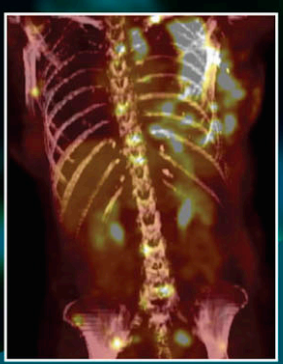
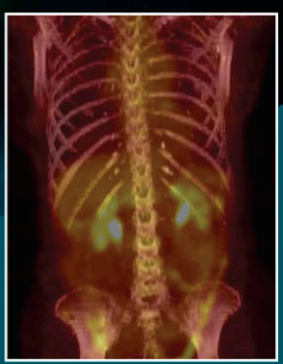
# Handbook of Metastatic Breast Cancer

## Second Edition





Edited by
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## Second Edition

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# 1 Introduction

**Stephen R. D. Johnston and Charles Swanton**

Despite significant advances in the diagnosis and treatment of breast cancer, approximately one-third of patients still develop and subsequently die from metastatic breast disease. Globally, half a million deaths each year are attributable to metastatic breast cancer and the median survival time from the diagnosis of secondary disease is approximately 3 years. The range is very wide however, with some patients having more indolent disease that they can live with for 10–15 years, while for others with widespread metastatic disease, the prognosis may only be a matter of months from the time of diagnosis. While this may represent the extent and distribution of metastatic disease, in part it reflects the biological diversity of breast cancer, with some women having disease that exhibits extreme sensitivity to hormonal treatments, whereas others with so-called triple-negative breast cancer may display relative resistance to all systemic therapies. In recent years, the increasing recognition that different molecular subtypes of breast cancer exist has substantially changed not only the way we classify and treat the disease, but also the impact that certain novel therapeutics in the metastatic setting can have on specific types of breast cancer.

In a rapidly evolving field in modern medicine where cancer genetics, molecular profiling, and targeted therapeutics have all had a huge impact over the last 5 years, it is timely to update the first edition of this "Handbook of Metastatic Breast Cancer" that was first published in 2006. Although the principles of treating the disease remain unchanged, there have been sufficient advances in several aspects of clinical management to merit a second edition that includes the most up-to-date information and results of clinical trials, and discusses the impact of these developments on the management of patients with metastatic breast cancer. There is a new chapter that discusses the molecular taxonomy of breast cancer, focusing on the relevance of gene expression signatures and predictive and prognostic biomarkers in the treatment of metastatic breast cancer. In addition, there are three new chapters on specialist systemic treatment options, including targeting HER2+ and issues relating to trastuzumab-resistant metastatic breast cancer, management of triple-negative sporadic and BRCA germline metastatic disease, and the role of angiogenesis inhibitors in the treatment of advanced breast cancer. We have provided significant updates to the existing chapters that discuss various systemic treatments for breast cancer, including endocrine therapy, chemotherapy, targeted therapies, and bisphosphonates. In addition we have updated the information on diagnostic imaging and tumour assessment, including the role of positron emission tomography and other functional imaging modalities.

The principal aim of treatment for secondary breast cancer remains to increase the duration of symptom-free survivorship and limit treatment-related toxicity, and thereby ensure the maximum quality of life for most of the patients. It is acknowledged that metastatic breast cancer can affect many parts of the body and this requires a wide range of treatments to control local symptoms. Therefore, it is strongly recommended that these patients are now managed by a specialist, multidisciplinary secondary breast cancer team which works closely with palliative care specialists and associated medical specialities as required. These aspects of multimodality management should underpin modern day services for women with secondary breast cancer, and we have updated the chapters from allied professionals that discuss local

treatment options for neurological, thoracic, orthopaedic, and hepatic complications in advanced secondary breast cancer.

The high prevalence of the disease, together with the relatively long natural history for many patients, means that in the United Kingdom approximately 100,000 women are living with a diagnosis of secondary breast cancer each year. However, for these women the true impact of living with an incurable condition and coping with an uncertain future is something that often goes unrecognised by health-care professionals. The diagnosis of metastatic breast cancer is always a devastating event for any patient who has received previous therapy for early breast cancer that was given with the hope and expectation of cure. Therefore, when secondary disease returns it is associated with the realisation that "cure" is no longer possible. The information needs of patients are now very complex, made more challenging and sometimes confusing by the vast volume of information available to patients via the Internet. This means that specialist information and support services for patients and their families are vitally important, and in many centres this is now provided by clinical nurse specialists in secondary breast cancer. The role of these support services is discussed in a new chapter.

While at present metastatic breast cancer cannot be cured, modern systemic and loco-regional treatment can be very effective in maximising the duration of a patient's quality time without disease-related symptoms, which if significant in itself will often manifest as prolonged survival. With the introduction of more effective therapies over the last two decades, there has been a substantial improvement in clinical outcomes for women with metastatic breast cancer compared to treatment therapies available 30 years ago. Indeed many patients can now expect to live with metastatic secondary breast cancer for several years. However, many challenges continue to remain in the development of novel therapies proving that a given intervention on its own impacts on overall survival; this is because with so many effective therapies to offer patients with advanced disease, randomised trials against no therapy or "best supportive care" are impossible and indeed unethical to conduct in this disease. Furthermore, because breast cancer in general is relatively sensitive to the various drug- and radiation-based therapies that are available, with multiple lines of treatment often being used during the course of a patient's illness, subsequent therapies given in sequence will undoubtedly have a major impact on patient outcome. This makes the likelihood of a novel therapy in the first-line setting having a significant impact on overall survival almost impossible to demonstrate. Because of this, "progression-free survival" has become in some instances a recognised primary endpoint that is used to demonstrate to regulatory authorities the clinical utility of any given novel therapeutic. For most patients and their health-care professionals, an effective therapy that controls disease without toxicities, from which life expectancy may be prolonged, remains the most important objective in the management of the disease.

As outcomes for women with secondary breast cancer continue to improve, there are now genuine grounds for optimism, despite the sense of uncertainty and loss of control that many women inevitably feel once diagnosed. During the past two decades there have been significant advances in the diagnosis and treatment of early breast cancer, reflected by the significant improvement in mortality from the disease observed both in the United States and Europe since the early 1990s (1). The United Kingdom has witnessed perhaps the largest single improvement in survival rates form breast cancer, with a 40% reduction in mortality since 1990 (2). Reasons for this progress are multifactorial and have been attributed to the possible impact of screening and detection of earlier-stage disease, better multidisciplinary management of breast cancer by dedicated specialists, together with a more widespread use of systemic adjuvant therapies including combination chemotherapy and hormonal treatment. Furthermore, the introduction of novel targeted therapies, in particular

biological therapies such as trastuzumab for HER2-positive disease, has altered the natural history of advanced breast cancer, with an unprecedented impact on survival from such treatments. As such, it is likely that many patients will now live considerably longer with their secondary breast cancer under control, although cure in this setting still remains an elusive goal.

We hope that the updated second edition of this handbook will be a useful source of information for all health-care professionals involved in the management of patients with metastatic breast cancer. Sharing knowledge helps improve practice, which ultimately benefits women afflicted by this disease.

