Optimizing the Tailored Treatment of Breast Cancer

By

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Graduate Institute of Health Policy, Management and Evaluation

University of Toronto

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Abstract

Background: Breast cancer is a diverse disease. Over the past 3 decades it has been increasingly appreciated that therapy should be targeted to specific patient and tumour characteristics. In recent years the evaluation of tailored therapy has been dominated by the development of new drug therapy which when successful has been marketed at a high price. There have been few successful attempts to optimize currently available therapies. This thesis explores the optimization of currently available therapies in three domains: efficacy, toxicity and supportive care.

Methods: Three independent studies were undertaken. First, a prospective cohort study was conducted to assess the impact of re-biopsy of recurrent breast cancer on physician choice of therapy and on patient satisfaction. The second study comprised a systematic review and meta-analysis of randomized trials exploring toxicities associated with different endocrine therapy options for early breast cancer with the aim of identification of patients who may be harmed by certain drugs. Finally, a randomized feasibility study was conducted to evaluate de-escalated intravenous bisphosphonates in women with low-risk metastatic breast cancer to bone.
Results: All studies met their objectives in showing that the tailored use of available therapies can be optimized. The prospective study of the impact of re-biopsy showed that treatment decisions were modified in 14% of women. Patient satisfaction with the process of re-biopsy was high. The meta-analysis of toxicities of endocrine therapy identified cardiovascular disease as a statistically significant toxicity of aromatase inhibitors, thereby suggesting that those with established cardiovascular disease or risk factors thereof should reduce their exposure to these drugs. Finally, the randomized feasibility study showed that it is possible to conduct randomized trials of de-escalated bisphosphonates in women with low-risk breast cancer and there was no signal that reducing the frequency of treatment was associated with untoward outcomes.

Conclusions: It is possible to optimize the tailored therapy of breast cancer using currently available treatments. This may lead to improved patient outcome while using existing resources. Further studies assessing the optimization of other treatments are warranted.
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Chapter 1: Introduction

I: Thesis Objectives

Breast cancer is a biologically diverse disease. In recent years there have been increasing attempts to tailor therapy to individual patient and tumour characteristics in order to improve the therapeutic index of treatments. However, most of the emphasis of this personalized medicine has centred on the development of novel therapeutics with little research being conducted to assess optimization of available therapies. The objectives of this thesis were therefore to conduct studies exploring methods to optimize the tailored use of available treatment in breast cancer in three separate domains: efficacy, toxicity and supportive care. These studies draw on several methods relating to clinical epidemiology including conduct of cohort and randomized studies, issues relating to recruitment and retention of subjects in prospective trials and finally the use of systematic reviews and meta-analysis.

II: Background

The first part of this chapter will provide an overview of the epidemiology of breast cancer and the history of clinical trials evaluating tailored therapies for this disease. It will also address problems in clinical trial methodology, with emphasis on
novel drug development, which has been suggested as being one explanation for the high cost of drugs when marketed\(^1\).

**A: Breast Cancer Epidemiology and Treatment**

Worldwide, breast cancer is the most common malignancy in women with an incidence of almost 1.4 million women each year\(^2\). In 2011, an estimated 23,400 Canadian women will be diagnosed with breast cancer and 5,100 will die of it\(^2\). Survival rates after breast cancer are prolonged and therefore, breast cancer is the most prevalent malignancy among Canadians accounting for over 20% of the ten-year prevalent cancer cases in Canada\(^3\).

The diagnosis of breast cancer has a substantial impact on healthcare utilization. Most patients are treated with surgery and many subsequently receive cytotoxic chemotherapy and endocrine therapy\(^4,5\). In the clinical setting, breast cancer is made up of a series of clinically distinct subsets, each with different treatment options and variable natural course. The important biologic differences between breast cancer subsets reflect the expression of the canonical biomarkers—estrogen receptor (ER), progesterone receptor (PgR), and the human epidermal growth factor receptor-2 (HER2). These receptors define the treatment options for both early and advanced disease. Patients with tumours that express ER or PR are usually treated with antiestrogen therapies, whereas patients with tumours that either have receptor overexpression or gene amplification of HER2 being treated with anti-HER2 therapies.
Patients with tumours lacking all three markers, so-called “triple-negative” breast cancers are typically candidates for chemotherapy alone.

Despite increased numbers of breast cancer diagnoses, the proportion of breast cancer patients dying of the disease has fallen, likely due to the combined effects of increased breast cancer awareness, the establishment of population-based screening for early disease as well as advances in systemic and loco-regional therapy\(^2\). A woman diagnosed with breast cancer in the developed world now has an 80% chance of remaining disease free for the rest of her life\(^2\). The recognition that breast cancer survivors are now more likely to die of other causes and the likelihood that breast cancer therapy may increase the risk of developing diseases which are common causes of death has led to some focus being shifted to the short and long-term toxicities of breast cancer therapy.

\(B: \text{Current Knowledge Base}\)

In order to provide patients with high quality treatment, clinicians need to translate the results of clinical trials in to practice. Part of this process needs to include the assessment of the quality of the studies’ methodology and execution, and the generalizability of the trials’ results. Prior studies have shown that in the last 20 years there has been an increase in the number of trials in oncology and that the sample size of these trials has increased\(^6;7\). There has also been both relative and absolute increases in sponsorship of clinical trials by the pharmaceutical industry\(^8\). This finding is of some
concern especially since authors of trials with for-profit sponsorship are more likely to strongly endorse novel therapies\textsuperscript{9}. Adding to this concern is the finding that over the same period of time, the magnitude of absolute benefit from treatment has become smaller\textsuperscript{10}.

Since 1975, randomized trials of new therapies in common tumours such as breast cancer and colorectal cancer have shown decreasing absolute benefits\textsuperscript{10}. These findings were consistent regardless of whether absolute benefits were measured as absolute differences in survival at pre-set cut-offs (such as differences in median survival or in the proportion of patients surviving at 12-month) or as the average duration of life gained. In trials conducted in patients with metastatic disease, new and expensive treatments have generally failed to show improvements in survival despite rapidly increasing costs\textsuperscript{10;11}. This rate of healthcare inflation is unsustainable\textsuperscript{12}.

A number of possible explanations may underlie these findings. First, experimental therapy in clinical trials is now more likely to be compared to active therapy rather than placebo or best supportive care. Second, progress in the early detection of cancers has led to a stage shift where most cancers are diagnosed at earlier stages where prognosis is improved regardless of any administered therapy. Consequently, the impact of any treatment is likely to have a smaller absolute magnitude. Third, there has been little progress in the development of accurate predictive factors for both benefit and for toxicity. Therefore, suboptimal patient
selection may have resulted in some experimental therapies appearing to have modest or no benefit in whole trial populations despite showing substantial activity in sub-groups of patients. Finally, the cost of anti-cancer drug treatment is not related to the benefit of the drug and is likely driven by the potential for maximizing profit margins for pharmaceutical companies\textsuperscript{13}. This is especially concerning as modern drug development is reliant on industry with around 70\% of all randomized trials in oncology now being sponsored by pharmaceutical companies\textsuperscript{14}.

\textit{C: Limitations of current data evaluating individualized breast cancer therapy}

In recent years, a number of late-stage clinical trials that have evaluated molecular targeted agents in an unselected population with common cancers have failed\textsuperscript{15}. In breast cancer examples include the anti-angiogenic drugs bevacizumab (reviewed in \textsuperscript{16}) and sunitinib\textsuperscript{17} as well as the bone agent zoledronic acid\textsuperscript{18}. It has been estimated that almost a third of all phase III trial failures occur with cancer drugs\textsuperscript{19}. In many trials responses were seen in a proportion of treated patients, but \textit{a priori} identification of such patients was not possible due to the lack of robust and validated biomarkers for this purpose. This observation has been a major limitation of drug development in cancer. Furthermore, a generalized lack of reporting of rare, but potentially serious toxicities of cancer drugs means that clinicians and patients are often not adequately informed of potential harms of therapy\textsuperscript{20}. As a result, cancer treatments are often not optimized for an individual patient. Improving the ability to individualize
therapy has potential advantages. First, it is likely to improve patient outcomes since specific therapies would be given to those who are more likely to derive benefit from them and not to those for whom any modest benefit is balanced by increased toxicity or even net harm. This improvement might also be derived from currently available therapy rather than relying on expensive new drug development. Second, assuming that predictive markers have good discriminatory accuracy, their effective application could allow for the more cost-effective use of treatments.

III: Overview of the Thesis

The first study is a cohort study assessing whether re-biopsy of recurrent disease and re-analysis of predictive biomarkers changes the choice of currently available therapy in metastatic breast cancer compared with the assessment of these markers from the primary breast tumour. The second study is a meta-analysis of randomized trials exploring toxicities from adjuvant endocrine therapy in early breast cancer to help identify patients who will develop differential toxicity from currently available endocrine therapy thereby allowing such therapies to be avoided. Finally, the third study is a randomized feasibility trial evaluating whether the supportive care agent pamidronate can be given less frequently than the standard monthly schedule in patients with low-risk bone metastases. It is hoped these studies will provide data to better inform clinicians about optimal strategies to individualize therapy in breast cancer.
IV: Methodological Considerations

The first study used a simple pre-post cohort design. In this design, the control population comprises patients prior to their biopsy and the comparison group is the same population as initially defined, but measured post-biopsy. In this design, there is no equivalent reference population. Such a quasi-experimental design has relatively good internal validity, but in achieving this sacrifices external validity. For example, the process of pre-testing may influence the result of the post-test and can exaggerate the observed effect. Other limitations include the effects of differential testing or regression to the mean. However, these were not felt to be of importance in the clinical setting which this study was conducted. Pre-post cohort designs do have good external validity if applied to a study sample which is representative of the general population with the disease under study. Furthermore, the study objectives related to a specific clinical question (does re-biopsy affect treatment choice?) where robust external validity was more important than weak internal validity.

The second study used meta-analysis to accurately define toxicities of adjuvant endocrine therapy in breast cancer. Assessment of adverse events using meta-analysis is of particular use for interventions where the margin between benefits and harms is narrow. For example if the beneficial effects of treatment is modest, the analysis of
adverse events is important as substantial toxicity may lead to equipoise. More comprehensive analysis of toxicity is also warranted where a number of efficacious treatments are available and these differ in safety and tolerability profiles.

Adverse effects are most reliably assessed using randomized trials, but many such events are too uncommon or require too long follow-up to be observed within randomized trials. Meta-analysis can use one of several strategies for addressing adverse effects. These can include assessment of toxicity as a supplemental analysis when assessing efficacy or conducting a separate review for adverse effects. The latter method is appropriate for a group of similar interventions (e.g. different aromatase inhibitors in early breast cancer) whose adverse effect profile might be expected to be similar. When conducting an adverse event based analysis, it is important to decide on either a narrow or broad focus. A narrow focus entails a detailed analysis of a small number of the serious adverse effects that are of special concern to patients or health professionals. It has the advantage of being less complex and of focusing on issues that have a major impact on the treatment decisions. Such methods are limited as they are only suitable for adverse events that are known in advance. In study 2, a narrow focus was chosen as only a select number of key toxicities were of interest.

Finally, in study 3, a randomized feasibility study was conducted. In line with the definition of feasibility studies, a small study aimed at helping to design further confirmatory studies, was conducted. Feasibility studies can have various purposes such
as testing study procedures, assessing the validity of tools, estimating the recruitment and retention rates, and estimating parameters such as the variance of the outcome measure, which is needed to calculate sample size. Published recommendations for the conduct of such studies state that the statistical analysis should be mainly descriptive and that results from hypothesis testing must be interpreted with caution\textsuperscript{21}. Therefore, the main objective of this study was to assess the feasibility of conducting a trial of de-escalated bisphosphonate therapy in patients with low-risk bone metastases. The study also aimed to provide data regarding recruitment and retention of trial participants for future larger trials. Hypothesis testing of non-inferiority of de-escalated therapy compared with standard therapy was an exploratory objective.

The remainder of the thesis presents the results of the each study in the form of three separate papers (Chapters 2-4), as summarized below. The final chapter (Chapter 5) provides an in-depth discussion of the relevance of the findings to health care providers, limitations of the study, and recommendations for future research.
Chapter 2: (Paper 1): Prospective Study Evaluating the Impact of Tissue Confirmation of Metastatic Disease in Patients With Breast Cancer.

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Introduction

Discordance in tumour characteristics between primary and metastatic breast cancer has been described for over 30 years\textsuperscript{22,23}, but data describing such discordance have been considered unreliable\textsuperscript{24}. Therefore, most practice guidelines recommend that decisions regarding systemic therapy for women with metastatic disease be based on the properties of the primary breast cancer\textsuperscript{25} and confirmatory biopsy of suspected metastatic lesions is not recommended consistently.

When compared to the primary tumour, expression of the estrogen (ER) and progesterone (PgR) receptors in metastatic breast cancer can be discordant in up to 40\%
of women\textsuperscript{26}. Lower rates of discordance are described for HER2\textsuperscript{27}. Most studies describing such discordance are retrospective and have limitations including selection bias and use of different techniques to evaluate receptors in the primary tumour and metastatic tissue. Such studies cannot evaluate success rates of biopsy of metastatic lesions and cannot accurately inform the impact of receptor discordance on clinical management.

In a previous pilot prospective study in which 35 women with suspected new metastases underwent biopsy; it was found that 40% had discordance of receptors and this led to a change in management in 20% of all patients\textsuperscript{28}. Other prospective studies include high proportions of women with operable, loco-regional recurrences, and have not evaluated the effects of discordance on patient survival\textsuperscript{29}. Retrospective analyses of primary and recurrent breast cancers suggest that receptor discordance is associated with poorer survival\textsuperscript{30-32}, perhaps due to the use of inappropriate targeted therapy or the identification of tumours with a more unstable phenotype and therefore more aggressive behaviour.

The present study built on a prior pilot study to address prospectively the success rates of biopsy of metastatic lesions in women with distant metastatic disease where a change in treatment is contemplated. It evaluated whether such biopsies altered management and examined the impact of receptor discordance on disease progression and survival in a prospective cohort of patients. It was hypothesized that in
the presence of discordance, if treatment were modified according to the results of the metastatic biopsy, similar outcome would be observed as in those with concordant disease.

Patients and Methods

Study Population

This prospective cohort study took place at a single large cancer hospital. Women with recurrent or progressive metastatic breast cancer were eligible. Availability of archival primary tumour was mandatory. There were no restrictions relating to the number of prior lines of systemic therapy. Exclusion criteria included operable loco-regional recurrence with no evidence of metastatic disease, clotting disorder precluding biopsy, rapidly progressive disease or history of non-breast second malignancies. The study was approved by the local Research Ethics Board.

Trial Endpoints

The primary endpoint of this study was the proportion of patients in whom results of the metastatic biopsy led to a change in management. The secondary goals were to define the discordance rates in ER, PgR and HER2 between primary and metastatic tissue, to assess procedural success rate, risks and patient satisfaction with performing a metastatic biopsy. Finally, an exploratory objective of evaluating progression-free (PFS) and overall survival (OS) was included.
**Trial Design**

Eligibility was assessed and consent obtained. The treating oncologists completed a questionnaire, prior to obtaining a biopsy from a metastatic lesion, to determine their treatment plan and the proposed start date. Once biopsy results were available, oncologists were again surveyed to determine if their treatment plan had changed based on the biopsy results and to determine the actual start date of treatment. Procedural success rate was assessed as the number of matched histopathological examinations of primary and metastasis as a proportion of biopsies undertaken. Delay of therapy was evaluated as the duration between the proposed and actual treatment start dates. Patient satisfaction was assessed using questionnaires, which were administered to all consenting women at the time of clinic follow-up: these evaluated side effects resulting from the biopsy procedure and willingness to recommend metastatic biopsy to other women (see appendix A). Women were followed and investigations were carried out at the clinician’s discretion; the protocol did not stipulate frequency of assessment. PFS was calculated as the duration between biopsy and first documented evidence of progressive disease or death from any cause. OS was defined as the duration from biopsy to death from any cause. A post-hoc, exploratory analysis of the effect of discordance on survival after diagnosis of metastatic disease was also conducted.
**Biopsy Procedures**

For superficial metastases, fine needle aspiration (FNA), core, punch, or excisional biopsies were performed using palpation guidance only. For internal lesions, the most amenable site of biopsy was determined in consultation with an interventional radiologist and FNA, core biopsy or aspiration of fluid were carried out under radiological guidance. When aspiration was undertaken, smears of tumour cells were prepared. Samples were fixed in 10% formalin within 20 minutes of the biopsy and processed using the same protocol as for other tissues (including formalin fixation for ≥8 hours). In order to optimize analysis of receptor expression, biopsies of metastatic bone lesions were not decalcified whenever possible\(^3\).

**Tissue Processing**

A single pathologist (NM) and a single cytopathologist (WG) evaluated all biopsies. Confirmation of malignancy and evaluation of hormone receptor and HER2 expression were analyzed from all available samples and compared to corresponding results for the primary tumour. All primary and metastatic tissues were analyzed using the same standardized methodology, but matched primary and metastatic samples were not necessarily tested using the same batch of reagents. Primary tumour tissue that was not reported in a central, university-affiliated laboratory or which did not use the latest antibodies and/or hybridization techniques was re-analyzed. In an attempt to reduce the waiting time for results, tissue from biopsy of metastases were processed immediately and archival primary samples processed when these became available.
Consequently, paired samples were not necessarily assessed at the same time using the same batch of reagents. Furthermore, pathologists were not systematically blinded to the receptor status of the primary tumour.

