2016b 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). *Journal of Clinical Oncology. Conference* **34**.
Hammond MEH, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, Fitzgibbons PL, Francis G, Goldstein NS, Hayes M, et al. 2010 American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer (Unabridged Version). *Archives of Pathology & Laboratory Medicine* **134** e48-e72.
Jeselsohn R, Buchwalter G, De Angelis C, Brown M & Schiff R 2015 ESR1 mutations as a mechanism for acquired endocrine resistance in breast cancer. *Nature reviews. Clinical oncology* **12** 573-583.
Johnston S, Basik M, Hegg R, Lausoontornsiri W, Grzeda L, Clemons M, Dreosti L, Mann H, Stuart M & Cristofanilli M 2016 Inhibition of EGFR, HER2, and HER3 signaling with AZD8931 in combination with anastrozole as an anticancer approach: Phase II randomized study in women with endocrine-therapy-na < ve advanced breast cancer. *Breast Cancer Research and Treatment* **160** 91-99.
Johnston S, Pippen Jr J, Pivot X, Lichinitser M, Sadeghi S, Dieras V, Gomez HL, Romieu G, Manikhas A, Kennedy MJ, et al. 2009 Lapatinib combined with letrozole versus letrozole and placebo as first-line therapy for postmenopausal hormone receptor - Positive metastatic breast cancer. *Journal of Clinical Oncology* **27** 5538-5546.
Kaufman B, Mackey JR, Clemens MR, Bapsy PP, Vaid A, Wardley A, Tjulandin S, Jahn M, Lehle M, Feyereislova A, et al. 2009 Trastuzumab plus anastrozole versus anastrozole alone for the treatment of postmenopausal women with human epidermal growth factor receptor 2-positive, hormone receptor-positive metastatic breast cancer: Results from the randomized phase III TAnDEM study. *Journal of Clinical Oncology* **27** 5529-5537.
Martin M, Loibl S, Von Minckwitz G, Morales S, Martinez N, Guerrero A, Anton A, Aktas B, Schoenegg W, Munoz M, et al. 2015 Phase III trial evaluating the addition of bevacizumab to endocrine therapy as first-line treatment for advanced breast cancer:The Letrozole/Fulvestrant and Avastin (LEA) study. *Journal of Clinical Oncology* **33** 1045-1052.
Milani A, Geuna E, Mittica G & Valabrega G 2014 Overcoming endocrine resistance in metastatic breast cancer: Current evidence and future directions. *World Journal of Clinical Oncology* **5** 990-1001.
Moher D, Liberati A, Tetzlaff J, Altman DG & The PG 2009 Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLOS Medicine* **6** e1000097.