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# 2 The prognostic and predictive value of gene expression signatures in breast cancer

**Hatem A. Azim, Jr., Debora Fumagalli, and Christos Sotiriou**

## INTRODUCTION

Gene-expression profiling with the use of DNA microarray allows measurement of thousands of messenger RNA transcripts in a single experiment. Results of such studies have confirmed that breast cancer (BC) is not a single disease, but rather a group of molecularly distinct subtypes (1). In this regard, four main molecular classes of BC have been identified which are as follows (2–5): luminal-A cancer, which is mostly low proliferative oestrogen receptor (ER) positive; luminal-B, which is mostly high proliferative ER positive; basal-like cancer, which is negative for ER, progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) and finally HER2-positive cancer which is characterised by the amplification of the HER2 gene.

Gene-expression profiling has also been used to develop genomic tests with the aim to provide prognostic tools which are better than classical clinicopathological parameters. In addition, such tests could serve as predictive tools to systemic therapies.

Two main approaches have been adopted to develop such signatures. The "first-generation signatures," were developed focusing on epithelial cancer cells. These include MammaPrint®: Agendia, Oncotype Dx®: Genomic Health, MapQuant Dx®: Ipsogen, and Theros®: Biotheranostics (6–10). These signatures were found to be useful for determining the risk of relapse in ER-positive BC, yet much less informative for the ER-negative and HER2-positive subgroups which are assigned to the high-risk category in almost all cases (11). More recently, another group of signatures were developed, referred to as "second-generation signatures," in which other factors are taken into consideration in addition to genomics information derived from epithelial cancer cells. After conducting a comprehensive gene expression profiling of each cell type, Allinen et al. have shown that at the transcriptome level, changes occur in epithelial as well as in myoepithelial and stromal cells that are already evident at the carcinoma *in situ* stage (12,13). The appreciation of this fact has resulted in the development of second-generation signatures derived from stromal cells (14,15), the immune system (16,17), and cancer-related pathways (16,18).

In this chapter, we discuss the prognostic and predictive value of first- and second-generation gene expression signatures emphasising their potential role in improving prognostication and selection of therapy for patients with early BC.

## PROGNOSTIC VALUE OF GENE EXPRESSION SIGNATURES

### First-Generation Signatures

In 2002, the Dutch group published two landmark publications addressing the prognostic value of the MammaPrint, an assay that measures the expression of 70 genes and accordingly categorises patients into good and poor risk groups (6,19). In the earlier study, the assay could accurately predict the prognosis of 78 untreated women with node-negative disease and tumour size <5 cm (6). This was followed by a validation study on a series of 295 patients including those with node-positive disease (19). In the latter study, the initial results were confirmed and the assay was found to assign patients more accurately to the low-risk category compared with other clinical prognostic tools like the St. Gallen criteria and the National Institutes of Health

consensus criteria (20,21). A second validation study was later published on a larger number of patients and the 70-gene signatures outperformed the clinicopathological risk assessment by the Adjuvant! Online (AOL) program (22). In this study, 87 of the 302 (29%) patients had discordant results. Of these, 68% had tumours that were rated as clinically high risk according to the clinicopathological criteria but low risk according to the gene signature; while 32% were rated as clinically low risk but high risk according to the gene signatures. Indeed, in these cases, the genomic test was more accurate in predicting prognosis. In the former group (low genomic risk, high clinical risk), the 10-year overall survival rate was 89% while in the latter group (high genomic risk, low clinical risk), it was 69%.

The genomic grade index ((GGI), MapQuant Dx) is another signature that was developed by Sotiriou et al. to explore whether gene-expression profiling could be used to grade tumours more accurately than the conventional histological grade, particularly those tumours with intermediate grade (GII) (8). In a study involving 570 patients, GGI was able to discern among GII tumours two risk groups with a significant difference in relapse-free-survival rates (high vs. low risk; HR: 2.83; 95% CI 2.13–3.77; $p < 0.001$). In a multivariate model that included all known clinicopathological parameters, GGI demonstrated strong prognostic information (HR: 1.38; 95% CI 1.43–2.78; $p < 0.001$) while histological grade was non-informative. In this analysis, tumour size, and lymph node status were also significantly associated with prognosis.

Oncotype Dx is another assay that measures the expression of ER and HER2, as well as that of ER-regulated transcripts and several proliferation genes. Using this assay, a recurrence score (RS) is calculated based on the expression of 16 cancer-related genes and 5 reference genes and accordingly a risk group is determined (low, intermediate, or high). Paik et al. have carried out a large retrospective analysis to examine the prognostic value of RS in predicting distant recurrence in patients with node-negative, tamoxifen-treated BC patients who were enrolled in the National Surgical Adjuvant Breast and Bowel Project clinical trial B-14 (9). The 10-year distant recurrence rates were 7%, 14%, and 30% for low-, intermediate-, and high-risk groups respectively. In a multivariate model, the recurrence score provided significant prognostic power independent of age and tumour size ($p < 0.001$) and was predictive for overall survival ($p < 0.001$). In a later study, Dowsett and colleagues examined the prognostic performance of the RS in predicting relapse at a median period of 9 years in patients enrolled in the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial (23). In this study, RS was significantly associated with time to distant relapse in multivariate analysis both for node-negative (HR: 5.25; 95% CI 2.84–9.73; $p < 0.001$) and node-positive disease (HR: 3.47; 95% CI 1.64–7.38; $p = 0.002$). Of note, tumour size was also significantly associated with time to distant relapse in the multivariate analysis in patients with node-negative (HR: 2.78; 95% CI 1.70–4.57; $p < 0.001$) and node-positive (HR: 2.04; 95% CI 1.20–3.48; $p = 0.006$) disease. There was no treatment interaction observed in this study, suggesting that the assay had similar prognostic power in patients treated with anastrozole and tamoxifen. Furthermore, the RS also showed a significant prognostic value beyond that provided by AOL ($p < 0.001$). In another study by the South West Oncology group, RS was found to be prognostic in patients with node-positive BC treated with tamoxifen (HR: 2.64; 95% CI 1.33–5.27; $p = 0.006$) (24). Importantly, the RS was only significant in predicting early relapses (i.e., during the first 5 years) ($p = 0.029$), with no addition prediction beyond 5 years ($p = 0.58$), although the cumulative benefit remained significant at 10 years. This observation was consistent for distant relapse, BC-specific survival, and overall survival.