ER staining was carried out using the Ventana SP1 antibody and PgR using Novocastra Clone 16. A positive result was defined as ≥1% of tumour cell nuclei staining positively with any intensity\textsuperscript{34,35}. HER2 was assessed exclusively by fluorescent in-situ hybridization (FISH) using the PathVysion HER-2 DNA Probe Kit (Vysis). HER2 and CEP17 signals were enumerated from 60 tumour nuclei. In borderline cases an additional 60 nuclei were enumerated. The threshold for over-expression was a HER2/CEP17 ratio of >2.2. To allow consistent comparison with FISH performed on primary tumours, HER2 FISH on cytology specimens was performed on paraffin sections of pelleted cells. For bone metastases, material not requiring decalcification was used for FISH. To assess receptor discordance, all results were dichotomized into either positive or negative using the methods described above. Quantitative changes in receptor expression were analyzed descriptively.

Statistical Analyses

Pilot data suggested that biopsy was associated with a change in therapy in approximately 20% of patients\textsuperscript{28}. Assuming this estimate, 121 patients were required to obtain a confidence interval with a half-width of 7.5% for the proportion of patients having a change in management. All results were presented descriptively as medians or
proportions. Two pre-specified sub-group analyses compared the assessment of receptor discordance by (a) number of lines of prior therapy with the expectation of higher discordance for those with more lines of therapy; and (b) the duration between initial assessment of the primary breast tumour and biopsy of a metastasis, with the anticipation of higher rates of discordance for those with longer durations. Differences between subgroups were assessed by an interaction test\textsuperscript{36}. Two post-hoc subgroup analyses assessed whether the site of metastatic biopsy or the receptor profile of the original tumour influenced receptor discordance. For the survival analysis, three separate analyses were undertaken. First, the effect of discordance in any receptor on both progression-free (PFS) and overall survival (OS) was assessed. Secondly, to assess the prognostic impact of undertaking a biopsy from a metastatic lesion, the probability of 12-month survival was evaluated for the following subgroups defined by the primary tumour assessment: a) ER or PgR-positive and HER2-negative, b) HER2-positive and c) triple negative. An exploratory analysis was also conducted to assess the median PFS in the group for whom re-biopsy led to a change in therapy compared with the median PFS for all biopsied patients. Patient characteristics in comparison groups were compared using chi-squared, Fisher’s exact or Wilcoxon rank sum tests as appropriate. PFS and OS were estimated using the Kaplan-Meier product limit method and different sub-groups were compared using the log-rank statistic. Hazard ratios (HR) and their confidence intervals (CI) were computed using the Mantel-Haenszel method. Two-sided tests with p-values <0.05 were considered statistically significant. Corrections were not made for multiple comparisons.
Results

Patient and Clinician Characteristics

Over a 2-year period, 151 women were approached, 137 consented and 121 underwent biopsy. A flow diagram is shown in Figure 1 and patient demographics are shown in Table 1. Treatment decisions were made by five different clinicians with 86 cases (71%) being made by one clinician. The other four clinicians contributed to 16%, 8%, 3% and 2% of cases respectively.

Procedural success rate

The sites of biopsy and the analyzable yield for determination of receptor status are shown in Table 2. In total, 117 of the 121 biopsies (96.7%) confirmed recurrent breast cancer. In 3 women (2.5%), biopsies showed benign disease, and their follow-up imaging showed either resolution or stability of the index lesion. In one participant (0.8%) a second malignancy (basal cell carcinoma) was discovered. Determination of ER and PgR by immunohistochemistry (IHC) was possible in 94 women (80.3%). Reasons for inability to perform IHC are shown in Figure 1. Fine needle aspiration, paracentesis or thoracocentesis were statistically significantly less likely to provide sufficient cells for examination than core biopsies (13 of 46 samples compared with 9 of 66 samples, p=0.03). Core biopsies from bone and bone marrow trephines were associated with a non-significantly lower yield of adequate tissue (8 of 29 samples compared with 14 of 78
samples, p=0.16). Of the 94 biopsies in which IHC was undertaken, HER2 FISH analysis was successful in 83 (88.3%).

*Risks of biopsy*

Biopsy was associated with a median delay to commencing treatment of 15 days (range 2-56). There was one serious adverse event related to biopsy: bleeding from a punch biopsy of the skin leading to admission. This resolved with conservative measures and the patient was discharged the next day.

*Receptor Discordance*

Discordance in one or more receptors between the original pathology report of the primary and the metastatic biopsy was found in 37.6% of women (see Figure 2). The most common change was loss of PgR. Quantitative changes in ER and PgR are shown in Figure 3.

Of the 94 patients in whom metastases could be analyzed for receptor status, 52 primary tumours (55%) were initially reported at external pathology departments or using outdated techniques and were therefore re-analyzed and compared to the original pathology report. The median time from initial analysis of the primary to metastatic biopsy was 81 months (range 9-182) in those re-analyzed, while it was 20 months (range 0.5-35) in those not re-analyzed. For 3 tumours (5.8%) the initial report suggested ER-negative disease and the re-analysis found ER-positive disease. In one of these tumours
the initial assessment used a biochemical assay for ER. In the other two tumours, immunohistochemistry was utilized, but with a different/older antibody. For 6 tumours (11.5%) there was discordance in PgR (4 negative-positive and 2 positive-negative pairs). In two samples, the initial assessment was conducted using a biochemical assay while in the other four, immunohistochemistry was utilized, but with a different/older antibody. For 2 tumours (3.8%) there was discordance in HER2 with one changing from positive to negative and the other from negative to positive. In both of these cases, HER2 was initially reported using immunohistochemistry and subsequent assessment was with FISH. These findings are similar to those reported in large studies comparing local and central reports of receptor status\textsuperscript{37,38}.

True receptor discordance was not seen in any triple negative patients: two of 23 women reported to have triple negative tumours based on their original pathology showed apparent receptor discordance in one or more receptor but re-analysis of the primary tumour of these women confirmed ER-positive disease consistent with that in the metastasis.

*Change in Therapy*

Seventeen women (14%, 95% CI 8.4-21.5%) had a change in treatment as compared to the pre-biopsy therapeutic plan. These cases were reported by four different clinicians. Changes in management included the addition of trastuzumab in women with gain of HER2 over-expression (n=6), the use of chemotherapy in place of
endocrine therapy in those with loss of ER (n=5), no change to previous treatment in those with benign disease or second primary (n=4) and provision of endocrine therapy in place of chemotherapy for those gaining ER (n=2). Where loss of ER led to use of chemotherapy, clinical factors such as response to prior lines of endocrine therapy were considered in conjunction with results of the biopsy. There was no evidence that probability of change in treatment was influenced by the number of prior lines of treatment received or the time-interval between evaluation of primary breast cancer and metastasis (table 3). There was no evident difference in the likelihood of change in therapy between different sites of metastatic biopsy.

Patient Satisfaction

Nine women deteriorated clinically and were unable to complete questionnaires, 4 women could not be contacted and 18 declined to complete the questionnaire. Of 90 women completing the questionnaire, 31 (34%) described anxiety pre-biopsy and 53 (59%) described pain post-biopsy; 29 women (32%) described mild, 17 (19%) moderate and 7 (8%) severe pain. Seventy-nine women (87.8%) would recommend metastatic biopsy to others with breast cancer.

Survival Analysis

Follow-up was available for all 94 patients with matched primary and metastatic tissue. After a median follow-up of 12.2 months, 77 patients (80%) had progressed and 38 (40%) had died. There were no differences in PFS between patients with metastases
that were concordant or discordant with the primary tumour (Figure 4). Median PFS was 6.3 months for the concordant group and 6.5 months for the discordant group. The Hazard Ratio (HR) for discordance was 0.88 (95% CI 0.55-1.41; p=0.59). In an exploratory analysis, among those for whom re-biopsy led to a change in therapy, the median PFS was 9.7 months compared with 5.7 months for all biopsied patients. Overall survival was not significantly different between patients with metastatic disease that was concordant or discordant with the primary tumour (Figure 5). Median OS was 27.6 months for the concordant group and 30.2 months for the discordant group. The HR for discordance was 0.94 (95% CI 0.49-1.80; p=0.85).

Based on the results of the receptor profile of the primary tumour, the 12-month survival following biopsy was 73.1% for hormone receptor-positive and HER2-negative tumours (N=67), 92.3% for HER2-positive tumours (N=13) and 37.5% for triple negative tumours (N=14). When assessed by the results of biopsy of a metastatic lesion, the 12 month survival for these subgroups was 83.0%, 75.0% and 36.4% respectively. These differences were driven predominantly by discordance in HER2. Those tumours with increasing HER2 expression i.e. HER2 negative primary and HER2-positive metastasis (N=6) had poorer OS whereas those tumours that were HER2-positive for the primary and HER2-negative in the metastasis (N=2) had an improved outcome.

Properties of biopsies from metastatic sites showed a trend towards better prediction of response to endocrine therapy than properties of the primary tumour. In
those patients with an ER- and PgR-positive primary tumour, loss of PgR expression in
the metastasis (N=15) was associated with worse PFS on endocrine therapy compared
with those that maintained PgR expression (N=15) (12-month PFS 27% and 47%
respectively, Figure 6).

Discussion

The choice of systemic therapy in advanced breast cancer is dependent on the
appropriate targeting of ER, PgR and HER2 receptors. The receptor status of metastatic
disease is usually assumed to be the same as that of the primary tumour, but there is
evidence for discordance in receptor status between primary and metastatic tumour
(reviewed in \textsuperscript{39}).

A number of variables related to tissue processing and analysis can influence
biopsy results and may lead to receptor discordance. In a overview of variables
potentially influencing the interpretation of immunohistochemical assessment of FNA
cytology and formalin-fixed paraffin-embedded tissue, 15 variables were shown to
impact results. These included fixation delay; fixative type; time in fixative; reagents and
conditions of dehydration, clearing and paraffin impregnation, and conditions of slide
drying and storage\textsuperscript{40}. Analytic variables which can lead to discordance include the use of
inconsistent techniques for assessment of the primary and recurrent lesion. For
example, older samples may have been analyzed for ER and PgR using a less sensitive
biochemical assay rather than immunohistochemistry, potentially resulting in artefactual discordance. For HER2, differences between the use of immunohistochemistry and FISH may also lead to inconsistent interpretation. When similar methods are used, inter-observer and inter-laboratory variability can still influence results. However, even when controlling for these potential sources of error, discordance between primary and recurrence samples is still evident suggesting that true biological differences between tumours resulting from heterogeneity of clonal populations remains a likely contributor to discordance. This finding is supported by molecular research showing breast cancer to be a biologically diverse disease and that primary breast cancers exhibit marked intra-tumoural heterogeneity even at a genetic level.

Clinicians use apparent receptor status in order to make decisions about treatment. The impact of receptor discordance on treatment decisions is not well described and few studies have determined the success rates of biopsy of metastatic lesions. Therefore, the usefulness of biopsy of metastases is disputed. Results of this study show that biopsy of metastatic sites is technically feasible, and that hormone receptors and HER2 expression can be determined from most biopsies. Receptor discordance was more common with hormone receptors than with HER2. Loss of PgR expression was the most common change, but this rarely had any impact on choice of therapy. Changes in therapy were reported in approximately 14% of women. These findings are similar to those published previously. No baseline factors were
identified that increased the likelihood of change in therapy, although triple negative tumours tended not to be discordant. There was also some discordance between the initial pathology report and the re-analysis of archival primary tissue, and in two women, change in therapy could have been guided by re-evaluation of the primary tumour. The limitations described above can influence the interpretation of receptor expression of both the primary and the recurrent tumour. It appeared that clinicians applied more weight to a switch from negative to positive receptor expression compared to positive to negative pairs. This suggests that there is greater concern regarding the potential for false negative receptor determination compared with false positive results.

The presence or absence of PgR expression has a prognostic impact in ER-positive early breast cancer\textsuperscript{48,49} and data from this study suggest that this may also be true in advanced breast cancer. The biological basis of PgR loss remains unclear, but it likely reflects a non-functional ER pathway or hyperactive growth factor signalling\textsuperscript{50}. Loss of PgR is also described as a consequence of endocrine therapy\textsuperscript{51}. It is possible that with progression or as a consequence of pre-treatment, tumours may lose PgR expression and become more resistant to hormonal therapy.

Changes in receptor profile should be interpreted with caution as variations in tissue processing can lead to erroneous results. Inadequate fixation can lead to false negative results for ER expression\textsuperscript{52,53}. Inadequate sampling of a heterogeneous cancer
can also lead to inaccurate results. Clinicians should consider responses to previous therapy before withholding targeted therapy in women with loss of receptor expression. Failure to detect tumours changing from receptor negative to positive is likely to have a greater impact on treatment decisions than failure to detect tumours changing from receptor positive to negative.\textsuperscript{54}

Discordance between primary and metastatic lesions was not associated with apparent differences in PFS or OS if treatment was modified accordingly. The power of this study to detect such differences was, however, low. Poor survival associated with receptor discordance in retrospective studies\textsuperscript{30-32} may be due to inappropriate use of targeted therapy in discordant cases. Indeed, among patients who had a change in treatment, median PFS was numerically longer than for all patients undergoing biopsy. However, there was limited power to detect such differences statistically. Biopsy of metastatic lesions may predict sensitivity to endocrine therapy: women with ER-positive and PgR-positive primaries, who were treated with endocrine therapy after their biopsy, had a higher likelihood of being progression-free at 12 months if PgR was maintained in the metastasis.

Delaying systemic therapy to await biopsy of a metastatic lesion and its characterization is a concern. In this study, the median delay associated with biopsy was 15 days (range 2-56). This was caused partly by batching of samples for HER2 FISH analysis. Approximately 1 in 3 patients described pre-biopsy anxiety and nearly 60%
described pain associated with their biopsy, but other procedure-related complications were rare. Acceptability of metastatic biopsy was high, with almost 90% willing to recommend metastatic biopsy to other women with breast cancer.

About 80% of biopsies from metastases could be analyzed for ER, PgR and HER2; FNA and biopsy of bone and bone marrow were associated with lower yield. Higher sensitivity from fine needle aspiration biopsy might have been obtained if immediate sample preparation and evaluation were available. Such evaluation might also allow expert triage of tumour material for ancillary studies and rigorous standardization of specimen preparation. Re-biopsy of patients in whom initial attempts did not provide adequate tissue was not routinely carried out in the present study. Such practice would almost certainly increase overall yield, but further delay commencing therapy.

Biopsy of metastatic disease does have limitations. While improvements in interventional radiology mean that most tissue is now accessible by minimally invasive methods, the choice of the location for biopsy is usually determined by the most accessible site. Such practice could potentially lead to sampling bias as different cancer clones may have a predilection for different metastatic sites. However, post-mortem data suggest that heterogeneity of hormone receptors among different metastatic sites is rare. Bone is the most common site of metastasis in breast cancer and biopsies of bone metastases provide a lower analyzable yield for IHC than other tissues. These factors limit the applicability of the results in some patients.
In this study, 46 of the 121 biopsies (38%) were analyzed using cytological rather than histological examination. Immunocytochemical study of FNA specimens has been reported to be a good test for the determination of ER and PgR status with concordance between cytology and histology of 98% for ER and 91% for PgR\(^{59}\). The use of cytology for HER2 assessment is more controversial. There are only limited data validating the use of FISH to assess HER2 copy number in breast carcinoma cells present in cytologic specimens. However the available studies have found strong and consistent correlation with HER2 status as defined from histological samples\(^{60-62}\). For the purposes of this study all FISH assays on material obtained by FNA biopsy were performed on paraffin sections of formalin fixed cell pellets (cell blocks), and the same thresholds were used as for any other paraffin sections because the same nuclear truncation effects pertain. A criticism of the use of FISH on FNA biopsy samples taken from primary tumours is that it is not possible to distinguish in-situ from invasive carcinoma cells. In the present study this limitation does not apply, since only FNA samples of metastatic disease were assessed. Another criticism of the use of FNA for assessment of HER2 status in primary breast tumours is that the small sample size cannot reliably take into account tumour heterogeneity. However the same number of nuclei (60) were assessed in both the primary tumour and metastasis so that a different result likely reflects clonal divergence rather than measurement error.
This study has shortcomings. First, it assessed the impact of biopsy on clinicians’ choice of therapy rather than improvement in outcome. Furthermore, most treatment decisions were reported by a single clinician and this may have introduced bias thereby weakening the external validity of the results. A randomized trial would be the optimal study design to evaluate effects of a biopsy on outcome, but this would be difficult to conduct, and the methods used were felt to be the most feasible. Second, while strict protocols were followed in the handling of the metastatic biopsies, the handling of the primary specimens was generally not known. Therefore, many of the pre-analytic variables described above may have influenced our estimate of receptor discordance. Third, primary tumour samples reported in a central laboratory were not re-analyzed and comparison with biopsies of metastases was therefore subject to inter-observer variability. This reflects clinical practice and should not therefore detract from the impact of the results. Fourth, the results are probably influenced by some selection bias as clinicians likely recruited patients which they felt were more likely to benefit from re-analysis of their recurrent disease based upon clinical parameters. Important selection factors likely included the natural history of the disease, timing of relapse, site(s) of recurrence, co-morbidities and previous and proposed treatments. Finally, data on survival should be considered exploratory as power to detect differences between concordant and discordant cases was limited and follow-up imaging was conducted at the clinician’s discretion rather than at fixed interval. This is pragmatic, but may lead to uncertainty in the definition of time to events.
In conclusion, this prospective study shows that clinicians alter immediate management in one of seven patients after biopsy of metastases. If treatment is modified according to results, discordant and concordant cases appear to have similar outcome. A decision to biopsy a metastatic site should be based on patient and tumour-related factors such as available treatment options, response to prior therapies and natural history of the disease. It is possible that the probability of change in therapy resulting from biopsy is exaggerated in this cohort due to selection bias as described above. For example, patients who may benefit most from re-biopsy of recurrent disease may be selected by the presence of inconsistency in their course of disease compared to that suggested by their initial receptor expression. Clinicians should consider carefully the method and site of biopsy to maximize analyzable yield and select those patients who may benefit most from re-analysis of their receptor expression.
Table 1 – Patient Demographics.