Musolino A, Campone M, Neven P, Denduluri N, Barrios CH, Cortes J, Blackwell K, Soliman H, Kahan Z, Bonnefoi H, et al. 2017 Phase II, randomized, placebo-controlled study of dovitinib in combination with fulvestrant in postmenopausal patients with HR+, HER2(-) breast cancer that had progressed during or after prior endocrine therapy. *Breast Cancer Research* **19**.
Osborne CK, Neven P, Dirix LY, Mackey JR, Robert J, Underhill C, Schiff R, Gutierrez C, Migliaccio I, Anagnostou VK, et al. 2011 Gefitinib or Placebo in Combination with Tamoxifen in Patients with Hormone Receptor–Positive Metastatic Breast Cancer: A Randomized Phase II Study. *Clinical Cancer Research* **17** 1147.
Piccart M, Baselga J, Noguchi S, Burris H, Gnant M, Hortobagyi G, Mukhopadhyay P, Taran T, Sahmoud T & Rugo H 2012 Final progression-free survival analysis of BOLERO-2: A phase III trial of everolimus for postmenopausal women with advanced breast cancer. *Cancer Research. Conference: 35th Annual CTRC AACR San Antonio Breast Cancer Symposium. San Antonio, TX United States. Conference Start* **72**.
Pietras RJ 2006 Biologic Basis of Sequential and Combination Therapies for Hormone-Responsive Breast Cancer. *The Oncologist* **11** 704-717.
Reinert T & Barrios CH 2015 Optimal management of hormone receptor positive metastatic breast cancer in 2016. *Therapeutic Advances in Medical Oncology* **7** 304-320.
Robertson JFR, Bondarenko IM, Trishkina E, Dvorkin M, Panasci L, Manikhas A, Shparyk Y, Cardona-Huerta S, Cheung K-L, Philco-Salas MJ, et al. 2016 Fulvestrant 500 mg versus anastrozole 1 mg for hormone receptor-positive advanced breast cancer (FALCON): an international, randomised, double-blind, phase 3 trial. *The Lancet* **388** 2997-3005.
Roche PC & Ingle JN 1999 Increased HER2 With U.S. Food and Drug Administration-Approved Antibody. *Journal of Clinical Oncology* **17** 434-434.
Tryfonidis K, Basaran G, Bogaerts J, Debled M, Dirix L, Thery J, Tjan-Heijnen V, Weyngaert D, Cufer T, Piccart M, et al. 2016 A European Organisation for Research and Treatment of Cancer randomized, double-blind, placebo-controlled, multicentre phase II trial of anastrozole in combination with gefitinib or placebo in hormone receptor-positive advanced breast cancer (NCT00066378). In *European journal of cancer (Oxford, England : 1990)*, pp 144-154.
Valero V, Bacus S, Mangalik A, Rabinowitz I, Arena F, Kroener J, Curcio E, Watkins C, Magill P & Cristofanilli M 2009 Molecular marker correlates of clinical outcome in a phase II study of gefitinib or placebo in combination with anastrozole in postmenopausal women with hormone receptor-positive metastatic breast cancer. *Cancer Research. Conference: 31st Annual San Antonio Breast Cancer Symposium. San Antonio, TX United States. Conference Start* **69**.
Wolff AC, Lazar AA, Bondarenko I, Garin AM, Brincat S, Chow L, Sun Y, Neskovic-Konstantinovic Z, Guimaraes RC, Fumoleau P, et al. 2013 Randomized phase III placebo-controlled trial of letrozole plus oral temsirolimus as first-line endocrine therapy in postmenopausal women with locally advanced or metastatic breast cancer. *Journal of Clinical Oncology* **31** 196-202.
Wright GL, Blum J, Krekow LK, McIntyre KJ, Wilks ST, Rabe AC, Vukelja SJ, Andersen JC, Wang Y, Asmar L, et al. 2011 Randomized phase II trial of fulvestrant with or without dasatinib in postmenopausal patients with hormone receptor-positive metastatic breast cancer previously treated with an aromatase inhibitor. *Cancer Research. Conference: 34th Annual CTRC AACR San Antonio Breast Cancer Symposium. San Antonio, TX United States. Conference Start* **71**.

Yardley DA, Noguchi S, Pritchard KI, Burris HA, Baselga J, Gnant M, Hortobagyi GN, Campone M, Pistilli B, Piccart M, et al. 2013 Everolimus Plus Exemestane in Postmenopausal Patients with HR+ Breast Cancer: BOLERO-2 Final Progression-Free Survival Analysis. *Advances in Therapy* **30** 870-884.

Table/Figure Legends:

Table 1. *Some targeted therapy agents that have been used in treating breast cancer in combination with other forms of cancer treatment.*

Table 2. Summary of included phase II/III studies that address combination of ET and TA (mTOR inhibitors; CDK4/6 inhibitors; PI3K inhibitor; HER2 inhibitors; VEGFR inhibitor; EGFR inhibitor and TKI). Figures with * and ** represent figures from the same study.

Table 3. *Summarised findings of different parameters from each phase III studies. The table is formatted as followed: (**experimental arm vs comparative arm**). Regarding toxicities column, selected toxicity was chosen by availability from study and prevalence*.

*** The changing of unit will be stated in the cell**

ctDNA = circulating tumour DNA

Hr = Hazard ratio

OR = Odd ratio

p = p-value

Table 4. *Summarised findings in different parameters from each phase II studies. The table is formatted as followed: (**experimental arm vs comparative arm**). Regarding toxicities column, selected toxicity was chosen by availability from study and prevalence.*

*** change of unit will be stated in the cell**

Hr = Hazard ratio

OR = Odds ratio

p = p-value

Figure 1. *A hypothetical comparison of combination therapy (Treatment A) and sequential therapy (Treatment B and C).*

ET *= Endocrine therapy agent*

TA *= Targeted agent*

ET/TA *= Combination of endocrine therapy agent and targeted agent*

Blocked arrow *= Duration of effective treatment from ET/TA*

Dashed arrow *= Duration of effective treatment from ET*

Straight arrow *= Duration of effective treatment from TA*

Figure 2. *A flow diagram displaying the study selection process that addressed for combinations of ET with targeted agents adapted from PRISMA (Moher, et al. 2009)*

Figure 3. *A flow diagram displaying the study selection process that addressed for sequential use of ET with targeted agents adapted from PRISMA (Moher et al. 2009)*