Interestingly, a meta-analysis of publically available gene expression and clinical data from almost 3000 breast tumours showed similar prognostic performance of these signatures despite the limited overlap of genes (25). Of note, testing with more

than one signature did not appear to improve the prognostic performance. Importantly, tumour size, and lymph node status remained independently prognostic, which highlights the importance of considering the known clinical prognostic parameters even in the era of gene expression signatures. While no one can question the prognostic performance of such signatures, one could argue on the real added value in terms of prediction of overall survival when these signatures are added to the known clinicopathological prognostic tools like AOL, Nottingham Prognostic Index and others. In the ATAC trial, it was clear that the RS added significantly to the prognostic prediction of AOL ($\Delta x^2 = 21.9$, $p < 0.001$), yet the high costs and advanced technology needed to perform such signatures remain a major hurdle. To address this point, Dutch investigators examined the cost effectiveness of the use of MammaPrint compared to the clinically available tools; namely the St. Gallen consensus and AOL (26). For this analysis, they developed a model to compare long-term consequences of the use of the three prognostic tools in patients with node-negative BC. The three strategies were found to be on average equally effective; with the St. Gallen strategy being more costly, followed by the MammaPrint, then the AOL strategy. However, the MammaPrint yielded more quality-adjusted life years (12.44) than the AOL and St. Gallen strategies (12.20 and 11.24). Currently, two large phase III trials, microarray in node-negative and 1 to 3 positive lymph node disease may avoid chemotherapy (MINDACT) and a cancer research trial assigning individualized options for treatment (Rx) (TAILORx), are ongoing to validate the use of such signatures in daily BC management (27,28). They would also address other important technical and analytical issues such as those related to shipping, reproducibility, and standardisation of these new molecular tools.

Hence, a critical look at the prognostic performance of first-generation signatures suggests that they improve the prognostic prediction of patients with early BC, especially those with ER-positive/HER2-negative disease. However, clinical predictors, particularly tumour size and lymph node status, should still be considered in determining patients' prognosis. Another point that deserves emphasis is that although these predictors perform well in identifying early relapses, they fail to predict late relapses (24). This suggests that different molecular mechanisms are likely to be involved during the development of early and late distant metastases (29).

### Second-Generation Signatures

As mentioned earlier, first-generation signatures were developed focusing on epithelial cancer cells but it is arguable that our understanding of the complexity of BC could improve by also considering the role of tumour-surrounding stroma and host-related factors like the immune system. Second-generation signatures were developed aimed at overcoming the drawbacks highlighted earlier with first-generation signatures. These include the ability to predict the prognosis of basal-like and HER2 molecular subtypes and to accurately predict late relapse.

Finak and colleagues isolated tumour stroma and matched normal stroma from breast tumours and derived a 26-gene signature called the stroma-derived prognostic predictor (SDPP) (14). In this study, the prognostic power of SDPP was tested in a multivariate Cox regression model with all clinicopathological prognostic factors across four datasets. The SDPP was highly prognostic independently of ER, HER2, lymph node status, grade, age, and systemic therapy. Interestingly and unlike the first-generation signatures, it was able to predict outcomes in the HER2-positive molecular subtype. The HR for the poor-outcome group identified by the SDPP in the HER2-positive cohorts was, on average, 2.6 times greater than for the whole populations, indicating increased utility of the predictor in this cohort. Furthermore, the SDPP predicted outcome with greater accuracy (75.6%) than MammaPrint (61.0%) and was 5.96 times more likely to identify a true poor-outcome group of patients in the HER2- positive cohort (positive diagnostic likelihood ratio of 6.86 for SDPP vs. 1.15 for MammaPrint).

A Cambridge University team provided some very interesting work in the interrogation of the immune system with the aim to identify a group of ER-negative tumours that had a good prognosis (16). In this study, they identified an immune-response-related 7-gene module and showed that downregulation of this module conferred a greater risk of distant relapse (HR 2.02; 95% CI 1.2–3.4; p = 0.009) in the ER-negative population, which was independent of lymph node status and lymphocytic infiltration. These results were further validated in two independent datasets. These results emphasise the point that ER-negative disease is heterogeneous in terms of expression of complement and genes involved in immune response pathways that help to identify patient subgroups with distinct prognosis. Another group from Germany further confirmed the role of immune signatures in identifying a subgroup among ER-negative tumours with favorable prognosis (17). Furthermore, Yau and co-workers recently reported a 14-gene signature that was able to predict prognosis of patients with triple-negative (basal-like) BC (30). This signature showed positive correlation with three immune-related signatures (STAT1, IFN, and IR), and further analysis identified 8 out of 14 genes as being functionally linked to immune/inflammatory chemokine regulation.

In an attempt to better understand the performance of the different signatures across the different BC subtypes, a meta-analysis conducted by Desmedt and co-workers has shown that stroma and immune signatures are the most relevant in determining clinical outcome in patients with HER2-positive tumours, while for ER-negative/HER2- negative (i.e., basal-like) tumours, only the immune response module is associated with prognosis (11).

Hence, second-generation signatures appear to improve the prognostic power beyond that achieved by first-generation signatures. It must be acknowledged that studies conducted using the second-generation signatures are fewer and require further validation. However, these signatures hold promise in improving prognostication particularly in HER2 and basal molecular subtypes in which first-generation signatures failed to provide prognostic information.

## THE PREDICTIVE VALUE OF GENE EXPRESSION SIGNATURES

Identification of biomarkers to predict response to a particular drug remains an important challenge for oncologists, since commonly used therapeutic agents are ineffective in many patients, and the side effects are frequent and considerable. At present, only two validated predictive biomarkers are used in the clinic: ER and HER2. Despite having an optimal negative predictive value, their positive predictive value is rather limited, and they do not provide information regarding regimen selection in the adjuvant setting. Moreover, their determination shows a substantial variation both within and between laboratories, and thus has a relatively poor reproducibility (31).

In the last years, different investigators attempted to define gene expression signatures that are able to predict response to chemo, endocrine, and targeted therapy. Of note, most of these signatures were developed in the neoadjuvant and adjuvant setting, but their findings could be potentially applicable to patients with advanced disease as well.

### Predicting Response to Chemotherapy

Several chemotherapy regimens are used in the primary treatment of BC. Interestingly, the retrospective application of the different gene signatures previously discussed showed that they are able to assign patients to diverse risk categories that benefit differentially from chemotherapy (32–36).

Paik and colleagues reported a significant interaction between a higher RS and greater benefit to adjuvant cyclophosphamide, methotrexate, and 5-florouracil (CMF)

regimen (test for interaction: $p = 0.038$) (32), suggesting that Oncotype DX could potentially be used to predict response to chemotherapy. Another report similarly showed that GGI is associated with sensitivity to neoadjuvant paclitaxel plus fluorouracil, adriamycin, and cyclophosphamide (T/FAC) chemotherapy in both ER-negative and ER-positive patients (35). However, it has been pointed out that the predictive component of these first-generation signatures relies on their ability to measure proliferation, a biological feature known to be associated with chemosensitivity in BC. This may limit the predictive features of these signatures to the detection of "generic" chemosensitivity rather than to the chemotherapy-regimen-specific sensitivity.

In this regard, Hess and colleagues evaluated gene expression profiling as a potential tool to predict the pathological complete response (pCR) to sequential anthracycline–paclitaxel preoperative chemotherapy (37). Diverse predictors of pCR were developed from 82 patients and their accuracy was validated on 51 independent patients with stage I–III BC treated with (T/FAC) chemotherapy. Among several identified predictors that performed equally well, a 30-probe set Diagonal Linear Discriminant Analysis (DLDA-30) classifier was selected for independent validation. It showed a significantly higher sensitivity (92% vs. 61%) than a clinical predictor including age, grade, and ER status. In a recent publication by the same group (38), the performance of DLDA-30 was evaluated in a prospective, randomised neoadjuvant clinical trial comparing T/FAC and FAC, both given for six cycles. While the assay was predictive of response to T/FAC with an apparent regimen specificity, its performance was similar to that of the clinical prediction model tested in their first study. This suggests that DLDA-30, as other genomic predictors developed with a similar strategy, interrogates mostly gene expression information associated with clinical phenotype (mainly ER, HER2, and proliferation), advocating the need for a different approach to develop clinically useful genomic predictive tools.