* Includes only those patients with availability of matched primary and metastatic tissue.
† Refers to comparison between concordant and discordant groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All biopsied patients (n=121)</th>
<th>Concordant Group* (n=53)</th>
<th>Discordant Group* (n=41)</th>
<th>p †</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>59</td>
<td>58.5</td>
<td>59</td>
<td>0.20</td>
</tr>
<tr>
<td>Range</td>
<td>29-83</td>
<td>35-83</td>
<td>36-72</td>
<td></td>
</tr>
<tr>
<td><strong>Adjuvant treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>63 (52.1%)</td>
<td>20 (36.4%)</td>
<td>21 (51.2%)</td>
<td>0.19</td>
</tr>
<tr>
<td>Endocrine therapy</td>
<td>90 (75%)</td>
<td>28 (50.9%)</td>
<td>17 (41.5%)</td>
<td>0.50</td>
</tr>
<tr>
<td>Trastuzumab</td>
<td>5 (4.1%)</td>
<td>2 (3.6%)</td>
<td>2 (4.9%)</td>
<td>0.85</td>
</tr>
<tr>
<td><strong>Advanced Disease Status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newly diagnosed metastatic</td>
<td>56 (46.3%)</td>
<td>11 (20.0%)</td>
<td>7 (17.1%)</td>
<td></td>
</tr>
<tr>
<td>One prior line of treatment in metastatic setting</td>
<td>21 (17.4%)</td>
<td>15 (27.3%)</td>
<td>10 (24.4%)</td>
<td>0.65</td>
</tr>
<tr>
<td>Two or more lines of treatment in metastatic setting</td>
<td>44 (36.4%)</td>
<td>29 (52.7%)</td>
<td>24 (58.5%)</td>
<td></td>
</tr>
<tr>
<td><strong>Duration of metastatic disease (months)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>35</td>
<td>18</td>
<td>24</td>
<td>0.35</td>
</tr>
<tr>
<td>Range</td>
<td>0-274</td>
<td>0.5-79</td>
<td>0.5-108</td>
<td></td>
</tr>
<tr>
<td><strong>Palliative treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median lines of endocrine therapy</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0.10</td>
</tr>
<tr>
<td>Median lines of chemotherapy</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.26</td>
</tr>
</tbody>
</table>
Table 2 – Sites of biopsy and analyzable yield of each site. Analyzable yield is the proportion of biopsies for which analysis for ER, PgR and HER2 could be completed. CNS, central nervous system. FNA, fine needle aspiration. CT, computed tomography. US, ultrasound. *, soft tissue includes subcutaneous tissue, muscle and peri-osseous tumour.

<table>
<thead>
<tr>
<th>Site</th>
<th>n (%)</th>
<th>Method of sampling</th>
<th>Analyzable Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph Node</td>
<td>25 (20.7%)</td>
<td>US-guided core biopsy – 2 (8%)</td>
<td>17 (68%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US-guided FNA – 8 (32%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unguided FNA – 15 (60%)</td>
<td></td>
</tr>
<tr>
<td>Cutaneous</td>
<td>24 (19.8%)</td>
<td>Unguided FNA – 2 (8.3%)</td>
<td>24 (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unguided punch biopsy – 21 (87.5%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Excision biopsy – 1 (4.2%)</td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>20 (16.5%)</td>
<td>CT-guided core biopsy – 18 (90%)</td>
<td>15 (75%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT-guided FNA – 2 (10%)</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>19 (15.7%)</td>
<td>US-guided core biopsy – 19 (100%)</td>
<td>19 (100%)</td>
</tr>
<tr>
<td>Soft tissue *</td>
<td>10 (8.3%)</td>
<td>US-guided core biopsy – 3 (30%)</td>
<td>7 (70%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT-guided core biopsy – 2 (20%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unguided FNA – 5 (50%)</td>
<td></td>
</tr>
<tr>
<td>Bone marrow</td>
<td>9 (7.4%)</td>
<td>Trephine biopsies – 9 (100%)</td>
<td>6 (66.7%)</td>
</tr>
<tr>
<td>Paracentesis</td>
<td>7 (5.8%)</td>
<td>Pleural drainage – 4 (57.1%)</td>
<td>5 (71.4%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ascitic drainage – 3 (43.9%)</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>5 (4.1%)</td>
<td>CT-guided FNA – 5 (100%)</td>
<td>4 (80%)</td>
</tr>
<tr>
<td>CNS</td>
<td>2 (1.7%)</td>
<td>Lumbar puncture – 2 (100%)</td>
<td>2 (100%)</td>
</tr>
</tbody>
</table>
Table 3 – Proportion of women with a change in originally planned therapy by subgroup.

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>n (%)</th>
<th>Test of interaction p</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>17 (14.0%)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Lines of therapy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newly metastatic</td>
<td>7 (12.5%)</td>
<td></td>
</tr>
<tr>
<td>One prior line of therapy in metastatic setting</td>
<td>2 (9.5%)</td>
<td></td>
</tr>
<tr>
<td>Two or more prior lines of therapy in metastatic setting</td>
<td>8 (18.2%)</td>
<td></td>
</tr>
<tr>
<td>Two lines (n=14)</td>
<td>2 (14.3%)</td>
<td>0.72</td>
</tr>
<tr>
<td>Three lines (n=8)</td>
<td>1 (12.5%)</td>
<td></td>
</tr>
<tr>
<td>Four lines (n=4)</td>
<td>1 (25%)</td>
<td></td>
</tr>
<tr>
<td>Five lines (n=4)</td>
<td>1 (25%)</td>
<td></td>
</tr>
<tr>
<td>Six or more lines (n=14)</td>
<td>3 (21.4%)</td>
<td></td>
</tr>
<tr>
<td><strong>Duration from primary breast cancer diagnosis and biopsy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First Quartile (&lt;35 months)</td>
<td>4 (11.4%)</td>
<td></td>
</tr>
<tr>
<td>Second Quartile (36-67 months)</td>
<td>4 (15.4%)</td>
<td>0.15</td>
</tr>
<tr>
<td>Third Quartile (68-118 months)</td>
<td>7 (24.1%)</td>
<td></td>
</tr>
<tr>
<td>Fourth Quartile (&gt;118 months)</td>
<td>2 (6.5%)</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1 – Flow diagram for the study. FISH, fluorescent in-situ hybridization.
Figure 2 – Changes in ER, PgR and HER2 between the original pathology report of the primary tumour and the metastasis.
Figure 3 – Waterfall plot showing absolute change in hormone receptor expression. A positive score confirms increased expression of receptor from the primary to the metastasis and a negative score reduced expression. A, Estrogen receptor. B, Progesterone receptor. Dark color, concordance with primary. Light color, discordance with primary.
Figure 4 Progression-free survival by concordance or discordance of receptor status
Figure 5 Overall survival by concordance or discordance of receptor status
Figure 6 Progression-free survival for patients with ER-positive and PgR-positive primary breast cancer after treatment with endocrine therapy and grouped by PgR expression of metastasis.
Chapter 3: (Paper 2) Toxicity of adjuvant endocrine therapy in postmenopausal breast cancer patients: A Systematic Review and Meta-Analysis

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Introduction

The development of aromatase inhibitors has provided an alternative form of adjuvant endocrine therapy for postmenopausal women with breast cancer. Several large randomized trials have assessed the benefit of the third-generation aromatase inhibitors anastrozole, letrozole, and exemestane compared with 5 years of tamoxifen. These trials have shown that aromatase inhibitors are associated with reduced recurrence rates and improved disease-free survival but have not demonstrated a statistically significant improvement in overall survival. Despite their large size, the individual trials had limited statistical power to detect small (but potentially important) differences in overall survival in the absence of extended follow-up. An individual patient data meta-analysis of the
randomized trials by the Oxford Early Breast Cancer Trialists' Collaborative Group that had greater statistical power confirmed that use of aromatase inhibitors was associated with improved disease-free survival and also showed a statistically significant albeit modest improvement in overall survival only for patients who took aromatase inhibitors for 2–3 years after 2–3 years of tamoxifen\(^68\). No overall survival benefit was seen in those who received aromatase inhibitors as initial (i.e., up-front) therapy.

The lack of an overall survival benefit with aromatase inhibitors despite improvements in disease-free survival is unexpected. It was therefore hypothesized that the relative toxicity of aromatase inhibitors compared with tamoxifen\(^69;70\) may explain these findings. Some have suggested that aromatase inhibitors should be used for 2–3 years after or before tamoxifen (i.e., in a switching strategy) to maximize benefits and offset the toxic effects\(^69;71\). Unfortunately, most individual trials that have assessed aromatase inhibitors vs. tamoxifen had suboptimal statistical power or duration of follow-up to detect differences in toxicities between these strategies. Furthermore, while meta-analyses conducted by the Oxford Overview have assessed toxicity of tamoxifen compared to no treatment, no such assessments have been done for the comparison of aromatase inhibitors to tamoxifen. This study was designed to evaluate and compare serious or life-threatening adverse events reported in randomized trials comparing different adjuvant endocrine therapy strategies in postmenopausal women with early-stage breast cancer by using a meta-analysis.
Methods

Search Strategy

Relevant trials were identified using a computerized search of the following databases: MEDLINE (host: OVID), 1996–April week 2, 2010; EMBASE (host: OVID), 1980–2010 week 16; American Society of Clinical Oncology Annual Meetings, 2000–2009; and San Antonio Breast Cancer Symposium Annual Meetings, 2000–2009. The search was restricted to English language articles. The terms “adjuvant,” “aromatase inhibitor,” and “tamoxifen” and “breast cancer” and similar terms were cross-searched by using the following search algorithm: ((aromatase inhibitor OR anastrozole OR letrozole OR exemestane) AND (tamoxifen) AND (adjuvant) AND (Breast neoplasm MeSH OR ((breast OR mammary) AND (carcinoma OR malignan* OR neoplasm OR tumour)))) AND (randomized controlled trial [pt]OR controlled clinical trial [pt]OR randomized controlled trial [mh]OR double-blind method [mh] OR single-blind method [mh] OR clinical trial [pt] OR clinical trials [mh] OR (“clinical trial”) [tw] OR singl* [tw] OR doubl* [tw] OR trebl* [tw] OR tripl* [tw] AND (mask* [tw] OR blind* [tw])) OR comparative study [mh] OR evaluation studies [mh] OR follow up studies [mh] OR prospective studies [mh] OR control* [tw] OR prospective* [tw] OR volunteer* [tw] NOT (animals [mh] NOT humans [mh]). Included studies were randomized phase III clinical trials that compared aromatase inhibitors with tamoxifen as initial adjuvant therapy in postmenopausal women with early stage breast cancer. Trials that had treatment durations of 5 years in total were included irrespective of duration of post-intervention follow-up. Trials that had treatment durations longer than 5 years (extended adjuvant) were
excluded as were trials conducted in premenopausal or perimenopausal women. Published articles and abstracts presented at annual meetings were included in the meta-analysis. All data were from intent-to-treat analyses. The primary objective of this study was to assess toxicity; therefore, data from all randomly assigned patients regardless of their hormone receptor status was included.

Data Extraction

Data on pre-specified serious or potentially life-threatening adverse events as defined by the individual trials were extracted. Definitions of adverse effects differed between studies. In some studies toxicity was defined by individual investigators\textsuperscript{64,66,67}, while others used common criteria such as the National Cancer Institute Common Terminology Criteria for Adverse Events\textsuperscript{65,72,73} or the European Organisation for Research and Treatment of Cancer Common Toxicity Criteria\textsuperscript{74}. To address this limitation, toxicities of interest of any grade were included. In addition, information on deaths without breast cancer recurrence was assessed. Data on adverse events and death without recurrence were extracted from the primary publications and from any associated online appendices by two authors (EA and BS) using a standardized data extraction form. Discrepancies were resolved by consensus. Six adverse events associated with a high risk of mortality were prespecified in this analysis: cardiovascular disease (including myocardial infarction, angina, and cardiac failure), cerebrovascular disease (including cerebrovascular accident and transient ischemic attack), venous thrombosis (defined as any venous thrombo-embolic episode), bone fracture (any), endometrial carcinoma alone, and other secondary cancers
(defined as any invasive cancer excluding endometrial carcinoma and contralateral breast cancer). Hypercholesterolemia was assessed in a preplanned exploratory analysis because it is a well-documented adverse effect of aromatase inhibitors and is associated with both cardiovascular and cerebrovascular disease. All grades of hypercholesterolemia were included in the meta-analysis. Adverse events such as hot flashes or arthralgia were excluded.

Data Synthesis

The meta-analysis was carried out in three treatment cohorts: 1) 5 years of an aromatase inhibitor vs. 5 years of tamoxifen (i.e., up-front treatment with aromatase inhibitor vs. with tamoxifen), 2) tamoxifen for 2–3 followed by an aromatase inhibitor for 2–3 years vs. 5 years of tamoxifen (i.e., switching vs. tamoxifen), and 3) tamoxifen for 2–3 years followed by an aromatase inhibitor for 2–3 years vs 5 years of an aromatase inhibitor (i.e., switching vs. aromatase inhibitor). In general, the aromatase inhibitor group included trial arms that received aromatase inhibitors alone or aromatase inhibitors for longer durations compared with the other intervention arm, and the tamoxifen group included trial arms that received tamoxifen alone or aromatase inhibitors for shorter durations compared with the other treatment arm.

Statistical Analysis

The magnitude of association between aromatase inhibitors and individual adverse event per person was expressed as an odds ratio (OR) and a 95% confidence interval (CI).
Initial analyses were conducted separately for each treatment cohort described above. The pooled data for all three cohorts was also analyzed. All data were analyzed using RevMan 5 analysis software (The Cochrane Collaboration, Copenhagen, Denmark). Pooled estimates of odd ratios were computed using generic inverse variance\textsuperscript{75} and a fixed-effects model\textsuperscript{76}. Studies were weighted by their individual squared standard errors. Data were not adjusted for different durations of follow-up between studies. Differences in summary estimates of the OR between the three cohorts (subgroups) were assessed using methods described by Deeks et al\textsuperscript{77}. Absolute risks of each adverse event were calculated as the number of events per person over the follow-up period of the individual trial. The difference in absolute risk between the aromatase inhibitor group and the tamoxifen group was also presented as the number needed to harm, which quantifies the number of patients that would need to be treated with a particular intervention to cause an adverse event in one patient who would not otherwise have experienced the adverse event. Positive values indicate higher absolute risks in the aromatase inhibitor group whereas negative values indicate higher absolute risks in the tamoxifen group. The probability of toxicity in the switching vs. tamoxifen cohort was adjusted to account for the longer survival in this group. Meta-analysis has shown a consistent reduction in the probability of death of around 0.44\% per year\textsuperscript{68}. Therefore, the frequency of toxicity in this group was discounted by 0.44\% for every year of follow-up. Such adjustment was not conducted for other groups as there is no data supporting differential survival in these groups. Statistically significant heterogeneity was defined as a $\chi^2$ P value less than 0.1 or an $I^2$ statistic greater than 50\%. All other statistical tests were two sided, and statistical significance was defined as $P$ less than .05.
Results

A total of 377 potentially relevant articles were identified in the primary literature search, of which seven were reports of randomized phase III trials enrolling 30,023 patients that met the inclusion criteria. Articles were excluded if they did not compare aromatase inhibitors to tamoxifen (n = 172), were review articles (n = 169), were cost-effectiveness analyses (n = 18), or were early analyses of included trials (n = 9). Two trials contributed data to the analysis of up-front aromatase inhibitors vs up-front tamoxifen: Arimidex, Tamoxifen, Alone or in Combination (ATAC; n = 6241 patients)\textsuperscript{67} and Breast International Group 01-98 (BIG 1-98; n = 4922 patients)\textsuperscript{65}. Four publications comprising five trials contributed data to the analysis of switching from tamoxifen to aromatase inhibitors vs tamoxifen: a combined analysis of the Austrian Breast and Colorectal Cancer Study Group trial 8 and the German Adjuvant Breast Cancer Group/Arimidex–Nolvadex (ABCSG8/ARNO 95; n = 3226 patients)\textsuperscript{66}, the Intergroup Exemestane Study (IES; n = 4724 patients)\textsuperscript{72}, the Italian Tamoxifen Anastrozole trial (ITA; n = 448 patients)\textsuperscript{64}, and the National Surgical Adjuvant Study Breast Cancer 03 trial (N-SAS BC03; n = 696 patients)\textsuperscript{73}. The sequencing arms of BIG 1-98 were not included because there were different follow-up times for the different arms of the trial. One trial, the Tamoxifen Exemestane Adjuvant Multinational trial (TEAM, n = 9766 patients)\textsuperscript{74}, provided data for the analysis of switching from tamoxifen to aromatase inhibitors vs aromatase inhibitors. The study designs and characteristics of the included studies are shown in Figure 1 and Table 1, respectively.
The number of adverse events, follow-up time, and the number of evaluable patients for each adverse event are shown in Table 2 while Table 3 presents the difference in absolute risk for each adverse event between the aromatase inhibitor group and the tamoxifen group and the number needed to harm.