### A "Second Generation" of Predictive Signatures

In the past decade, our group led a prospective neoadjuvant clinical trial in which ER-negative BCs were treated with anthracycline monotherapy with the objective to evaluate the predictive value of topoisomerase IIα and to develop a gene expression signature to identify patients who do not benefit from anthracyclines (39). An "anthracycline-based score (A-Score)" was developed that combined three different signatures associated with the efficacy of anthracyclines: a topoisomerase IIα signature, a stroma signature, and an immune-response signature. The "A-Score" turned out to have a high negative predictive value both in the overall population and in the two subgroups of HER2-positive and HER2-negative patients.

Similar to prognostic signatures, the development of a "second-generation" of predictive signatures that are generated in targeted populations and that explore the role of tumour microenvironment (15) or pathway activation (40) is possibly a better way to move forward in defining clinically useful predictive tools.

### Predictors of Response to Endocrine Therapy

Several randomised trials have assessed the value of endocrine therapy in early and advanced stage ER-positive BC (41). As one can expect, numerous investigators have tried to develop gene expression signatures that are able to predict sensitivity or resistance to both tamoxifen and aromatase inhibitors (AIs) (42–44). In a recent study, Symmans and colleagues defined a genomic index for sensitivity to endocrine therapy (SET index) from genes co-expressed with the oestrogen receptor gene (ESR1) (44). They hypothesised that the measurement of gene expression related to ER within a BC sample represents intrinsic tumour sensitivity to adjuvant endocrine therapy. The association of SET index and ESR1 levels with distant relapse risk was evaluated in 437 microarray profiles of newly diagnosed ER-positive BC. Several cohorts were

included, including a group which received 5 years of adjuvant tamoxifen and another group which received neoadjuvant chemotherapy followed by tamoxifen and/or AI. This is in addition to two cohorts which received no adjuvant systemic therapy. The SET index (165 genes) was found to be significantly associated with the risk of distant relapse and death in both tamoxifen-treated and chemo-endocrine–treated cohorts independently from pathological response to chemotherapy. Yet, it was not prognostic in the two untreated cohorts. No distant relapse or death was observed after tamoxifen treatment if node-negative and high SET index or after chemo-endocrine therapy if intermediate or high SET index.

## ALTERNATIVE STRATEGIES TO DEFINE MULTIGENE PREDICTORS

### Development of *In Vitro* Signature Analysis

An alternative "associative" strategy that has been used to generate predictive multigene assays derives from *in vitro* signature analysis. In this approach, gene expression data and *in vitro* drug response information from cell line panels are used to generate drug-specific associative pharmacogenomic response predictors that can be applied to human data (45). However, several investigators failed to reproduce in humans the discriminating power of purely cell line derived drug-specific predictors (46–48). This highlights the difficulties of associative analyses that do not interrogate gene function, deriving from cell line models, to capture patient-related differences in drug metabolism and the influence of tumour microenvironment in response to treatment.

### RNA Interference Technology

Recently, the use of the RNA interference (RNAi) technology has allowed the identification of genes influencing resistance and sensitivity to diverse cytotoxic drugs used in clinical practice (49–51). Starting from a small number of overexpressed and amplified genes from chromosome 8q22 significantly associated with early disease recurrence despite anthracycline-based adjuvant chemotherapy and using RNAi knockdown, Li and colleagues were able to identify two genes (YWHAZ and LAPTM4B) which sensitized tumour cells to anthracyclines when either was depleted (50). The overexpression of either of them was on the contrary associated with drug resistance. Of note, these functional genomic data could be combined with other molecular data, such as gene expression signatures, and increase their strength (52).

Finally, a kinome RNAi screen recently identified a ceramide and a mitotic module that influenced response to paclitaxel across multiple cell lines, including an ER-negative BC cell line. This module of six genes, called "functional metagene," was tested in two retrospective cohorts of ER-negative patients treated with T/FAC neoadjuvant chemotherapy. The functional metagene was shown to predict pCR to paclitaxel-specific regimens but not regimens that did not contain a paclitaxel backbone (49).

## UNRESOLVED ISSUES AND FUTURE PERSPECTIVES

It is worthy of note that advances in molecular technologies in the last years allowed the identification of different molecular events, such as DNA mutation and chromosomal rearrangements, which could influence the response to cancer treatment (53,54). Lately, several investigators have focused on the influence of epigenetic modifications on BC behaviour and came up with epigenetic signatures that could potentially be combined with gene expression signatures and improve their performance (55).

Despite the promises, none of the signatures generated so far have been approved for use in the clinical setting. The confidence in the results obtained remains

limited given the small sample sizes and multiple comparisons (56). In addition, most of the available studies have been carried out in unselected BC populations. If different molecular classes have different sensitivity to chemotherapy, using data from "all comers" will likely yield predictors that primarily discriminate between molecular classes and have less strength to predict response within a class. Hence, properly powered studies with innovative design in a clearly defined patient population will likely provide more robust conclusions on the predictive validity of such signatures in the clinic. The adoption of an integrative approach that takes into consideration the complex interplay of factors involved in response to therapy, which might include functional RNA interference approaches, could contribute immensely to the development of a new class of predictive signatures with clinical impact.

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# 3 Endocrine therapy for advanced disease

**Stephen R. D. Johnston**

## INTRODUCTION

In the United Kingdom, breast cancer affects up to 1 in 8 women during their lifetime; with an annual incidence that has now reached more than 41,000, the death rate is approximately 12,000 per year (1). Approximately 5–10% of newly diagnosed breast cancer patients have locally advanced/metastatic disease at the outset, and 20–70% of patients (depending on their tumour biology, initial stage of disease and subsequent therapy) will develop recurrent/metastatic disease in the future. It is estimated that in the United Kingdom over 100,000 women are living with advanced/metastatic breast cancer (MBC) at any one time. Once the metastatic disease is diagnosed it cannot be cured, and the overall median survival from the time metastatic disease is confirmed is between 2 and 3 years.

The optimal management of patients with metastatic disease remains a challenge, with systemic drug treatments such as chemotherapy, endocrine therapy, biological targeted therapy, and supportive therapies being the mainstay of care. The decision as to which is the most appropriate treatment option is based on a number of patient- and disease-related factors. Approximately two-thirds of human breast carcinomas express oestrogen receptors (ERs) and thus may be dependent on oestrogen for their growth, and for patients in whom their breast cancer (either primary tumour or biopsy of accessible metastatic disease) is positive for ER and/or progesterone receptor (PgR) endocrine therapy is an important treatment option with minimal toxicity. For patients with ER-/PgR-positive breast cancer and an estimated low risk of rapid progression of their advanced disease (i.e., soft tissue and/or bone metastasis as their dominant site, absence of life threatening visceral involvement, disease-free interval greater than 2 years, and limited sites of metastatic involvement), endocrine therapies can be very effective in the treatment of their advanced/metastatic disease (Table 1). For example, locally advanced ER-positive disease within the breast of elderly women is often slow growing and extremely hormone sensitive. Excellent clinical responses can be achieved with simple well-tolerated endocrine therapy such as tamoxifen, albeit maximal response and tumour shrinkage may take between 6 and 9 months to occur (Fig. 1A, B). However, sites of visceral metastases such as the liver may also respond well to endocrine therapy provided an appropriate selection of patients is undertaken. For example, post-menopausal patients with strongly ER/PgR-positive disease with a long treatment-free interval of many years after completion of adjuvant tamoxifen, may then develop metastatic disease within the liver but with a limited number of tumours and preserved organ function (i.e., normal liver function tests), lack of any symptoms from their advanced disease, and a good overall performance status. Such patients can have an excellent clinical response to endocrine therapy alone with, for example, aromatase inhibitors (AIs), which may last for 18–24 months before their disease progresses and patients require chemotherapy (Fig. 1C, D). Therefore, appropriate selection of patients that are suitable for initial endocrine therapy is therefore crucially important in order to maximise the benefits from such treatments.

In this chapter the evidence for the current endocrine therapy options that are available for advanced disease are reviewed in more detail, together with the emerging strategies that might be used in future to further enhance their effectiveness.

**TABLE 1** Clinical Parameters Utilised in Decision Making Regarding Systemic Therapy Options in Advanced Breast Cancer

| |
|---|
| Patient Factors |
| Age |
| Menopausal status |
| Performance status |
| Severity and nature of symptoms |
| Presence/absence of visceral disease |
| Prior adjuvant systemic therapies |
| Organ function (i.e., liver/renal functions) |
| Disease-Related Factors |
| Tumour biology (ER/PgR status; HER2 status) |
| Duration of treatment-free period (i.e., sensitive vs. resistant disease) |
| Dominant site of disease (i.e., bone/soft tissue vs. visceral metastasis) |
| Number of sites of metastasis |
| Tumour burden |

*Abbreviations*: ER, oestrogen receptor; PgR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

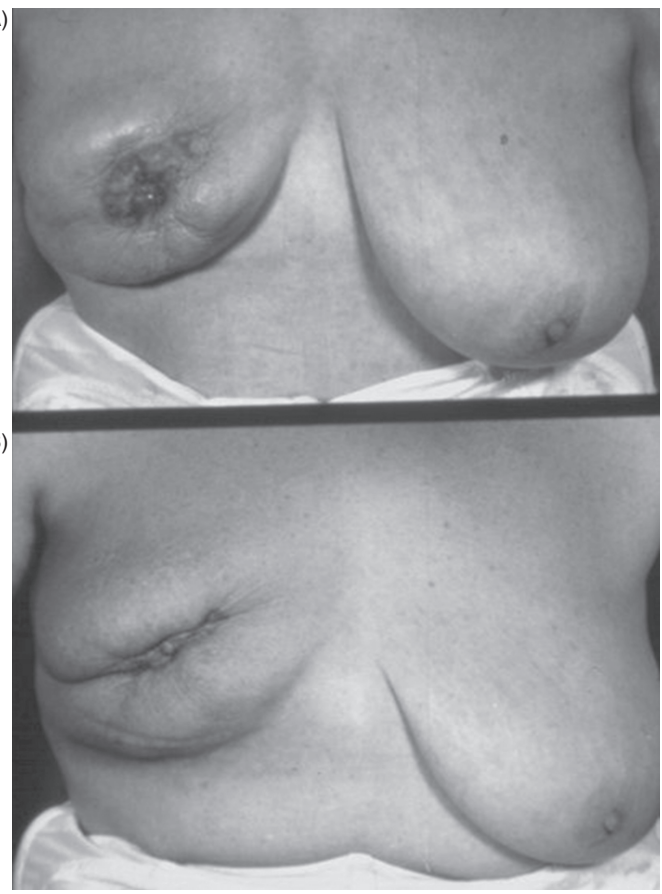


**FIGURE 1** Locally advanced disease of the breast before (**A**) and 6 months after (**B**) therapy with tamoxifen, showing a substantial tumour shrinkage. Metastatic disease within the liver with three isolated tumours developing many years after prior adjuvant tamoxifen. (*Continued*)

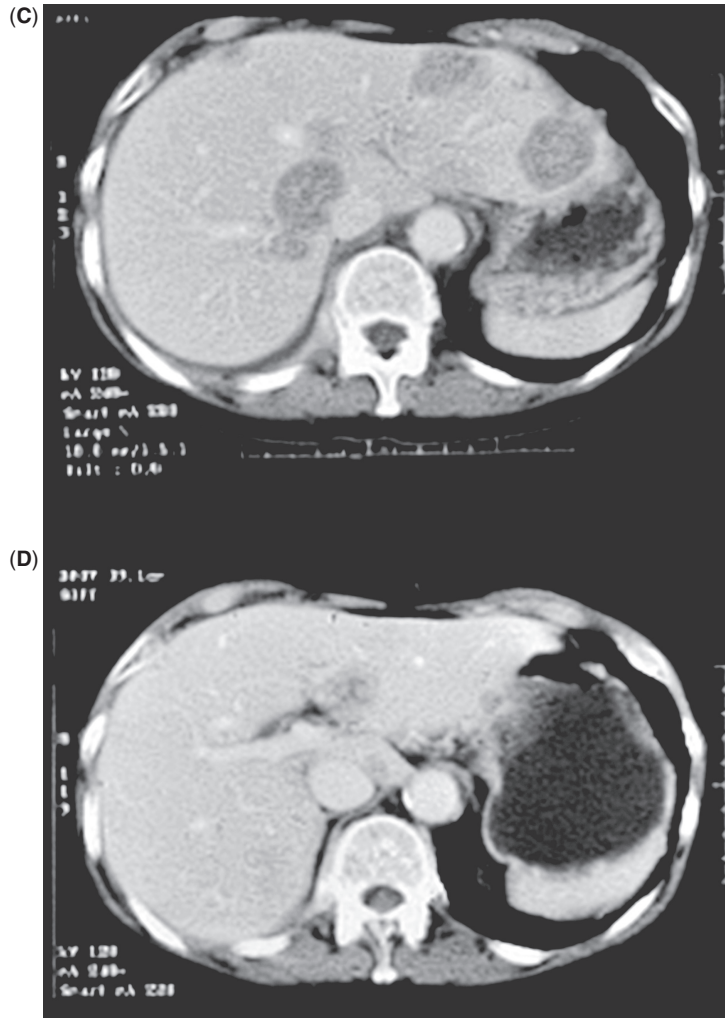


**FIGURE 1** (*Continued*) Before (**C**) and after (**D**) 6 months, therapy with an aromatase inhibitor.

## ENDOCRINE THERAPY OPTIONS FOR MBC

Historically, tamoxifen has been the approved "gold standard" endocrine therapy for the treatment of MBC, both of pre- and postmenopausal women. Tamoxifen is a non-steroidal ER antagonist which inhibits breast cancer growth by the competitive antagonism of oestrogen at the receptor site (Fig. 2). However, its actions are complex due to partial oestrogenic agonist effects which in some tissues (i.e., bone) can be beneficial (2), but in others may be harmful increasing the risk of thromboembolism and uterine cancer (3). Although this being an effective treatment for advanced breast cancer, the partial agonist effects may account for the development of tamoxifen resistance after prolonged treatment. Furthermore, the majority of women with ER-positive breast cancer who then develop metastatic disease have already been treated with tamoxifen in the adjuvant setting. In the past, tamoxifen therapy was used again if tamoxifen had been stopped several years previously, but now alternative endocrine approaches that deprive tumours of circulating oestrogens are utilised in preference.