**Cardiovascular Disease**

Longer durations of aromatase inhibitor use were associated with increased odds of cardiovascular disease compared with tamoxifen use. Combined analysis of the two trials that evaluated up-front aromatase inhibitors vs. up-front tamoxifen\(^{65,67}\) showed a statistically significant association between aromatase inhibitor use and cardiovascular disease (OR = 1.30, 95% CI = 1.06 to 1.61, \(P = .01\)) (Figure 2, A). Combined analysis of trials that evaluated switching vs. up-front tamoxifen\(^{64,66,72,73}\) showed a non–statistically significant association between aromatase inhibitor use and cardiovascular disease (OR = 1.15, 95% CI = 0.93 to 1.41, \(P = .20\)) (Figure 2, A). After adjustment for differential survival between the aromatase inhibitor group and the tamoxifen group, the odds ratio was 1.12 (95% CI = 0.92 to 1.35, \(P = .26\)). Finally, the one trial\(^{74}\) that evaluated up-front aromatase inhibitors vs. switching showed a statistically significant association between aromatase inhibitor use and cardiovascular disease (OR = 1.37, 95% CI = 1.05 to 1.79, \(P = .02\)) (Figure 2, A). A pooled analysis of the data for all three cohorts showed that longer duration of aromatase inhibitor use was associated with a statistically significant increase in the odds of developing cardiovascular disease compared with tamoxifen alone or shorter duration of aromatase inhibitor use (OR = 1.26, 95% CI = 1.10 to 1.43, \(P < .001\)) (Figure 2, A). Adjustment
for differential survival between the aromatase inhibitor group and the tamoxifen group did not change the pooled odds ratio for all cohorts. There was no evidence for heterogeneity ($\chi^2 P=0.90, I^2=0\%$) In absolute terms, 4.2% of patients in the aromatase inhibitor group and 3.4% of patients in the tamoxifen group suffered a cardiovascular event (difference in absolute risk = 0.8%, number needed to harm = 132) (Table 2 and Table 3). Increased odds of cardiovascular events in the aromatase inhibitor group vs. tamoxifen group were seen in all treatment cohorts, although the magnitude was numerically, but not statistically significantly lower for the treatment cohort where aromatase inhibitors were administered after 2–3 years of treatment with tamoxifen (ORs of 1.15 vs 1.30 and 1.37, test of subgroup differences, $P = .53$).

*Cerebrovascular Disease*

Neither the individual studies nor the pooled data showed any statistically significant difference in the odds of cerebrovascular disease between the two treatment groups (OR = 1.01, 95% CI = 0.81 to 1.26, $P = .93$) (Figure 2, B). Adjustment for differential survival between the aromatase inhibitor group and the tamoxifen group did not change the pooled odds ratio for all cohorts. There was no evidence for heterogeneity ($\chi^2 P=0.21, I^2=34\%$). Cerebrovascular disease was an uncommon adverse event: it occurred in 1.4% of patients in the aromatase inhibitor group and in 1.5% of patients in the tamoxifen group (difference in absolute risk = −0.1%, number needed to harm = −974) (Table 2 and Table 3).
Venous Thrombosis

Longer durations of aromatase inhibitor use were associated with decreased odds of venous thrombosis compared with tamoxifen. A pooled analysis of the data for all three cohorts revealed a 45% reduction in the relative odds of venous thrombosis for the aromatase inhibitor group compared with the tamoxifen group (OR = 0.55, 95% CI = 0.46 to 0.64, \( P < .001 \)) (Figure 2, C). Adjustment for differential survival between the aromatase inhibitor group and the tamoxifen group did not change the pooled odds ratio for all cohorts. There was no evidence for heterogeneity (\( \chi^2 P = 0.59, I^2 = 0\% \))

The incidence of thrombosis was 1.6% and 2.8% in the aromatase inhibitor and tamoxifen groups, respectively (difference in absolute risk = −1.3%, number needed to harm = −79 (Table 2 and Table 3). The test of subgroup differences for upfront aromatase inhibitors vs switching from tamoxifen to aromatase inhibitors was not statistically significant (\( P = .67 \)), suggesting that that the relative harm of 2–3 years of tamoxifen was not reduced by switching to aromatase inhibitors.

Bone Fractures

Longer durations of aromatase inhibitor use were associated with increased odds of bone fractures compared with tamoxifen use. A pooled analysis of the data for all three cohorts showed that a longer duration of aromatase inhibitor use was associated with a 47% increase in the odds of bone fractures compared with tamoxifen (OR = 1.47, 95% CI = 1.34 to 1.61, \( P < .001 \)) (Figure 2, D). After adjustment for differential survival between the
aromatase inhibitor group and the tamoxifen group, the odds ratio decreased slightly to 1.45 (95% CI = 1.33 to 1.60) but remained statistically significant (P < .001). There was no evidence for heterogeneity (χ² P=.43, I²=0%). In absolute terms, fracture incidence was 7.5% and 5.2% in the aromatase inhibitor and tamoxifen groups, respectively (difference in absolute risk = 2.2%, number needed to harm = 46) (Table 2 and Table 3). The test of subgroup differences for upfront aromatase inhibitors vs switching from tamoxifen to aromatase inhibitors was not statistically significant (P = .97), suggesting that there was no difference in the relative harm of aromatase inhibitors between upfront use of aromatase inhibitors use of aromatase inhibitors after switching from tamoxifen.

Endometrial Carcinoma

Analysis of the pooled data showed that longer duration of aromatase inhibitor use was associated with a 66% reduction in the relative odds of endometrial carcinoma compared with tamoxifen (OR = 0.34, 95% CI = 0.22 to 0.53, P < .001) (Figure 2, E). Adjustment for differential survival between the aromatase inhibitor group and the tamoxifen group did not change the pooled odds ratio for any of the cohorts. There was no evidence for heterogeneity (χ² P=.43, I²=0%). Endometrial carcinoma was a very rare event: it occurred in 0.1% of the aromatase inhibitor group and in 0.5% of the tamoxifen group (difference in absolute risk = −0.4%, number needed to harm = −258) (Table 2 and Table 3). The test of subgroup differences for upfront aromatase inhibitors vs switching from tamoxifen to aromatase inhibitors was not statistically significant (P = .35).
Other Second Cancers

Analysis of the pooled data showed no statistically significant difference in the odds of developing other second cancers between the aromatase inhibitor group and the tamoxifen group (OR = 0.98, 95% CI = 0.85 to 1.14, \( P = .83 \)), and adjustment for differential survival between the groups did not change the pooled odds ratio for any of the cohorts. The absolute rates of other cancers were 4.7% for aromatase inhibitor–treated patients and 4.8% for those receiving tamoxifen alone (Table 3). The test of subgroup difference for upfront aromatase inhibitors vs switching from tamoxifen to aromatase inhibitors was statistically significant (\( P = .02 \)), suggesting that switching from tamoxifen to aromatase inhibitors may decrease the odds of second cancers. Consequently, there was also evidence for heterogeneity (\( \chi^2 P=.05, I^2=58\% \)).

Hypercholesterolemia

Hypercholesterolemia at any point during treatment or follow-up was assessed formally by only four studies (2,3,13,15) and was not graded consistently among those studies. Analysis of the pooled data showed that longer duration of aromatase inhibitor use was associated with a statistically significant increase in the odds of hypercholesterolemia compared with tamoxifen (OR = 2.36, 95% CI = 2.15 to 2.60, \( P < .001 \)). This effect was most apparent when upfront aromatase inhibitor use was compared with tamoxifen alone (OR = 3.14, 95% CI = 2.78 to 3.55, \( P < .001 \)) (3), less marked when upfront aromatase inhibitor use was compared with switching from tamoxifen to aromatase inhibitors (OR = 1.71, 95% CI = 1.38 to 2.13, \( P < .001 \)) (15), and least evident when switching from tamoxifen to aromatase
inhibitors was compared with tamoxifen alone (OR = 1.27, 95% CI = 1.01 to 1.59, \( P = .04 \)) (2,13). The test of subgroup differences for upfront aromatase inhibitor use vs switching from tamoxifen to aromatase inhibitors was statistically significant (\( P < .001 \)), suggesting that shorter durations of aromatase inhibitors might reduce the odds of hypercholesterolemia. Consequently, there was also evidence for heterogeneity (\( \chi^2 P<.001, I^2=95\% \))

**Death without Recurrence**

In a pooled analysis, use of up-front aromatase inhibitors was associated with a non-statistically significant higher odds of death without recurrence compared with use of tamoxifen alone or switching from tamoxifen to aromatase inhibitors (OR = 1.11, 95% CI = 0.98 to 1.26, \( P = .09 \)). Conversely, switching from tamoxifen to aromatase inhibitors was associated with decreased odds of death without recurrence compared with up-front tamoxifen for 5 years (OR = 0.75, 95% CI = 0.58 to 0.98, \( P = .04 \)) (Figure 3). The test of subgroup differences for upfront aromatase inhibitor use vs switching from tamoxifen to aromatase inhibitors was statistically significant (\( P = .03 \)), which suggests that switching to aromatase inhibitors after 2–3 years of tamoxifen may reduce the odds of death without recurrent breast cancer compared with the use of either tamoxifen or aromatase inhibitors alone. Analysis of all data combined revealed no association between longer duration of aromatase inhibitor use and the odds of death without recurrence (OR = 1.04, 95% CI = 0.93 to 1.16, \( P = .51 \)) (Figure 3). Compared with those treated with 5 years of either tamoxifen or aromatase inhibitors, those treated with a switching strategy had statistically significant
reduction in the odds of death without breast cancer recurrence (OR = 0.87, 95% CI = 0.77 to 0.99, \( P = .03 \)). Adjustment for differential survival between the aromatase inhibitor group and the tamoxifen group did not change the pooled odds ratio. There was no evidence for heterogeneity (\( \chi^2 P = .23, I^2 = 26\% \)). In absolute terms, 4.2% of patients who received longer durations of aromatase inhibitor died without breast cancer recurrence compared with 4.1% of patients treated predominantly with tamoxifen (difference in absolute risk = 0.1%, number needed to harm = 610) (Table 2 and Table 3).

**Discussion**

This systematic review and meta-analysis shows that, compared with tamoxifen, the use of aromatase inhibitors in postmenopausal women with early-stage breast cancer increases the odds of developing cardiovascular disease and bone fractures and decreases the odds of venous thrombosis and endometrial carcinoma. There were no differences in the odds of cerebrovascular disease, other second cancers, or death without breast cancer recurrence between treatment strategies.

Aromatase inhibitors can reduce recurrence of breast cancer, and pooled trial data show that they improve disease-free survival\(^68\). However, in most trials, the improvement in breast cancer–specific outcomes has not resulted in subsequent improvement in overall survival. For example, when compared to tamoxifen alone, upfront use of aromatase inhibitors is associated with an improvement in the probability of recurrence of 3.8% at 8 years (number needed to treat \([\text{NNT}] = 27\)). In contrast, there was little difference in all-
cause mortality (NNT = 500). Similar data were observed when aromatase inhibitor use after initial tamoxifen was compared to tamoxifen alone (NNT = 30 versus 46 respectively). The lack of association between disease-free survival and overall survival requires careful evaluation of the toxicities of different endocrine therapy options. Tamoxifen and aromatase inhibitors have distinct toxicity profiles; however, individual trials have not shown a statistically significant difference in overall toxicity between patients treated with these therapies. Although randomized trials may have enough statistical power to detect differences in common toxicities between treatment groups, they typically lack statistical power to detect differences in rare but potentially serious adverse events. A previous attempt to pool trial data to assess the differential toxicity of aromatase inhibitors and tamoxifen was based on a limited number of studies with relatively short follow-up. The current study included a larger number of studies and longer follow-up.

These data show that aromatase inhibitors and tamoxifen have different toxicity profiles (Figure 2 and Table 3). Many of the included adverse events were rare; therefore, if clinical decisions are made based on these data, they should be based on absolute risk (or number needed to harm) rather than relative risk, which can be confusing especially for endpoints of varying frequencies. Furthermore, as individual patients may have experienced more than one adverse event, absolute risks for the different adverse events should not be summed as this is not equivalent to the number of patients with one or more event. For cardiovascular events, pooled data showed that longer durations of aromatase inhibitor use are associated with a statistically significantly higher odds of developing such events.
compared with tamoxifen alone or a shorter period of aromatase inhibitor use after an initial period of tamoxifen therapy (OR = 1.26, P < .001). The effect size demonstrated in individual trials was consistent among all included studies, and this finding was independent of whether patients received upfront aromatase inhibitors or whether aromatase inhibitors were given after 2–3 years of tamoxifen. Although the effect sizes corresponded to only a small increase in the absolute risk of cardiovascular disease in the overall population of women who received adjuvant hormonal therapy (<1%, number needed to harm = 132), it is possible that specific subpopulations of patients are at higher risk. For example, the US Food and Drug Administration–revised label for anastrozole states that in women with pre-existing heart disease in the ATAC trial (7.5% of the total trial population), the incidence of cardiovascular events was 17% with anastrozole and 10% with tamoxifen and urges physicians to consider the risks and benefits of anastrozole therapy in such patients. This important information has not been published in the scientific literature. Data from this study suggest that the increased risk of cardiovascular events in women with pre-existing heart disease is not confined to anastrozole, but may be a class effect for aromatase inhibitors.

There are several possible explanations for the finding of an increased number of cardiovascular events with aromatase inhibitors compared with tamoxifen. First, data from male mice show that aromatase inhibitors may have a direct effect on endothelium that may predispose the mice to the development of atherosclerosis. Second, hypercholesterolemia is a well-known risk factor for the development of cardiovascular
disease. The pooled analysis showed that aromatase inhibitor use was associated with a statistically significantly increased odds of hypercholesterolemia compared with tamoxifen use (OR = 2.36, \( P < .001 \)). This effect was most apparent with longer durations of aromatase inhibitor use, suggesting that the cumulative exposure to aromatase inhibitors is what may be important. However, the possible effect of inter-study differences in duration of follow-up cannot be excluded given that vascular events are likely to accumulate for an extended period after a rise in cholesterol. Increases in hypercholesterolemia and serious cardiovascular events with aromatase inhibitors compared with tamoxifen were also demonstrated in a combined analysis of the up-front and sequencing arms of the BIG 1-98 study\(^82\). Finally, randomized and observational comparisons of tamoxifen vs. placebo or no treatment have shown that tamoxifen is associated with a reduction in cardiovascular events\(^83\)-\(^86\). Therefore, any differences in cardiovascular events between aromatase inhibitors with tamoxifen may be explained by this finding. Unfortunately, data on the independent effect of aromatase inhibitors on cardiovascular events is unreliable as reporting of such events in women participating in trials of aromatase inhibitors or placebo is variable. For example, data from the National Cancer Institute of Canada MA.17 trial of letrozole vs placebo after an initial 5 years of tamoxifen showed little difference in cardiovascular events between the study arms\(^87\). Moreover, cardiovascular adverse events were not reported at all in two other randomized trials also conducted in the extended adjuvant setting\(^88\);\(^89\). A prolonged protective effect of tamoxifen in patients receiving aromatase inhibitors after 5 years of initial tamoxifen therapy cannot be excluded\(^90\).
Oncologists should consider carefully the risks and benefits of aromatase inhibitors in patients with pre-existing heart disease or related risk factors. This is especially important in the extended adjuvant setting, where predictive factors for benefit remain scarce and the potential harm from ongoing aromatase inhibitor therapy may outweigh any small reductions in the recurrence of breast cancer.