Within the last 5 years third-generation potent oral AIs have become a standard treatment option for postmenopausal patients with ER-positive advanced/metastatic breast cancer. Oral AIs such as anastrozole (Arimidex™), letrozole (Femara™), and exemestane (Aromasin™) all reduce serum oestrogen levels in postmenopausal women by preventing the conversion of adrenal androgens into oestrogens (Fig. 2). Oestrogens are normally synthesised in the ovary in premenopausal women, but following the menopause, mean plasma oestradiol (E2) levels fall from about 400–600 pmol/L to around 25–50 pmol/L. These residual oestrogens come solely from peripheral aromatase conversion, particularly in subcutaneous fat, and plasma E2 levels correlate with body mass index in postmenopausal women (4). As discussed below, in postmenopausal women with advanced breast cancer several clinical trials have demonstrated that AIs are more effective and better tolerated than tamoxifen as first-line management of MBC. Since the late 1990s AIs have become the new "gold standard" for first-line endocrine treatment in postmenopausal women with advanced breast cancer.

For premenopausal women with ER-positive advanced breast cancer, oestrogen deprivation through ovarian ablation has been the main endocrine approach when tamoxifen has been used previously in the adjuvant setting. This can be achieved either by surgical oophorectomy, radiation of the ovaries, or medical ablation with luteinising hormone-releasing hormone (LHRH) agonists such as goserelin (Zoladex™) (Fig. 2). Such an approach can be effective in premenopausal women with endocrine-sensitive advanced disease, and at the time of further progression the addition of AIs to LHRH agonists has been a successful additional second-line option.

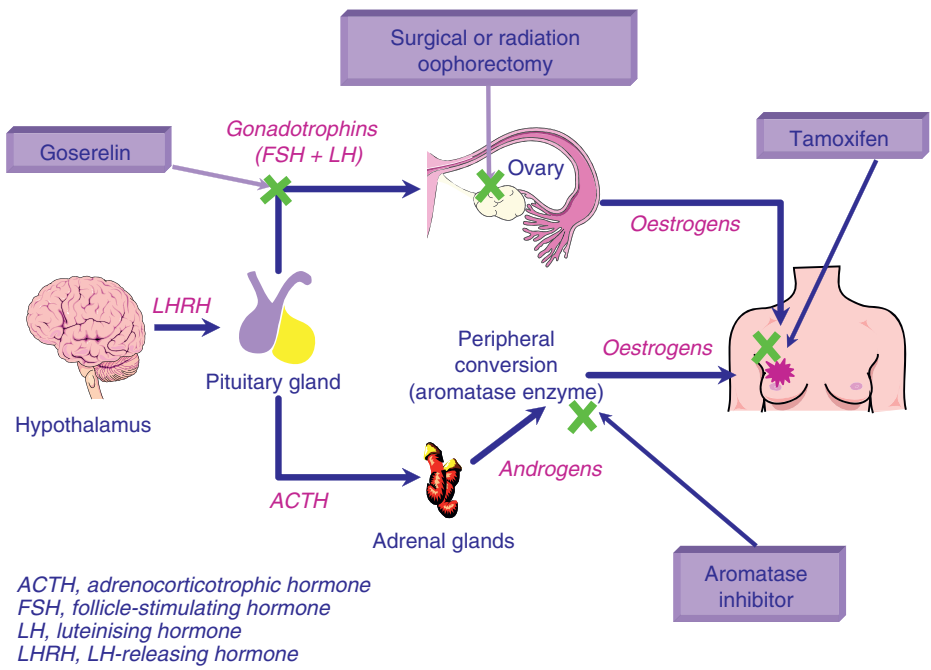


**FIGURE 2** Source of oestrogens in pre- and postmenopausal women, together with endocrine therapy options to either antagonise oestrogens (tamoxifen), or induce oestrogen deprivation via aromatase inhibition (postmenopausal) or ovarian ablation (premenopausal) via surgical, radiation, or medical means.

As discussed below, for women initially presenting with endocrine-sensitive advanced disease who have not received prior tamoxifen, tamoxifen combined with LHRH agonists appears to be a more effective strategy than tamoxifen alone.

Recently oestrogen suppressive therapies with either AIs or LHRH agonists have started to move into the adjuvant setting for post- and premenopausal women, respectively. This has led to new questions about the optimal sequence of endocrine therapies for subsequent use in advanced disease. The ER downregulator fulvestrant (Faslodex™) is a novel treatment option for women with progressive disease following prior tamoxifen therapy, and current trials are investigating whether fulvestrant is a suitable treatment option for postmenopausal women following progression with an AI. Research in endocrine therapy has been focusing on understanding the mechanisms of acquired resistance and the molecular pathways which allow ER-positive cells to escape from endocrine therapy. As discussed at the end of this chapter, several new strategies that combine endocrine therapies with various signal transduction inhibitors are now being investigated in ongoing clinical trials in advanced breast cancer. The ultimate goal will be to overcome and/or prevent the development of endocrine resistance in ER-positive breast cancer, and thus further enhance the benefits of existing endocrine therapy.

## CLINICAL EFFICACY OF AIS IN ADVANCED BREAST CANCER

### Pharmacology

Anastrozole and letrozole are third-generation non-steroidal AIs that have similar pharmacokinetics with half-lives of approximately 48 hours allowing a once-daily schedule (5,6). Exemestane is a steroidal aromatase inactivator with a longer half-life of 27 hours (7) (Fig. 3). All three compounds are orally active, reducing serum oestrogen levels in postmenopausal women by preventing conversion of adrenal androgens (androstenedione and testosterone) into oestradiol (E1) and oestrone (E2) via the cytochrome P450 enzyme aromatase. Based on the clinical trials outlined below, all