No statistically significant difference between aromatase inhibitors and tamoxifen was found for the odds of cerebrovascular disease. However, the possibility that differences in risk of cerebrovascular disease may become more apparent with longer follow-up of trial participants cannot be excluded. The overlapping risk factors for the development of cardiovascular and cerebrovascular disease, including hypercholesterolemia, which is increased by aromatase inhibitors, might ultimately lead to an increase risk of cerebrovascular disease as well as of cardiovascular disease with aromatase inhibitors. The inconsistent association between aromatase inhibitors and cardiovascular and cerebrovascular disease is unexpected, but has two possible explanations. First, in the natural history of atherosclerosis, cardiovascular disease tends to present before cerebrovascular disease. It is possible that the limited follow-up in the included studies did not allow for cerebrovascular events to be adequately captured. Second, the etiology of cerebrovascular disease involves a balance between atherosclerosis and thrombosis.91:92. It is possible that any increase in risk of the former with AIs would be balanced by an increase in the latter with tamoxifen.
Data from this study suggest that when excluding studies where upfront use of aromatase inhibitors was not evaluated, (i.e. where a switch to aromatase inhibitors after 2–3 years of tamoxifen was compared to tamoxifen alone), such up-front use is associated with an increased odds of death without breast cancer recurrence compared with the use of tamoxifen alone or a switch to aromatase inhibitors after 2–3 years of tamoxifen. This finding may explain why up-front use of aromatase inhibitors improves disease-free survival but not overall survival. Furthermore, these data suggest that switching to aromatase inhibitors after 2–3 years of tamoxifen is associated with a reduction in the number of deaths without breast cancer recurrence compared with the use of either tamoxifen or aromatase inhibitors alone. One explanation for this finding is a reduction in risk of cumulative toxicities brought about by switching from one agent to another. These hypothesis-generating data appear to support the use of switching strategies as a way to reduce cumulative toxicities and may be particularly relevant among older women. For example, a secondary analysis of the ATAC trial showed that older age and increasing number of comorbidities were associated with a substantially increased risk of death without recurrence in women with lymph node–negative breast cancer for both tamoxifen and anastrozole treated patients.93

From a clinical viewpoint, data on cardiovascular adverse events suggest that the use of aromatase inhibitors for postmenopausal women with ischemic heart disease should be considered on an individual basis. In patients who are at a lower risk of breast cancer
recurrence for whom the absolute benefits of aromatase inhibitors are reduced, use of these agents should be avoided. In those who are at higher risk of breast cancer recurrence, the absolute benefit of aromatase inhibitors is greater and therefore these agents should be used in sequence with tamoxifen. Conversely, patients with a personal or family history of thrombo-embolic disease should probably avoid tamoxifen\textsuperscript{94}. These data may help physicians to better counsel patients with risk factors for toxicities from both treatments about their treatment options.

Pooled data have confirmed results reported previously in the individual studies that compared with tamoxifen, aromatase inhibitors are associated with an increased odds of bone fracture (OR = 1.47, \( P < .001 \)) and reduced odds of venous thrombosis (OR = 0.52, \( P < .001 \)) and endometrial carcinoma (OR = 0.34, \( P < .001 \)). Switching from one agent to another did not appear modify the relative risks of developing these adverse events. However, in most of the switching studies included in this meta-analysis, randomization to an aromatase inhibitor occurred after an initial 2–3 years of tamoxifen, and toxicities that occurred before randomization were not recorded. Furthermore, because the current analysis was based on intention to treat, any patients who crossed over from the tamoxifen arms to aromatase inhibitors may have reduced protection from toxicity associated with switching strategies.

This study has several limitations. First, this is a meta-analysis of the literature rather than of individual patient data and is based on reports of trials with different durations of follow-up; it was not possible to report actuarial rates of toxicity. The availability of
individual patient data may also allow for new statistical analyses to be conducted. In this setting, it would have been interesting to assess the interaction of age, co-morbidity and the effect of concomitant medications on adverse events. Second, collection of data for the purpose of this meta-analysis was dependent on the rigorous collection and reporting of adverse events by the investigators. The availability of such data is dependent on both adequate collection and complete reporting of adverse events. It is known that published studies have variable quality in both\(^9\). Furthermore, adverse events are usually collected in intervention trials only until an event of interest occurs, such as breast cancer recurrence (the primary endpoint in these trials). Consequently, the data presented in this analysis may over-estimate adverse event rates with aromatase inhibitors as fewer patients treated with these agents will relapse and therefore the pool of evaluable patients will be of greater magnitude. Adverse events after breast cancer recurrence remain of interest because most women with hormone receptor–positive breast cancer survive for several years, even with metastatic disease. Such data were not available for the trials included in this meta-analysis. Third, information on the potentially confounding baseline host factors (e.g., obesity, hypertension, diabetes, family history of events of interest) or the use of concurrent medications was not available and therefore their effects on the odds of the adverse events could not be quantified. However, as most included studies were large randomized trials, it is likely that such baseline factors would be balanced between groups. Fourth, the analysis included all grades of toxicity. Higher-grade toxicities have more profound early implications; however, the influence of grading of toxicity on long-term health outcomes is unclear. In keeping with the objective to explore serious and/or potentially life-threatening
toxicities, it was felt that inclusion of all grades of toxicity was important. Fifth, a number of different treatment strategies were evaluated in the included studies (upfront aromatase inhibitors vs. tamoxifen, switching vs. tamoxifen and switching vs. upfront aromatase inhibitors). Only one study assessed switching versus upfront aromatase inhibitors. The strength of this comparison could have been improved using a network meta-analysis where statistical inferences can be made to compare two treatments which have not been formally compared, but share a common treatment arm. However, in such methods, certain assumptions about homogeneity are necessary for valid estimates of indirect comparisons. As results from included studies showed some heterogeneity and as all comparison included well powered assessments, it was not felt that a network meta-analysis would add substantially to the analysis. Finally, it may not be possible to generalize the results of this analysis to patients treated outside of clinical trials. Trial participants are usually highly selected and may not be representative of patients treated in general practice. Trials usually have multiple exclusion criteria, which include particular co-morbidities or the use of certain concomitant medications. Therefore, it is likely that the probability of adverse events will be greater in general practice compared to that observed in clinical trials.

Despite these limitations, this study demonstrates a statistically significant and consistent increase in cardiovascular risk associated with the use of aromatase inhibitors. Although the increase in absolute risk is small in the general population, it is likely to be higher in patients with pre-existing cardiovascular disease or risk factors associated with it. Given the finding that aromatase inhibitor use is associated with a greater than twofold
increase in the odds of hypercholesterolemia, the risk of cardiovascular events may increase further with longer follow-up of patients on these trials. Furthermore, data suggest an increase in the risk of death without breast cancer recurrence associated with the use of either tamoxifen or aromatase inhibitors alone compared with the use of aromatase inhibitors after 2–3 years of tamoxifen. Switching from tamoxifen to aromatase inhibitors appears to be the optimal strategy for offsetting serious adverse events of individual drugs.

In conclusion, clinicians are urged to consider carefully the toxicity profiles of different endocrine therapy options for breast cancer and choose those that best suit their patients’ baseline health characteristics. Investigators participating in practice-changing clinical trials or in trial overviews should rigorously report not only efficacy data, but also data on less common and potentially serious toxicities.
Table 1. Characteristics of included studies

<table>
<thead>
<tr>
<th>Trial (reference)</th>
<th>Trial Funding</th>
<th>Median follow-up (mo)</th>
<th>Treatment arms</th>
<th>Sample size</th>
<th>Age of patients (y)</th>
<th>Patients with tumour size &gt;2cm (%)</th>
<th>Node-positive patients (%)</th>
<th>Hormone receptor-positive patients (%)</th>
<th>Other adjuvant therapy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATAC67</td>
<td>Industry</td>
<td>100</td>
<td>A T</td>
<td>3125</td>
<td>Mean: 64.1</td>
<td>36</td>
<td>39</td>
<td>84</td>
<td>Radiotherapy: 63 Chemotherapy: 20</td>
</tr>
<tr>
<td>BIG01-9895</td>
<td>Industry</td>
<td>51</td>
<td>L T</td>
<td>2463</td>
<td>Median: 61</td>
<td>38</td>
<td>43</td>
<td>100</td>
<td>Radiotherapy: 72 Chemotherapy: 25</td>
</tr>
<tr>
<td>IES74</td>
<td>Industry</td>
<td>55.7</td>
<td>T→E T</td>
<td>2352</td>
<td>Median: 63.9</td>
<td>52</td>
<td>48</td>
<td>98</td>
<td>Chemotherapy: 33</td>
</tr>
<tr>
<td>ABCSG8/ARNO 95</td>
<td>Industry / Non-industry ‡</td>
<td>28</td>
<td>T→A T</td>
<td>1618</td>
<td>Median: 62</td>
<td>30</td>
<td>26</td>
<td>100</td>
<td>0+†</td>
</tr>
<tr>
<td>ITA64</td>
<td>Industry</td>
<td>64</td>
<td>T→A T</td>
<td>225</td>
<td>Median: 63</td>
<td>51</td>
<td>100</td>
<td>100</td>
<td>Radiotherapy: 49 Chemotherapy: 67</td>
</tr>
<tr>
<td>N-SAS BC0375</td>
<td>Non-industry</td>
<td>42</td>
<td>T→A T</td>
<td>347</td>
<td>Mean: 59.9</td>
<td>22</td>
<td>40</td>
<td>93</td>
<td>Chemotherapy: 53</td>
</tr>
<tr>
<td>TEAM74</td>
<td>Industry</td>
<td>61</td>
<td>T→E E</td>
<td>4868</td>
<td>Mean: 64.5</td>
<td>41</td>
<td>47</td>
<td>100</td>
<td>Radiotherapy: 70 Chemotherapy: 36</td>
</tr>
</tbody>
</table>

*ATAC = Anastrozole, Tamoxifen Alone or in Combination; A = anastrozole; T = tamoxifen; BIG = Breast International Group01-98/International Breast Cancer Study Group 18-98; L = letrozole; IES = Intergroup Exemestane Study; E = exemestane; ABCSG = Austrian Breast Cancer Study Group VIII; ARNO = German Adjuvant Breast Cancer Group/Arimidex-Nolvadex; ITA = Italian Tamoxifen Anastrozole Trial; N-SAS BC03 = National Surgical Adjuvant Study Breast Cancer 03 trial; TEAM = Tamoxifen Exemestane Adjuvant Multicenter Trial. ‡ No previous radiotherapy or chemotherapy was allowed. † ABCSG8 was funded by industry and ARNO 95 was non-industry funded.
<table>
<thead>
<tr>
<th>Trial (median follow-up)</th>
<th>Adverse event</th>
<th>No. of evaluable patients</th>
<th>No. of events on AI (%)</th>
<th>Number of events on switching (%)</th>
<th>No. of events on tamoxifen (%)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATAC (100 mo)</td>
<td>Cardiovascular disease†</td>
<td>A: 3092 T: 3094</td>
<td>130 (4.2)</td>
<td>NA</td>
<td>106 (3.4)</td>
<td>Forbes et al.67</td>
</tr>
<tr>
<td></td>
<td>Cerebrovascular disease</td>
<td>A: 3092 T: 3094</td>
<td>64 (2.1)</td>
<td>NA</td>
<td>91 (2.9)</td>
<td>Supplementary material</td>
</tr>
<tr>
<td></td>
<td>Venous thromboembolism</td>
<td>A: 3092 T: 3094</td>
<td>87 (2.8)</td>
<td>NA</td>
<td>141 (4.6)</td>
<td>Forbes et al.67</td>
</tr>
<tr>
<td></td>
<td>Bone fractures</td>
<td>A: 3092 T: 3094</td>
<td>521 (16.7)</td>
<td>NA</td>
<td>377 (12.1)</td>
<td></td>
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<tr>
<td></td>
<td>Endometrial carcinoma</td>
<td>A: 3092 T: 3094</td>
<td>5 (0.2)</td>
<td>NA</td>
<td>24 (0.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other second cancers</td>
<td>A: 3092 T: 3094</td>
<td>287 (9.3)</td>
<td>NA</td>
<td>264 (8.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Death without recurrence</td>
<td>A: 3125 T: 3116</td>
<td>279 (8.9)</td>
<td>NA</td>
<td>242 (7.8)</td>
<td></td>
</tr>
<tr>
<td>BIG 1-98 (51 mo)</td>
<td>Cardiovascular disease†</td>
<td>L: 2448 T: 2447</td>
<td>78 (3.2)</td>
<td>NA</td>
<td>55 (2.2)</td>
<td>Coates et al.65</td>
</tr>
<tr>
<td></td>
<td>Cerebrovascular disease</td>
<td>L: 2448 T: 2447</td>
<td>34 (1.4)</td>
<td>NA</td>
<td>35 (1.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Venous thromboembolism</td>
<td>L: 2448 T: 2447</td>
<td>50 (2.0)</td>
<td>NA</td>
<td>94 (3.8)</td>
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<tr>
<td></td>
<td>Bone fractures</td>
<td>L: 2448 T: 2447</td>
<td>211 (8.6)</td>
<td>NA</td>
<td>141 (5.8)</td>
<td></td>
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<tr>
<td></td>
<td>Endometrial carcinoma</td>
<td>L: 2448 T: 2447</td>
<td>4 (0.2)</td>
<td>NA</td>
<td>16 (0.7)</td>
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</tr>
<tr>
<td></td>
<td>Other second cancers</td>
<td>L: 2448 T: 2447</td>
<td>59 (2.4)</td>
<td>NA</td>
<td>66 (2.7)</td>
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<tr>
<td></td>
<td>Death without recurrence§</td>
<td>L: 2463 T: 2459</td>
<td>87 (3.5)</td>
<td>NA</td>
<td>87 (3.5)</td>
<td>Mouridsen et al.97</td>
</tr>
</tbody>
</table>

Supplementary material
<table>
<thead>
<tr>
<th>Trial (median follow-up)</th>
<th>Adverse event</th>
<th>No. of evaluable patients</th>
<th>No. of events on Al (%)</th>
<th>Number of events on switching (%)</th>
<th>No. of events on tamoxifen (%)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>IES (55.7 mo)</td>
<td>Cardiovascular disease†</td>
<td>E: 2352 T: 2372</td>
<td>NA</td>
<td>261 (11.1)</td>
<td>233 (9.8)</td>
<td>Coombes et al.72</td>
</tr>
<tr>
<td></td>
<td>Cerebrovascular disease</td>
<td>E: 2352 T: 2372</td>
<td>NA</td>
<td>32 (1.4)</td>
<td>33 (1.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Venous thromboembolism</td>
<td>E: 2352 T: 2372</td>
<td>NA</td>
<td>45 (1.9)</td>
<td>72 (3.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bone fractures</td>
<td>E: 2352 T: 2372</td>
<td>NA</td>
<td>162 (7.0)</td>
<td>115 (4.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Endometrial carcinoma</td>
<td>E: 2352 T: 2372</td>
<td>NA</td>
<td>5 (0.2)</td>
<td>10 (0.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other second cancers</td>
<td>E: 2362 T: 2380</td>
<td>NA</td>
<td>27 (1.1)</td>
<td>53 (2.2)</td>
<td>Coombes et al.98</td>
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<tr>
<td></td>
<td>Death without recurrence</td>
<td>E: 2352 T: 2372</td>
<td>NA</td>
<td>71 (3.0)</td>
<td>95 (4.0)</td>
<td>Coombes et al.72</td>
</tr>
<tr>
<td>ABCSG8/ARNO (28 mos)</td>
<td>Cardiovascular disease‡</td>
<td>A: 1618 T: 1606</td>
<td>NA</td>
<td>3 (0.2)</td>
<td>2 (0.1)</td>
<td>Jakesz et al.66</td>
</tr>
<tr>
<td></td>
<td>Cerebrovascular disease</td>
<td>NS</td>
<td>NA</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Venous thromboembolism</td>
<td>A: 1618 T: 1606</td>
<td>NA</td>
<td>3 (0.2)</td>
<td>12 (0.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bone fractures</td>
<td>A: 1618 T: 1606</td>
<td>NA</td>
<td>34 (2.1)</td>
<td>16 (1.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Endometrial carcinoma</td>
<td>A: 1618 T: 1606</td>
<td>NA</td>
<td>1 (&lt;0.1)</td>
<td>7 (0.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other second cancers</td>
<td>NS</td>
<td>NA</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Death without recurrence</td>
<td>A: 1618 T: 1606</td>
<td>NA</td>
<td>21 (1.3)</td>
<td>28 (1.8)</td>
<td></td>
</tr>
<tr>
<td>Trial (median follow-up)</td>
<td>Adverse event</td>
<td>No. of evaluable patients</td>
<td>No. of events on AI (%)</td>
<td>Number of events on switching (%)</td>
<td>No. of events on tamoxifen (%)</td>
<td>Source</td>
</tr>
<tr>
<td>--------------------------</td>
<td>----------------------------------------------</td>
<td>---------------------------</td>
<td>-------------------------</td>
<td>----------------------------------</td>
<td>-------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>ITA (64 mo)</td>
<td>Cardiovascular disease†</td>
<td>A: 223 T: 225</td>
<td>NA</td>
<td>17 (7.6)</td>
<td>14 (6.3)</td>
<td>Boccardo et al. 64</td>
</tr>
<tr>
<td></td>
<td>Cerebrovascular disease</td>
<td>NS</td>
<td>NA</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Venous thromboembolism</td>
<td>A: 223 T: 225</td>
<td>NA</td>
<td>5 (2.2)</td>
<td>10 (4.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bone fractures</td>
<td>NS</td>
<td>NA</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Endometrial carcinoma</td>
<td>A: 223 T: 225</td>
<td>NA</td>
<td>1 (0.4)</td>
<td>6 (2.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other second cancers</td>
<td>A: 223 T: 225</td>
<td>NA</td>
<td>5 (2.2)</td>
<td>4 (1.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Death without recurrence</td>
<td>A: 223 T: 225</td>
<td>NA</td>
<td>2 (0.9)</td>
<td>3 (1.3)</td>
<td></td>
</tr>
<tr>
<td>N-SAS BC03 (42 mo)</td>
<td>Cardiovascular disease†</td>
<td>A: 347 T: 349</td>
<td>NA</td>
<td>2 (0.6)</td>
<td>3 (0.9)</td>
<td>Aihara et al. 73</td>
</tr>
<tr>
<td></td>
<td>Cerebrovascular disease</td>
<td>NS</td>
<td>NA</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Venous thromboembolism</td>
<td>A: 347 T: 349</td>
<td>NA</td>
<td>1 (0.3)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bone fractures</td>
<td>A: 347 T: 349</td>
<td>NA</td>
<td>5 (1.4)</td>
<td>9 (2.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Endometrial carcinoma</td>
<td>A: 347 T: 349</td>
<td>NA</td>
<td>0 (0)</td>
<td>1 (0.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other second cancers</td>
<td>A: 347 T: 349</td>
<td>NA</td>
<td>7 (2.0)</td>
<td>7 (2.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Death without recurrence</td>
<td>A: 347 T: 349</td>
<td>NA</td>
<td>2 (0.6)</td>
<td>1 (0.3)</td>
<td></td>
</tr>
<tr>
<td>Trial (median follow-up)</td>
<td>Adverse event</td>
<td>No. of evaluable patients</td>
<td>No. of events on AI (%)</td>
<td>Number of events on switching (%)</td>
<td>No. of events on tamoxifen (%)</td>
<td>Source</td>
</tr>
<tr>
<td>--------------------------</td>
<td>--------------------------------------</td>
<td>---------------------------</td>
<td>-------------------------</td>
<td>----------------------------------</td>
<td>--------------------------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>TEAM (61 mo)</td>
<td>Cardiovascular disease†</td>
<td>E: 4898 T: 4868</td>
<td>132 (2.7)</td>
<td>96 (2.0)</td>
<td>NA</td>
<td>van de Velde et al.⁷⁴</td>
</tr>
<tr>
<td></td>
<td>Cerebrovascular disease</td>
<td>E: 4898 T: 4868</td>
<td>51 (1.1)</td>
<td>35 (0.7)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Venous thromboembolism</td>
<td>E: 4898 T: 4868</td>
<td>45 (0.9)</td>
<td>97 (2.0)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bone fractures</td>
<td>E: 4898 T: 4868</td>
<td>249 (5.1)</td>
<td>170 (3.5)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Endometrial carcinoma</td>
<td>E: 4898 T: 4868</td>
<td>7 (0.1)</td>
<td>17 (0.4)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other second cancers</td>
<td>NS</td>
<td>NS</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Death without recurrence</td>
<td>E: 4898 T: 4868</td>
<td>213 (4.3)</td>
<td>193 (4.0)</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

* ATAC = Anastrozole, Tamoxifen Alone or in Combination; A = anastrozole; T = tamoxifen; BIG = Breast International Group 01-98/International Breast Cancer Study Group 18-98; L = letrozole; IES = Intergroup Exemestane Study; E = exemestane; ABCSG = Austrian Breast Cancer Study Group VIII; ARNO = German Adjuvant Breast Cancer Group/Arimidex-Nolvadex; ITA = Italian Tamoxifen Anastrozole Trial; N-SAS BC03 = National Surgical Adjuvant Study Breast Cancer 03 trial; TEAM = Tamoxifen Exemestane Adjuvant Multicenter Trial; NS = not specified; NA = not applicable.

†Data on cardiovascular disease including cardiac failure.

‡Data on myocardial infarction only.

§Based on median follow-up of 76 months
Table 3. Absolute differences in adverse events and number needed to harm associated with one adverse event of each type. Positive values indicate higher absolute risks in the aromatase inhibitor group whereas negative values indicate higher absolute risks in the tamoxifen group.

<table>
<thead>
<tr>
<th>Trial (reference)</th>
<th>Cardiovascular disease</th>
<th>Cerebrovascular disease</th>
<th>Venous thrombosis</th>
<th>Bone fractures</th>
<th>Endometrial Carcinoma</th>
<th>Other second cancers</th>
<th>Death without recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absolute difference (%)</td>
<td>NNH</td>
<td>Absolute difference (%)</td>
<td>NNH</td>
<td>Absolute difference (%)</td>
<td>NNH</td>
<td>Absolute difference (%)</td>
</tr>
<tr>
<td>ATAC67</td>
<td>0.8</td>
<td>129</td>
<td>−0.8</td>
<td>−115</td>
<td>−1.8</td>
<td>−59</td>
<td>4.6</td>
</tr>
<tr>
<td>BIG01-9865</td>
<td>0.9</td>
<td>107</td>
<td>0</td>
<td>∞</td>
<td>−1.8</td>
<td>−56</td>
<td>2.8</td>
</tr>
<tr>
<td>IES72</td>
<td>1.3</td>
<td>79</td>
<td>0</td>
<td>∞</td>
<td>−1.2</td>
<td>−84</td>
<td>2.1</td>
</tr>
<tr>
<td>ABCSG8/ARNO 9566</td>
<td>&lt;0.1†</td>
<td>1643†</td>
<td>NS</td>
<td>NS</td>
<td>−0.6</td>
<td>−179</td>
<td>1.1</td>
</tr>
<tr>
<td>IITA64</td>
<td>1.3</td>
<td>72</td>
<td>NS</td>
<td>NS</td>
<td>−2.3</td>
<td>−40</td>
<td>NS</td>
</tr>
<tr>
<td>N-SAS BC0373</td>
<td>−0.3</td>
<td>−354</td>
<td>NS</td>
<td>NS</td>
<td>0.3</td>
<td>347</td>
<td>−1.2</td>
</tr>
<tr>
<td>TEAM74</td>
<td>0.7</td>
<td>139</td>
<td>0.4</td>
<td>311</td>
<td>−1.1</td>
<td>−91</td>
<td>1.6</td>
</tr>
<tr>
<td>Pooled</td>
<td>0.8</td>
<td>132</td>
<td>−0.1</td>
<td>−974</td>
<td>−1.3</td>
<td>−79</td>
<td>2.2</td>
</tr>
</tbody>
</table>

*Positive values indicate excess events with aromatase inhibitors and negative values indicate excess events with tamoxifen. NNH = number needed to harm (positive values indicate higher absolute risks in the aromatase inhibitor group whereas negative values indicate higher absolute risks in the tamoxifen group); NS = not specified.
†Myocardial infarctions only.
Figure 1. Designs of included studies.
Figure 2. Forest plots of odds ratios for adverse events. A) Cardiovascular events. B) Cerebrovascular events. C) Venous thrombosis. D) Bone fractures. E) Endometrial carcinoma. F) Other second cancers.

A

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>Weight (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upfront AI vs Tamoxifen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATAC</td>
<td>24.3</td>
<td>1.24 [0.95 to 1.61]</td>
</tr>
<tr>
<td>BIG 1-96</td>
<td>13.8</td>
<td>1.43 [1.01 to 2.03]</td>
</tr>
<tr>
<td>Subtotal</td>
<td>38.1</td>
<td>1.30 [1.06 to 1.61]</td>
</tr>
</tbody>
</table>

Test for overall effect: Z = 2.48 (P = .01)

Tamoxifen to AI vs Tamoxifen alone

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>Weight (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCSG8/ARNO</td>
<td>0.5</td>
<td>1.51 [0.25 to 9.02]</td>
</tr>
<tr>
<td>IES</td>
<td>34.2</td>
<td>1.15 [0.92 to 1.43]</td>
</tr>
<tr>
<td>ITA</td>
<td>3.1</td>
<td>1.22 [0.59 to 2.54]</td>
</tr>
<tr>
<td>N-SAS BC03</td>
<td>0.5</td>
<td>0.67 [0.11 to 4.03]</td>
</tr>
<tr>
<td>Subtotal</td>
<td>38.3</td>
<td>1.15 [0.93 to 1.41]</td>
</tr>
</tbody>
</table>

Test for overall effect: Z = 1.29 (P = .20)

B

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>Weight (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upfront AI vs Tamoxifen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATAC</td>
<td>31.5</td>
<td>0.77 [0.52 to 1.15]</td>
</tr>
<tr>
<td>BIG 1-96</td>
<td>21.5</td>
<td>0.96 [0.60 to 1.55]</td>
</tr>
<tr>
<td>Subtotal</td>
<td>53.0</td>
<td>0.84 [0.62 to 1.14]</td>
</tr>
</tbody>
</table>

Test for overall effect: Z = 1.09 (P = .28)

Tamoxifen to AI vs Tamoxifen alone

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>Weight (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IES</td>
<td>20.7</td>
<td>1.01 [0.62 to 1.64]</td>
</tr>
<tr>
<td>Subtotal</td>
<td>20.7</td>
<td>1.01 [0.62 to 1.64]</td>
</tr>
</tbody>
</table>

Test for overall effect: Z = 0.03 (P = .97)

Tamoxifen to AI vs AI alone

<table>
<thead>
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<th>Study or subgroup</th>
<th>Weight (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEAM</td>
<td>26.3</td>
<td>1.45 [0.94 to 2.23]</td>
</tr>
<tr>
<td>Subtotal</td>
<td>26.3</td>
<td>1.45 [0.94 to 2.23]</td>
</tr>
</tbody>
</table>

Test for overall effect: Z = 1.69 (P = .09)

Total

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>Weight (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamoxifen to AI vs AI alone</td>
<td>100.0</td>
<td>1.01 [0.81 to 1.26]</td>
</tr>
</tbody>
</table>

Test for overall effect: Z = 0.09 (P = .93)

Test for subgroup differences: $\chi^2 = 4.04$ (P = .13)
### C

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>Weight (%)</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Upfront AI vs Tamoxifen</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATAC</td>
<td>35.7</td>
<td>0.61 [0.46 to 0.80]</td>
<td></td>
</tr>
<tr>
<td>BIG 1-98</td>
<td>21.2</td>
<td>0.52 [0.37 to 0.74]</td>
<td></td>
</tr>
<tr>
<td>Subtotal</td>
<td>56.9</td>
<td>0.57 [0.46 to 0.71]</td>
<td></td>
</tr>
</tbody>
</table>

**Test for overall effect:** $Z = 5.06$ ($P < .001$)

#### Tamoxifen to AI vs Tamoxifen alone

<table>
<thead>
<tr>
<th></th>
<th>Weight (%)</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCSGB/ARNO</td>
<td>1.6</td>
<td>0.25 [0.07 to 0.88]</td>
<td></td>
</tr>
<tr>
<td>IES</td>
<td>18.3</td>
<td>0.62 [0.43 to 0.91]</td>
<td></td>
</tr>
<tr>
<td>ITA</td>
<td>2.2</td>
<td>0.49 [0.17 to 1.47]</td>
<td></td>
</tr>
<tr>
<td>Subtotal</td>
<td>22.1</td>
<td>0.57 [0.40 to 0.80]</td>
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</tr>
</tbody>
</table>

**Test for overall effect:** $Z = 3.22$ ($P = .001$)

### D

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<th>Study or subgroup</th>
<th>Weight (%)</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Upfront AI vs Tamoxifen</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATAC</td>
<td>42.4</td>
<td>1.45 [1.26 to 1.68]</td>
<td></td>
</tr>
<tr>
<td>BIG 1-98</td>
<td>18.1</td>
<td>1.54 [1.24 to 1.92]</td>
<td></td>
</tr>
<tr>
<td>Subtotal</td>
<td>60.5</td>
<td>1.48 [1.31 to 1.67]</td>
<td></td>
</tr>
</tbody>
</table>

**Test for overall effect:** $Z = 6.45$ ($P < .001$)

#### Tamoxifen to AI vs Tamoxifen alone

<table>
<thead>
<tr>
<th></th>
<th>Weight (%)</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCSGB/ARNO</td>
<td>2.4</td>
<td>2.14 [1.18 to 3.90]</td>
<td></td>
</tr>
<tr>
<td>IES</td>
<td>14.1</td>
<td>1.41 [1.10 to 1.80]</td>
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</tr>
<tr>
<td>N-SAS BC03</td>
<td>0.7</td>
<td>0.58 [0.18 to 1.71]</td>
<td></td>
</tr>
<tr>
<td>Subtotal</td>
<td>17.1</td>
<td>1.44 [1.15 to 1.80]</td>
<td></td>
</tr>
</tbody>
</table>

**Test for overall effect:** $Z = 3.18$ ($P = .001$)

#### Tamoxifen to AI vs AI alone

<table>
<thead>
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<th></th>
<th>Weight (%)</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEAM</td>
<td>22.3</td>
<td>1.48 [1.21 to 1.80]</td>
<td></td>
</tr>
<tr>
<td>Subtotal</td>
<td>22.3</td>
<td>1.48 [1.21 to 1.80]</td>
<td></td>
</tr>
</tbody>
</table>

**Test for overall effect:** $Z = 3.91$ ($P < .001$)

**Total**

|                      | 100.0      | 1.47 [1.34 to 1.61] |             |

**Test for overall effect:** $Z = 8.19$ ($P < .001$)

**Test for subgroup differences:** $\chi^2 = 0.05$ ($P = .97$)
### E

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>Weight (%)</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Upfront AI vs Tamoxifen</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ATAC</td>
<td>20.4</td>
<td>0.21 [0.08 to 0.54]</td>
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<tr>
<td>BIG 1-96</td>
<td>16.2</td>
<td>0.25 [0.08 to 0.74]</td>
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<tr>
<td><strong>Subtotal</strong></td>
<td>36.6</td>
<td>0.22 [0.11 to 0.46]</td>
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Test for overall effect: Z = 4.05 (P < .001)

**Tamoxifen to AI vs Tamoxifen alone**

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<th>Weight (%)</th>
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<td>ITA</td>
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<td><strong>Subtotal</strong></td>
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Test for overall effect: Z = 2.19 (P < .03)

**Tamoxifen to AI vs AI alone**

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<td>24.6</td>
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Test for overall effect: Z = 1.99 (P < .05)

Total

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Test for overall effect: Z = 4.80 (P < .001)

Test for subgroup differences: $\chi^2$ = 2.11 (P = .35)

### F

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Test for overall effect: Z = 0.65 (P = .51)

**Tamoxifen to AI vs Tamoxifen alone**

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<td>1.01 [0.35 to 3.03]</td>
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<td><strong>Subtotal</strong></td>
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Test for overall effect: Z = 2.33 (P < .02)

Total

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Test for overall effect: Z = 0.22 (P = .83)

Test for subgroup differences: $\chi^2$ = 5.61 (P = .02)
**Figure 3.** Forest plot of odds ratios for death without breast cancer recurrence.

<table>
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<th>Weight (%)</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
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<tr>
<td>ATAC</td>
<td>38.7</td>
<td>1.16 [0.97 to 1.39]</td>
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<tr>
<td>BIG 1-98</td>
<td>14.0</td>
<td>1.00 [0.74 to 1.35]</td>
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<td>Subtotal</td>
<td>52.7</td>
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<td>ITA</td>
<td>0.4</td>
<td>0.67 [0.11 to 4.05]</td>
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<tr>
<td>N-SAS BC03</td>
<td>0.2</td>
<td>2.02 [0.18 to 22.36]</td>
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<td>Subtotal</td>
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<td>0.75 [0.58 to 0.98]</td>
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<td>Test for overall effect: Z = 2.08 (P = .04)</td>
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<tr>
<td>TEAM</td>
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<td>Subtotal</td>
<td>28.7</td>
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<td>Test for overall effect: Z = 0.92 (P = .36)</td>
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<td><strong>Total</strong></td>
<td>100.0</td>
<td>1.04 [0.93 to 1.16]</td>
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<tr>
<td>Test for subgroup differences: $\chi^2 = 6.72$ (P = .03)</td>
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Chapter 4: (Paper 3) Randomized Feasibility Study of De-escalated (every 12 weeks) versus Standard (every 3-4-weeks) Intravenous Pamidronate in Women with Low-Risk Bone Metastases from Breast Cancer

This article was submitted for publication as:


Introduction

Bone is a common site of metastasis from breast cancer. Women with bone as the predominant or only site of metastatic spread tend to have longer survival than women with visceral metastases\(^99\). Despite their relatively favourable outcomes, these women are at risk of developing skeletal related events (SREs), defined as the need for radiation or surgery to bone, pathological fractures, spinal cord compression, or hypercalcemia\(^100\). The administration of bisphosphonates in combination with systemic anti-cancer therapy has been shown in randomized trials to delay the onset and reduce the frequency of SREs\(^101;102\). The optimal dosing frequency of bisphosphonates is not
known. Treatment is usually given every 3-4 weeks based on data derived from the treatment of hypercalcemia of malignancy."}^{103-105}

There is substantial variation in the frequency of SREs among patients with bone metastases from breast cancer."^{106} Despite this, practice guidelines recommend that bone-targeted agents be given at the same dose and frequency to all patients irrespective of their individual risk."^{107} However, it is likely that some patients may derive similar benefit from less frequent administration of these agents. Bisphosphonates exert a prolonged effect on bone."^{108} In patients with metastatic bone disease, the median duration of symptomatic benefit from a single dose of pamidronate therapy is approximately 12 weeks."^{109}

Assessment of the efficacy of bisphosphonate treatment can be difficult. Radiological tests such as bone scintigraphy or computed tomography are unreliable, and the clinician often bases treatment decisions on a combination of the patients’ symptoms and/or development of SREs to document progression of bone disease."^{110} More recently, there has been increasing interest in the use of biochemical marker of bone resorption and bone formation to help assess bone response to therapy."^{111} Bone resorption can be assessed biochemically by the determination of bone resorption markers in serum and urine. Evidence has shown that N-terminal cross-linking telopeptide of type I collagen (NTx) is a rapid and reliable predictor of response to bisphosphonate therapy."^{112} Several studies have demonstrated that urinary NTx falls in
response to bisphosphonate therapy in patients with bone metastases, and that this fall is associated with improved palliation, a decreased risk of SREs and improved survival\textsuperscript{113,114}. Data also show that an absolute value of urinary NTx of <100 nM/mM creatinine correlates with a lower risk of development of SREs, and that an absolute value $\geq$100 nM/mM creatinine corresponds with higher risk\textsuperscript{115}. More recently, serum C-telopeptide (CTx) has begun to replace urinary NTx due to its lower co-efficient of variability\textsuperscript{116}. In a large prospective cohort of women\textsuperscript{117}, it was possible to extrapolate that the low-risk cut-off of urinary NTx ($<$100mM/mM creatinine) corresponds to a value of $<$600ng/L of CTx (the threshold separating second and third tertiles). Alkaline phosphatase is the most frequently used biochemical marker of osteoblastic bone formation. However, four different isoforms of this enzyme exist in humans and these are produced in various tissues. Therefore, assays of total alkaline phosphatase are non-specific. The bone-specific isoform of alkaline phosphatase (BAP) has been shown to best reflect osteoblast differentiation during osteogenesis\textsuperscript{118}.