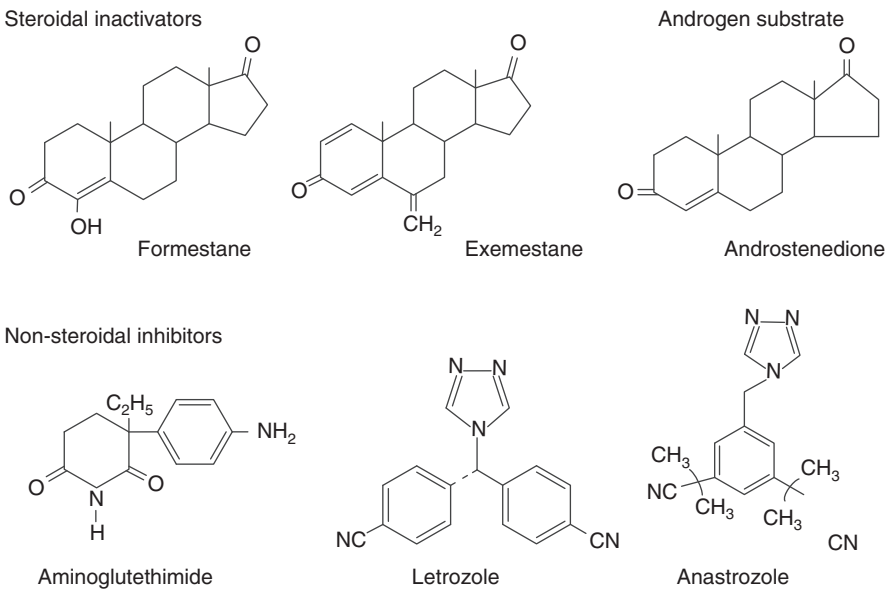


**FIGURE 3** Structures of steroidal and non-steroidal aromatase inhibitors.

three AIs are licensed and approved as endocrine treatment for postmenopausal women with ER-positive advanced breast cancer.

### Second-Line Therapy Post Tamoxifen

Between 1995 and 2000 the three third-generation AIs established themselves clinically when a series of randomised controlled trials (RCTs) in over 2000 women demonstrated clinical superiority over megestrol acetate (MA) as second-line therapy after tamoxifen (8–13) (Table 2). An analysis of two randomised phase III trials of 764 patients treated with either anastrozole or MA as second-line therapy after tamoxifen failure demonstrated an equivalent efficacy in terms of objective response rates (10.3% and 7.9%, respectively) and disease stabilisation for 6 months (25.1% and 26.1 %, respectively), although showed a better tolerability for anastrozole (8). A subsequent analysis following a median of 31 months follow-up showed a significant improvement in overall survival for anastrozole (hazard ratio (HR) 0.78, p = 0.02) (9). For letrozole, improvements were seen in objective tumour response rate (HR 1.82, p = 0.04) and time to treatment failure compared with MA, although no impact on survival was detected (10). In the trial with exemestane duration of objective response, time to disease progression, and overall survival were all significantly better than with MA (11). A subsequent second trial of letrozole (12), together with a study or the AI vorozole (no longer in development) (13) showed less substantial improvements over MA.

This was in contrast with previous trials with the second-generation inhibitors, fadrozole and formestane, which had all failed to show any such advantage (14,15).

**TABLE 2** Comparative Second-Line Trials of Third-Generation Aromatase Inhibitors vs. Megestrol Acetate

| Author | Comparators | n | Response (%) | Clinical benefit (%)[a] | Median time to progression (months) | Median overall survival (months) |
|---|---|---|---|---|---|---|
| Buzdar et al. (8,9) | Anastrozole 1 mg | 263 | 13 | 42 | | 27[b] |
| | Megestrol acetate | 253 | 12 | 40 | | 23[b] |
| Dombernowsky et al. (10) | Letrozole 2.5 mg | 174 | 24[b] | 35 | 5.6 | 25 |
| | Megestrol acetate | 189 | 16[b] | 32 | 5.5 | 22 |
| Buzdar et al. (12) | Letrozole 2.5 mg | 199 | 32 | 53 | 3.0 | 29 |
| | Megestrol acetate | 201 | 30 | 47 | 3.0 | 26 |
| Kaufmann et al. (11) | Exemestane 25 mg | 336 | 15 | 37 | 4.7[b] | Not reached[b] |
| | Megestrol acetate | 403 | 12 | 35 | 3.8[b] | 28[b] |
| Goss et al. (13) | Vorozole 2.5 g | 225 | 11 | | 2.7 | 26 |
| | Megestrol acetate | 227 | 8 | | 3.6 | 29 |

[a]Defined as the total percentage of patients responding or achieving stable disease for at least 6 months.
[b]Significant difference.

The improvements in clinical endpoints for the third-generation AIs, together with their consistent superior tolerability profile over MA (i.e., reduced weight gain and thromboembolic events), defined the AIs by the late 1990s as the standard endocrine treatment for advanced postmenopausal breast cancer following tamoxifen failure (16). In practice, however, developments in first-line endocrine therapy rapidly diminished the clinical relevance of these findings.

### First-Line Therapy vs. Tamoxifen

Subsequent trials in advanced breast cancer questioned whether AIs could challenge tamoxifen as the first-line endocrine agent of choice. Previously, no first- or second-generation AI had proved superior to tamoxifen (17–19). In addition to comparing tolerability, the potential of these studies with the new third-generation AIs was to see whether the nearly complete oestrogen blockade provided by these drugs could deliver greater control of hormone-sensitive breast cancer than tamoxifen, thus circumventing the problem of acquired resistance due to the partial agonist effects of tamoxifen (20).

The first published data came from two parallel multi-centre double-blind RCTs in which anastrozole was compared with tamoxifen as first-line therapy in ER-positive breast cancer (Table 3). The first study in 353 women showed that anastrozole significantly prolonged the time to disease progression from 5.6 to 11.1 months ($p = 0.005$) (21). While there was no significant difference in objective tumour response rate (21% anastrozole vs. 17% tamoxifen), the clinical benefit rate (defined as the proportion of patients who responded or had stable disease for at least 6 months) was significantly better for anastrozole (59% vs. 46%). By contrast, in the larger trial with 668 patients no difference was found between the treatments in terms of median time to progression [(TTP) 8.2 vs. 8.3 months], response rate (33% both arms), or clinical benefit rate (56% both arms) (22). The explanation for the different results may have involved a higher proportion of patients with unknown ER status in the second trial, and a subsequent combined analysis of women with just ER-positive disease from both trials confirmed a significant improvement in disease-free survival in favour of anastrozole (23). Short-term side effects such as hot flashes, vaginal dryness, and headaches were infrequent and similar in both trials in comparison with tamoxifen.

The largest single trial was conducted with letrozole in comparison with tamoxifen in over 900 women with advanced breast cancer (24). Patients treated with

**TABLE 3** Comparative First-Line Trials of Aromatase Inhibitors vs. Tamoxifen

| Author | Comparators | n | Response % | Clinical benefit %[a] | Median time to progression (months) |
|---|---|---|---|---|---|
| Nabholtz et al. (21) | Anastrozole | 171 | 21 | 59[b] | 11.1[b] |
| | Tamoxifen | 182 | 17 | 46 | 5.6 |
| Bonneterre et al. (22,23) | Anastrozole | 340 | 33 | 56 | 8.2 |
| | Tamoxifen | 328 | 33 | 56 | 8.3 |
| Mouridsen et al. (24,25) | Letrozole | 453 | 30[b] | 49[b] | 9.4[b] |
| | Tamoxifen | 454 | 20[b] | 38 | 6.0 |
| Paridaens et al. (26) | Exemestane | 182 | 46[b] | 66[b] | 9.9[b] |
| | Tamoxifen | 189 | 31 | 49 | 5.8 |

[a]Defined as the total percentage of patients responding or achieving stable disease for at least 6 months.