Reducing the frequency of intravenous bisphosphonate administration in those at low risk of skeletal complications has several potential advantages. It would reduce direct expenditures for drug costs and chair time in infusion clinics, as well as indirect costs to patients and their family related to the time needed to receive treatment. It could also reduce cumulative toxicities associated with bisphosphonates such as renal impairment\textsuperscript{119} and osteonecrosis of the jaw\textsuperscript{120}. 


This feasibility study was designed to explore whether it is feasible to conduct a randomized trial of de-escalated pamidronate (once every 12 weeks) in women with metastatic breast cancer and low biochemical markers of bone resorption (CTx levels <600 ng/L). Furthermore, unlike other ongoing trials\textsuperscript{121,122} in which prior treatment with 3-4 weekly bisphosphonates was needed for at least 9 months, this study assessed the role of less frequent therapy after only 3 months.

**Materials and Methods**

**Study Population**

Women with breast cancer and radiological or biopsy confirmed bone metastases and CTx levels in the low-risk range (defined as serum CTx levels in the lowest tertile [<600ng/L]\textsuperscript{123}) were eligible. Maximum suppression of bone resorption has been shown to occur after 3 months of bisphosphonate therapy\textsuperscript{124}, therefore, eligible patients must have received anti-resorption therapy for at least 3 months and had no change in systemic anti-cancer therapy or radiation therapy in the 28 days prior to randomization. Patients with baseline renal function above the institutional normal range were excluded due to the lack of data informing the dosing of pamidronate in these patients. The study was approved by the Research Ethics Board of Princess Margaret Hospital, Toronto, Canada.
**Trial Design**

A randomized, non-inferiority, feasibility study was conducted in a single large cancer center. Study participants were stratified according to baseline serum CTx (<400 ng/L and 400-600ng/L) and duration of prior bisphosphonate use (<6months and >6 months) and were then randomly allocated to receive pamidronate 90 mg intravenously every 3-4 weeks (control group) or every 12 weeks (de-escalated group).

Serum CTx and bone alkaline phosphatase (BAP) levels were measured every 12 weeks for 48 weeks. Patients also completed validated pain questionnaires (Brief Pain Inventory (BPI)\(^{125}\) and Functional Assessment of Cancer Therapy-Bone Pain (FACT-BP)\(^{126}\)) and were questioned on occurrences of toxicities of bisphosphonates (if any) at the same times. Those remaining in the low-risk CTx group continued to receive their allocated treatment. Those whose telopeptide levels rose above 600ng/L remained on study, but thereafter received treatment every 3-4 weeks. For patients receiving radiation therapy to bone or a change in systemic therapy during the study, data was collected until the time of radiation of systemic therapy change. Subsequent data were not analyzed to avoid potential confounding. All patients received concomitant vitamin D\(_3\) (800-1000 IU/day) and calcium (1200-1500mg/day) to prevent hypocalcaemia and/or secondary hyperparathyroidism.
Endpoints

The primary goal of this study was to assess feasibility of conducting a randomized trial of de-escalated pamidronate in a group of low-risk patients with bone metastases. Feasibility was defined as completion of follow-up in ≥90% of enrolled patients. Exploratory goals were to assess the proportion of patients remaining in the low risk CTx range throughout the 48 week study and to explore the absolute changes in CTx, BAP, BPI and FACT-BP pain scores and the number of symptomatic SREs in patients in each study group. Adverse effects were graded and recorded according to the NCI Common Terminology Criteria for Adverse Events, version 3.0.

An exploratory, post-hoc analysis to assess reasons for patients changing from low to high risk was also conducted. In this analysis, laboratory and imaging investigations which were conducted as standard of care were assessed for evidence of bone only progression or for progressive disease in extra-skeletal sites as well.

Biochemical Analyses

Serum samples were collected after an overnight fast, were allowed to clot for at least 30 minutes then centrifuged at 3,000 rpm for 10 minutes in a clinical centrifuge at room temperature. Samples were then analyzed immediately. Serum CTx was measured with an enzyme-linked immunosorbent assay (Beta-Cross Laps/serum assay, Roche Diagnostics Canada Inc, Laval, QC; detection limit 10ng/L, interassay variability, 5.4–
7.9%). Serum BAP was measured by immunoassay using a monoclonal antibody (Metra Biosystems, San Diego, CA; detection limit 0.7 IU/L, interassay variability, 5.2%).

**Statistical Analysis**

For this feasibility study, a pragmatic sample size was chosen based on the precision of the estimate of the primary endpoint (proportion of all patients completing follow-up). In order to obtain a confidence interval with a half-width of approximately 10% for the 90% of patients completing follow-up, 38 patients were needed to be enrolled and more than 34 to complete follow-up. Skewed data were log transformed and generalized estimating equations were used in a repeated measures analysis to compare CTx and BAP levels between groups over the 48 week period after an adjustment for baseline values. The main analysis was performed on all observed data under a plausible assumption about the missing data as described by White et al. Consistent with an alpha of 0.1, statistical significance of all testing other than the non-inferiority hypothesis was based on a two-sided test using a type I error rate of \( p = 0.10 \).

**Results**

**Patient enrolment**

Over a period of 16 months, 54 patients were approached to enter the study and 44 provided informed consent. Of these, six patients (13.6%) failed screening due to baseline CTx levels >600ng/L. Therefore, 38 patients were eligible for randomization to
the study with 19 participants allocated to each arm. Thirty-five patients (92.1%) were followed for the entire 48 week study period. One patient in the control group died of progressive breast cancer between 12 and 24 weeks and two patients in the de-escalated group withdrew consent (one after 12 weeks and one after 36 weeks). One patient reported fear of de-escalated therapy as the reason for withdrawal while the other transferred her care to another treatment centre where continued participation in the trial was not possible. Neither patient experienced increasing CTx, BAP, pain scores or a SRE during the study period (Figure 1). Missing data were therefore considered to be missing at random and the main analysis was conducted using all available data without imputation for missing values. Patient characteristics were well balanced in the two treatment arms (Table 1).

Biomarker changes

Five control group participants (26.3%) and 6 de-escalated group participants (31.6%) changed from low-risk to higher-risk CTx, an absolute difference of 5.3%. The one-sided [upper] 90% confidence interval for this absolute difference was 24.1%. Kaplan-Meier plots showing time to change from low-risk to high-risk CTx levels are shown in figure 2.

An exploratory analysis showed that there was evidence of progressive extra-skeletal disease in all patients not maintaining CTx in the low-risk range (n=11). No women with stable disease (n=20) or progressive metastases confined to bone (n=7)
had a change in CTx from the low to higher risk group. A quantitative increase in CTx over time in women receiving de-escalated therapy was observed (figure 3A). A quantitative difference in CTx over time was also apparent in the subgroup of women with stable disease and progressive metastases confined to bone. There was no apparent difference in levels of BAP over time between the two treatment groups. However, in the de-escalated arm, there was a numerical increase in BAP levels from week 24 onwards suggesting increased bone turnover beyond this time point (figure 3B).

**Clinical Endpoints**

Over the 48 week follow-up period, two symptomatic SREs were observed in each treatment group. Both required radiation therapy to control bone pain. There were a total of three serious adverse events (2 in the control group and 1 in the de-escalated group). All events related to hospitalization for control of symptoms related to disease progression. No bisphosphonate-specific toxicities such as renal impairment, osteonecrosis of the jaw or atrial fibrillation were observed in either arm of the study.

**Pain Scores**

Pain scores as measured by BPI and FACT-BP remained generally stable over time in both the control and the de-escalated groups (figures 3C and 3D). There were no apparent differences between groups in cumulative pain scores as measured by BPI or
by FACT-BP. There was no correlation between CTx and FACT-BP (Spearman rho 0.07, p=0.76) or with BPI (Spearman rho 0.19, p=0.41).

**Discussion**

The use of bisphosphonates provides symptomatic improvement to women with metastatic breast cancer, but does not influence either progression-free or overall survival\(^{128-130}\). In contrast to other supportive care measures, there have been few attempts to individualize the use of bisphosphonates in women with bone metastases from breast cancer. Indeed, current guidelines advise a “one size fits all” approach with the recommendation that all patients receive the same dose and frequency of bisphosphonates regardless of their underlying risks or needs\(^{107}\).

In this chapter a feasibility study assessing less frequent administration of intravenous pamidronate compared with standard 3-4 weekly dosing in women at low risk of skeletal events is reported. Results show that it is feasible to carry out a randomized trial in this setting. De-escalated therapy was not statistically inferior to standard scheduling as measured by the proportion of patients remaining at low risk of developing skeletal complications over the 48 week study period. However, based on the 90% one-sided confidence interval, up to 24% more women had a change in their risk group on de-escalated therapy compared to standard therapy. However, this exploratory study observed a time dependent increase in bone resorption biomarkers in
the de-escalated arm that was not statistically significant. These findings should be tested in a larger randomized trial. For a definitive non-inferiority trial with the primary endpoint being proportion of patients remaining at a low-risk telopeptide level, 112 patients in each arm would provide 80% power with an alpha of 0.05 using a one-sided test of 2 proportions assuming 10% of patients move from low to high risk status and a maximal allowable increase of 10% in this proportion (the non-inferiority boundary). For a non-inferiority trial with the primary endpoint being the proportion of patients with at least one SRE, 484 patients per arm would provide 80% power with a one-sided alpha of 0.05 assuming a 50% event rate and a similar non-inferiority boundary. In order to conduct such large trials, a multi-centre approach would be required and the greatest success would be achieved by conducting such trials within a co-operative group. Unfortunately, it is doubtful that the cost of such trials would be justified by a reduction in cost of treatment or related morbidity with de-escalated therapy.

There has been increasing interest in the use of bone resorption biomarkers in the risk assessment of patients with metastatic bone disease as well as for personalizing treatment in these patients. Urinary N-telopeptide (NTx) has historically been the preferred marker of bone resorption. However, data show that serum CTx is comparable to NTx and validation studies show that CTx is robust and reliable. Furthermore, CTx has been shown to be a sensitive biomarker in women with breast cancer. High levels of CTx have been associated with worse relapse-free survival.
and normalization of high levels has been shown to be associated with pain relief during treatment with pamidronate\textsuperscript{137}.

Among the 11 patients who did not maintain their CTx levels in the pre-specified low risk range (5 in the control arm and 6 in the de-escalated arm), all patients showed signs of disease progression in both skeletal extra-skeletal sites with none showing evidence of bone-only progression. As all patients had bone metastases, these results likely reflects failure of systemic anti-cancer therapy rather than of bone-specific treatment. In those with stable extra-skeletal or bone-only metastatic disease, there were no patients who changed from the low-risk CTx range to higher risk levels, although there was an absolute increase in CTx levels in those receiving de-escalated therapy.

There were differences in biomarkers between the two treatment arms. Patients receiving de-escalated therapy showed a consistent and maintained increase in absolute CTx levels compared to those receiving standard, 3-4 weekly treatment. Although the absolute values remained in the low risk group (<600 ng/L) in the absence of progression in other metastatic sites, it is possible that continued de-escalated therapy beyond 48 weeks may lead to further rises in CTx and to increased skeletal complications. BAP is a useful marker of active bone formation with only minor cross reactivity with liver-specific alkaline phosphatase\textsuperscript{138}. However, unlike CTx, BAP has a more complex association with bone turnover. BAP levels decrease in response to
initiation of anti-resorptive therapy\textsuperscript{139}, but conversely, in patients previously treated with anti-resorptive therapy, levels can rise in response to either anabolic therapy\textsuperscript{140} or second line bisphosphonates\textsuperscript{112}. No significant changes in BAP were observed, although there was a numerical increase in levels beyond 24 weeks in women receiving de-escalated therapy. The clinical relevance of these non-significant changes is unclear.

There were no differences in pain scores as measured by both BPI and FACT-BP between the two treatment schedules. Compared with baseline, there were trends toward increasing pain with time in both arms of the study although these were not statistically significant. Pain scores have been shown previously to correlate with bone resorption biomarkers such as CTx in patients with progressive bone metastases\textsuperscript{112,141}. In this study no consistent association between CTx and pain scores was identified. This may be explained by differences in the characteristics of patients in this study and those in previous trials. This study enrolled patients with low risk, and therefore less symptomatic bone metastases and in this group there may be less strong correlation between these biomarkers and pain. It should be noted however, that this study had insufficient power to detect differences in these secondary clinical outcomes.

No occurrences of bisphosphonate-specific toxicity were observed. Such toxicities are uncommon and occur after cumulative dosage of bisphosphonates\textsuperscript{119,120}, and it is therefore likely that the small sample size and limited follow-up did not allow
for detection of such adverse events. Nonetheless, it is likely that bisphosphonate-specific toxicity will occur less often in those receiving a less intensive dosing schedule.

This study has limitations. First, changes in systemic therapy or provision of radiation therapy might affect CTx values and pain scores, thereby contaminating the effects of randomization. Such effects were minimized by excluding women who received radiation therapy or a change in systemic therapy in the 28 days prior to randomization and censoring such patients when this occurred after randomization. Second, this was a small, single-center, unblinded, randomized feasibility study and intermediate endpoints such as biomarkers of bone resorption were used to define activity of bisphosphonate therapy. The use of clinically relevant endpoints such as number or frequency of SREs would be more relevant, but this would have required a much larger sample size and was not appropriate for this feasibility study. More definitive trials using endpoints that are clinically relevant to patients are ongoing. Unpublished data from one study showed that rates of SREs and time to such events were similar between those treated with monthly and 3-monthly zoledronic acid. However, rates of accrual in other studies have been below expectations. Clinician enthusiasm for this study was high and it is hoped that the presented results may encourage others to accrue patients to ongoing studies. Third, the criteria for non-inferiority in this study may be criticized. The pre-specified primary outcome used to assess differences between control and de-escalated groups was the proportion of patients whose biomarkers remained in the low-risk range. However, the
correlation between low levels of serum bone resorption biomarkers and favorable outcome is derived from studies in patients who had high levels at baseline and subsequently normalized in response to treatment. Telopeptide biomarkers may therefore have better positive predictive value for skeletal outcomes than negative predictive value\textsuperscript{144}. It is possible that positive outcomes relate more to the fall of resorption biomarkers (suggesting response to therapy) than to the resultant low levels of bone resorption. In this study, all patients had low baseline levels of CTx and few women showed substantial changes in bone resorption. This leads to uncertainty in the definition of “low-risk” status in this study. Finally, the increasing use of zoledronic acid or denosumab in many settings means that pamidronate might not reflect the standard of care. However, zoledronic acid did not show superiority over pamidronate in the only head-to-head trial\textsuperscript{145} and it is therefore likely that the reported results can be applied to zoledronic acid. The different pharmacokinetic and efficacy properties of denosumab compared to bisphosphonates\textsuperscript{146} mean that these results cannot be generalized to this agent.

In conclusion, randomized trials of de-escalated pamidronate in women with low-risk bone metastases are feasible even after limited pre-treatment with monthly bisphosphonates. Based upon our analysis, twelve-weekly treatment appears non-inferior in approximately 75% of patients, especially those with stable systemic disease and those with bone-only metastatic disease. This finding will require confirmation in a larger trial. Large randomized trials are needed to adequately advise clinicians and
patients on the optimal dose of bisphosphonates in women with metastatic breast cancer in a variety of clinical settings.
**Table 1 – Patient Characteristics.**

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<td>n=19</td>
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<td>Range</td>
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<td>Baseline FACT-BP score (total)</td>
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<tr>
<td>Range</td>
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<td>5-27</td>
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Figure 1 – CONSORT diagram.