[b]Significant difference vs. tamoxifen.

letrozole had a significantly higher objective tumour response rate (30% vs. 20%, $p < 0.001$), clinical benefit rate (49% vs. 38%, $p < 0.001$), and prolonged time to disease progression (TTP) (median TTP of 9.4 months vs. 6.0 months, HR 0.72, $p < 0.0001$). Of particular note in this trial, nearly 20% patients had received tamoxifen prior in the adjuvant setting, although had ceased more than a year (median 3 years) prior to the development of metastatic disease; in this subgroup, re-treatment with tamoxifen had a low response rate of 8% compared with a 32% response rate with letrozole. The improvements in clinical efficacy for letrozole resulted in an early improvement in survival during the first 2 years, with overall 64% of patients treated with letrozole alive at 2 years compared with 58% treated with tamoxifen ($p = 0.02$) (25), although with a longer follow-up this difference was lost. The explanation for this may relate to the high number (>50%) of patients who prospectively crossed over to the alternate treatment at the time of progression, as significantly more patients benefited from second-line letrozole after progression on tamoxifen than from second-line tamoxifen after letrozole. Again, there were no significant differences in toxicity between the two treatments.

Finally, a large European study in 383 patients has compared the efficacy and tolerability of the steroidal aromatase inactivator exemestane with tamoxifen as first-line therapy (26). After a median follow-up of 29 months, there was an improvement in progression-free survival from 5.8 months for tamoxifen to 9.9 months for exemestane (HR 0.84, $p = 0.028$ by Wilcoxon sensitivity test). There was a significantly higher objective response rate (ORR) with exemestane than tamoxifen (46% vs. 31%, ORR 1.85, $p = 0.005$). Likewise the clinical benefit rate was significantly higher (66% vs. 49%). Both treatments were well tolerated, with more grade 1 myalgia in the exemestane treated group, and more grade 2 edema, grade 1 hot flashes, vaginal bleeding, and sweating in the tamoxifen group.

Thus, the available data from the four RCTs of the inhibitors in advanced disease suggest consistent improved efficacy over tamoxifen, and as such all are approved as first-line endocrine therapy for post-menopausal women with ER-positive advanced breast cancer, especially where prior adjuvant endocrine therapy was with tamoxifen. Since 2001, the third-generation AIs have become the standard of care as first-line endocrine therapy in this setting.

### Tolerability in Advanced Disease

All the third-generation AIs are in general very well tolerated with a remarkably low incidence of serious short-term side effects, reflecting the extreme specificity of their action. The commonest include hot flashes, vaginal dryness, musculoskeletal stiffness/pain and headache, but are usually mild. Comparative trials in general show these to be very similar in nature and frequency to those of tamoxifen, and less troublesome than with the progestins. A better indication of the drug-specific side effects, particularly the long-term effects of AIs on bone and cognition over many years, has come from large-scale adjuvant trials. Furthermore, unlike the advanced breast cancer studies these adjuvant trials are not confounded by tumour-related symptoms and have reported that patients treated with AIs had a significantly lower incidence of hot flashes, vaginal bleeding, vaginal discharge, weight gain, and venous thromboembolism than with tamoxifen. However, musculoskeletal symptoms and fractures were more common than with tamoxifen.

### Comparisons Between Different Third-Generation AIs in Advanced Disease

Letrozole achieved greater aromatase inhibition than anastrozole in a cross-over pharmacodynamic trial (27), and the clinical data for its superiority over tamoxifen in advanced disease are more solid. Preliminary data from a comparative trial of these two inhibitors in advanced breast cancer after tamoxifen are confusing, with letrozole

achieving significantly more regressions overall than anastrozole, but not in the key subgroup with known ER-positive tumours (28). Overall current clinical evidence suggests that there are unlikely to be major direct clinical differences among the different AIs in advanced disease. There are no comparative data for exemestane with anastrozole or letrozole, although as discussed below further responses have been reported for this drug and the second-generation inhibitor formestane in patients relapsing after anastrozole, letrozole, or the other non-steroidal inhibitors suggesting a partial non-cross resistance (29,30).

## POSTMENOPAUSAL SECOND-LINE TREATMENT OPTIONS POST AIS

It has become important to develop effective endocrine therapies that will work following non-steroidal AIs, and to date clinical options have included treatment with tamoxifen (especially if this had not been used prior to the AI), use of the steroidal aromatase inactivator exemestane based on phase II data suggesting non-cross resistance), or the ER downregulator fulvestrant based on its novel endocrine mechanism of action. Likewise, other endocrine approaches including progestins, corticosteroids, oestrogens, and inhibitors of androgen biosyntheses are being evaluated. Evidence for each of these seven approaches of further endocrine therapies in advanced disease is reviewed below.

### Tamoxifen Following Prior Non-Steroidal AIs

There are few prospective data to show the true efficacy of tamoxifen in those who had progressed on a non-steroidal AI (i.e., anastrozole or letrozole). The largest available data come from the letrozole versus tamoxifen study where over 50% of the patients prospectively crossed over to an alternative treatment at the time of progression (25). Median overall survival from the date of cross-over was 19 months for patients who crossed to second-line tamoxifen, compared with 31 months for patients who crossed to second-line letrozole. The only other data come from retrospective questionnaire data from the combined analysis of the two international phase III anastrozole versus tamoxifen TARGET trials (21,22). This analysis suggested that of the 119 patients who went on to receive tamoxifen following progression on anastrozole, 58 (49%) derived clinical benefit and 12 (10%) had an objective response (31). A subsequent double-blind crossover study by the Swiss centres in the TARGET trial (SAKK 21/95 sub-trial) further investigated the clinical impact of the sequence anastrozole followed by tamoxifen, and reported that 8 of the 16 (50%) derived clinical benefit from tamoxifen (32). Thus, tamoxifen may have some efficacy as second-line therapy after AI therapy. However, data are sparse to confidently determine the optimal sequence. Furthermore, preclinical studies (discussed below) suggest that tamoxifen may be an agonist in cells resistance to long-term oestrogen deprivation (LTED), and more effective endocrine/signalling strategies may exist for use following failure of first-line AI therapy.

While the clinical data with the third-generation AIs suggest they are more effective if given as first-line therapy for advanced breast cancer; they are more expensive and in some health-care systems will only gain greater acceptance if they can also demonstrate cost effectiveness. Life table analyses have been used to compare the costs and benefits of treating post-menopausal women with advanced breast cancer with the first-line AI letrozole with the option of second-line tamoxifen, compared with first-line use with tamoxifen with the option of second-line letrozole. The results of a U.K.-based analysis showed that the mean cost of providing first- and second-line hormonal therapy was £4765 if letrozole was first-line therapy, compared with £3418 if tamoxifen was provided first (a difference of £1347) (33). However, patients who received letrozole as first-line therapy gained an additional 0.228 life