Approached to enter study
n=54

Consented to participate in study
n=44 (81.5%)

Eligible for randomization
n=38 (86.4%)

Completed 48 week follow-up
n=35 (92.1%)

Fear of de-escalated therapy n=6
Did not wish to complete questionnaires n=2
No reason provided n=2

Failed screening n=6
- Baseline CTx >600ng/L (n=6)

Withdraw consent n=2
Died n=1
Figure 2 – Kaplan-Meier plots showing time to change from low to high-risk CTx levels.
Figure 3 – Changes in endpoints over time. A, CTx. B, BAP. C, total FACT-BP. D, total BPI.
Figure 4 – Changes in serum C-Telopeptide over time for patients with metastatic disease confined to bone.
Chapter 5: Discussion

This chapter will summarize the results of the thesis in terms of its contribution to the literature and implications for women with breast cancer and the clinicians involved in their care. The limitations and strengths of the study will be discussed and suggestions for key areas of future research will be presented.

I: Contribution to the Literature

The choice of medical therapies is usually based on knowledge from different sources generated by epidemiological and clinical studies or evidence-based medicine. Large randomized studies remain the gold standard for the assessment of the benefit of specific interventions. However, such methodology is designed to determine the best approach for populations and not for specific individuals. In breast cancer, predictive markers for benefit from various cancer treatments in individual patients have been known since the 1980s. Expression of estrogen and progesterone receptors has been shown to predict for response to endocrine therapy\textsuperscript{147} and over-expression or amplification of the oncogene \textit{HER2/neu} confers both worse prognosis, but also response to the drug trastuzumab\textsuperscript{148,149}. Therefore testing for these markers is now the standard of care. The use of more modern techniques such as gene expression profiling has been lauded as having the potential to improve upon current strategies for personalized medicine\textsuperscript{150}; however, despite breast cancer subtypes based upon gene
expression being described over 10 years ago\textsuperscript{151}, gene expression profiling has provided only limited benefit in clinical care. Specifically, molecular profiling is not an approved method for determination of receptor status or of the likely benefit from targeted therapy.

There are various reasons for the lack of progress in personalized medicine for breast cancer in the last 30 years. First, most studies have explored markers of benefit alone with few published data on markers of safety and tolerability. Second, despite cancer being a heterogeneous disease\textsuperscript{152}, it has been assumed that important properties of cancer cells do not change with time and therefore patients have generally been assessed at the time of initial diagnosis, and their biomarkers were assumed to remain the same for the course of their disease. Finally, for most biomarkers which have been identified, outcomes have been validated to predict for the number of events within a defined population rather than the likelihood of an event in a specific individual within that population. Therefore, data supporting the use of such biomarkers tend to show good calibration, but discriminatory accuracy can be diminished.

This thesis comprises three studies which have attempted to address the above problems. Each study assessed independently the use of different predictive factors to identify patients for improved therapeutic window of available anti-cancer therapy. The studies also aimed to identify those at increased risk of toxicity and those for whom supportive care interventions can be optimized, thereby reducing hospital visits and
consequently reducing direct and indirect healthcare costs. In the first study (Chapter 2), the effects of re-biopsy of recurrent breast cancer was assessed in 121 women presenting with newly-discovered or progressive metastatic disease. Results showed that analysis of biopsy material for 3 predictive biomarkers (estrogen, progesterone and HER-2 receptors) influenced clinicians’ choice of therapy in 14% of women. All patients were then administered targeted therapy based upon the newly analyzed predictive factors. Follow-up of these patients did not show any detrimental effect of differences between the original and repeated biopsies on either progression-free or overall survival although power to detect such differences was low and small differences between groups cannot be excluded. Furthermore, in an exploratory analysis there was no evidence that those with discordant receptors leading to a change in therapy had worse PFS. This is in contrast to prior retrospective analyses where treatments were not changed based upon repeat biopsy where inferior survival was observed in those where predictive biomarkers differed. This suggests that repeat biopsy has the potential to optimize the individualized treatment of patients with recurrent or metastatic breast cancer. However, a definitive assessment of the impact of re-biopsy on patient outcomes would require formal evaluation in a randomized trial of repeat biopsy versus not. Such methodology is considered unfeasible and contentious.

In the second study (Chapter 3), a systematic review and meta-analysis were conducted. All phase III, randomized trials comparing combinations of tamoxifen and aromatase inhibitors for post-menopausal women with early breast cancer were
assessed for differences in serious toxicities. Results showed that there were
differentially higher rates of ischemic heart disease and bone fractures for those treated
predominantly with aromatase inhibitors and differentially lower rates of thrombo-
embolism and endometrial carcinoma. Rates of cerebrovascular disease and other
second cancers were similar. In those with established ischemic cardiovascular disease,
aromatase inhibitors were associated with a higher magnitude of harm than in
unselected patients. Finally, it appeared that using a switching strategy where tamoxifen
and aromatase inhibitors are used in sequence rather than alone was associated with a
reduction in the odds of death without breast cancer recurrence. These data suggest
that assessment of patients’ baseline health characteristics is important in the choice of
adjuvant endocrine therapy. By using these simple predictive factors there can be more
informed choice of personalized therapy.

In the final study (Chapter 4), the feasibility of conducting a randomized trial
assessing less frequent (de-escalated) versus standard scheduling of intravenous
pamidronate was evaluated. Women with breast cancer and bone metastases are a very
diverse group with high levels of heterogeneity in the risk of developing skeletal
complications and hence benefiting from drugs such as pamidronate. Despite this,
pamidronate therapy has been administered by convention at 3-4 weekly intervals in all
patients without assessing individual risk. The study reported in this thesis examined
whether the use of bone resorption biomarkers such as C-telopeptide could be used to
guide less frequent therapy in those women who are at lower risk and therefore less
likely to derive benefit from such therapy. Results showed that conducting a randomized trial in women with metastatic breast cancer and low-risk bone metastases is feasible. Preliminary data also suggest that baseline serum C-telopeptide may predict low risk status and that de-escalated therapy with bisphosphonates may be non-inferior to standard scheduling of therapy in over three-quarters of low-risk women. More definitive trials with better power to detect clinical differences are required and such trials are in progress.

This thesis reports three investigator-led, independent studies which have shown that use of predictive biomarkers can potentially optimize both the efficacy and the toxicity of breast cancer treatment as well as allowing supportive care measures to be better tailored to individual risk. All studies met their respective objectives of identifying a patient cohort which is more likely to benefit from either anti-cancer or supportive therapies. Therefore, these studies have the potential to improve patient outcomes at a lower cost than the development of new novel drugs. Moreover, these studies emphasize the potential for improvement of currently available therapies by improving patient selection.

As discussed in Chapter 1, the majority of clinical trials in oncology and particularly those for women with breast cancer are funded by the pharmaceutical industry. They evaluate predominantly new and potentially expensive therapy. A number of problems have been identified including the need for large phase III trials,
the limited clinical benefit obtained from some approved agents, the high cost at which novel therapies are marketed and the decreasing absolute benefit observed in many trials\textsuperscript{13}. Although data from this thesis have limitations especially with regard to methodological quality and internal validity, they do have the potential to change clinical practice at low absolute cost. Formal cost-effectiveness analysis and decision modeling based upon these results would help refine costs more accurately. Furthermore, while the primary data collection for study 2 was carried out by industry-funded research, the analyses of all three studies were conducted independently of involvement of the pharmaceutical industry and addressed questions hitherto unanswered even by overview committees such as the Early Breast Cancer Trialists Collaborative Group. All studies have provided data to suggest that anti-cancer and supportive treatment can be better tailored to patients thereby improving the therapeutic index of each therapy.

\section*{II: Clinical Implications}

The main findings of the studies included in this thesis are that the therapeutic index of currently available anti-cancer and supportive therapies can be improved and better personalized by the use of parameters which predict for better efficacy or reduced toxicity. The study reported in Chapter 2, showed that re-biopsy of recurrent breast cancer influenced physician choice of therapy once for every 7 biopsies performed. Although these data do not provide evidence of improvements in outcomes
important to patients (e.g. survival or quality of life), the frequent change in physician choice of therapy suggests that clinicians consider such changes important and value the information that such biopsies provide. It is likely that the results of this study will lead to more frequent biopsy of recurrent breast cancer and assessment of hormone and HER2 receptors. It is hoped that such tailored therapy may also improve outcomes of patients. It should be emphasized however, that change in therapy remained uncommon. If re-biopsy of metastatic disease and re-analysis of receptor expression were to become standards of care, the selection of patients with discordance between the predicted prognosis (based on receptor expression) and eventual outcome may enrich for those who may benefit from re-biopsy.

The study reported in Chapter 3 identified important toxicities associated with the use of different options for adjuvant endocrine therapy in post-menopausal women. As the majority of such patients die of causes other than their breast cancer, the influence of drugs on other co-existing diseases is important. The study identified longer use of aromatase inhibitors as being associated with increased risk of ischemic heart disease. The magnitude of this toxicity appeared to be greatest in patients with established ischemic heart disease or risk factors thereof. Consequently, it is hoped that a better defined risk profile of adjuvant endocrine therapy options in post-menopausal women will allow for more rational choices of therapy and may ultimately lead to fewer iatrogenic toxicities. This study also presented data suggesting that switching from tamoxifen to aromatase inhibitors after 2-3 years is associated with a reduction in
deaths without breast cancer recurrence (i.e. potentially toxic deaths). This switching strategy limits exposure of patients to individual anti-hormone therapies and has biological rationale. It is hoped that this study will lead to more patients receiving such treatment, which appears to have the best balance between efficacy and toxicity.

Finally, the study reported in Chapter 4 has shown that it is feasible to conduct a randomized trial of de-escalated pamidronate in patients with metastatic breast cancer to bone in whom bone resorption markers are at a level consistent with a low risk of skeletal related events. It is hoped that these data will provide stimulus to the completion of ongoing larger clinical trials of de-escalated bisphosphonate therapy and allow their timely conclusion. Furthermore, the preliminary data from this pilot study are similar to recently reported data from a larger randomized trial evaluating de-escalated zoledronic acid in more heavily pre-treated patients. The similarity of the data suggests that the efficacy of de-escalated zoledronic acid can likely be extrapolated to pamidronate and perhaps other bisphosphonates. It also appears that it is not necessary receive monthly bisphosphonates for 9 months prior to de-escalating to less frequent therapy. Similar results were observed from de-escalation after only 3 months. The results of this study, in combination with other more definitive data, should allow clinicians to more accurately inform their patients about the benefit of different treatment schedules and tailor therapy based on risk of skeletal-related events and other factors such as patient preferences.
III: Strengths and Limitations

A: Strengths

All three studies contributing to this thesis involved the analysis of prospectively collected data, that were rigorously and objectively collected. The study in Chapter 2 is to date the largest prospective assessment of the impact of biopsy of distant (as opposed to loco-regional) recurrences. It is also the first to report actuarial rates of success in yielding sufficient tumour material to be able to analyze predictive biomarkers. The study in Chapter 3 provides a comprehensive assessment of toxicities of adjuvant endocrine therapy. While some data have previously been available regarding the association of specific aromatase inhibitors with the potential for cardiovascular toxicity\textsuperscript{79,80}, this study identified cardiovascular disease as a risk factor for treatment of early breast cancer patients with aromatase inhibitors as a drug class. Furthermore, it also reported similar rates of cerebrovascular events for tamoxifen and aromatase inhibitors where previous data suggested high risk of such events with tamoxifen. This difference may be explained by initial data with aromatase inhibitors being based on short follow-up. Longer experience has shown higher rates of cerebrovascular disease. The use of meta-analytical techniques to identify uncommon, but potentially serious toxicity is a novel method for assessment of toxicity in trials of adjuvant cancer therapy although has been used in other branches of medicine\textsuperscript{141}. In the published overviews, meta-analysis was frequently used to evaluate efficacy with no reporting of adverse events\textsuperscript{4,5,68}. Other systematic reviews have included an assessment
of toxicity as a secondary aim after a primary evaluation of efficacy\textsuperscript{155}. However, with the increasing use of new targeted therapies where treatment is of modest benefit, the importance of adverse events has become more important and the use of meta-analysis has become more prevalent. This has been especially the case for the drug bevacizumab where in 2011 alone, at least 6 meta-analyses have primarily assessed adverse effects associated with the use of this drug in various solid cancers\textsuperscript{156-161}. Finally the study in Chapter 4 reported the use of biochemical markers of bone resorption as aids to tailor supportive bone-specific therapy in metastatic breast cancer. While results from this study are not definitive, they provide insight into the feasibility of conducting trials of de-escalated bisphosphonates in low-risk patients with metastatic breast cancer to bone. It is hoped that these data may help in designing larger and confirmatory clinical trials of de-escalated therapy in this setting.

The data presented in this thesis should be generalizable to a wide spectrum of breast cancer patients. The studies in Chapters 2 and 3 both had very broad inclusion criteria while the study in Chapter 4 included a clearly defined subgroup of eligible patients. Clinical trials usually have multiple exclusion criteria, which include particular co-morbidities and the use of certain concomitant medications\textsuperscript{162}; therefore, important differences in disease and host characteristics can exist between trial participants and patients treated in general practice.
B: Limitations

The studies described in this thesis have limitations. The study described in Chapter 2 used a non-randomized design and change in physician choice of therapy was the primary endpoint. This is a pragmatic endpoint, and as mentioned in the discussion of this study, randomized trials of biopsy of recurrent lesions are likely not ethical. Furthermore, the use of outcomes important to patients such as survival or quality of life may not be feasible. The chosen methodology was therefore felt to be the most appropriate for this setting. Further limitations include the limited power to detect survival outcomes based upon change in receptor status and the uncertainty in the definition of PFS resulting from follow-up imaging being conducted at the clinician’s discretion rather than at fixed interval.

The study described in Chapter 4 was predominantly designed to test the feasibility of undertaking a study where biomarkers inform scheduling of therapy of supportive care in metastatic bone disease. While it showed that such a biomarker adapted design was feasible, it was inadequately powered to assess the safety of de-escalated therapy.

Finally, while the study in Chapter 3 was comprehensive and included data from over 30,000 women, it was reliant on reported data. The capture and causation of toxicity in clinical trials relies on the judgement of investigators. Consequently, some adverse events which are infrequent but which might be clinically relevant may not be
reported in the publication of trials. Furthermore, data were collected using different methods which may have led to the pooled estimate being limited by heterogeneity. Lastly, as discussed above, while serious toxicities may be rare in a population of patients included in clinical trials, they may occur more commonly in susceptible subsets of patients who are more prevalent in general oncological practice.

IV: Future Research

This thesis highlights key areas for future investigation including:

1. **Comparison of molecular profiling of primary and recurrent breast cancer.**
   Analysis of tumour biopsy material by either exome or genome sequencing could have substantial impact on knowledge of the drivers of breast cancer progression and may shed light on key genes which are involved in this process. It may lead to a better understanding of why some tumours are able to metastasize and other cannot. This process also has the potential to allow for the identification of new targets which could be the basis for targeted drug development aimed at reducing the likelihood of breast cancer recurrence.

2. **The undertaking of secondary analyses may allow for more toxicity data to be reported.** Secondary analyses involve the use of existing data, collected for the purposes of a prior study, in order to pursue a research interest which is distinct from that of the original work. This may be a new research question or an alternative perspective on the original question. Such supplementary analyses
of clinical trials have been described for over 20 years\textsuperscript{164}, but the publication of such data remains rare. The cancer literature does however show successful uses of secondary analyses both for the assessment of prognosis and toxicity. For example, a secondary analysis of the Radiation Therapy Oncology Group (RTOG) protocol 9413\textsuperscript{165} showed that the concomitant use of androgen deprivation and pelvic radiation therapy led to an increase in late urinary and rectal toxicities. Investigators should be encouraged or even mandated to carry out further analyses especially if new safety signals are observed in post-marketing studies or become apparent in health outcomes research. Furthermore, it should be mandated that the collection of such data is undertaken using standardized and reproducible methods.

3. Well conducted health outcome research may allow for more accurate assessment of both efficacy and toxicity in the general population than may be evident from randomized trials. The use of large and linked databases can allow for assessment of specific outcomes (e.g. cardiovascular events after therapy with aromatase inhibitors) and provide both validation of data from clinical trials as well as a more refined assessment of the magnitude of effect of a drug when utilized in a less selected population. For example, an analysis of linked population-based data on breast cancer outcomes from Norway attempted to isolate the benefit of mammographic screening from other factors that may have changed over time, including increased breast-cancer awareness and improvements in treatment. In contrast with previously published data, results
showed statistically non-significant reductions in breast cancer-specific mortality associated with mammography. Around two-thirds of the improvement in mortality which was seen over a 20 year period was associated with improvements in the multi-disciplinary management of breast cancer rather than population based screening\textsuperscript{166}.

4. Definitive trials of de-escalated bisphosphonate therapy utilizing endpoints that are clinically relevant to patients need to be completed. While such trials are ongoing\textsuperscript{121,122,142}, the optimal methodology would be a biomarker strategy design. With this design patients are randomized to an experimental arm that uses a biomarker to select the therapy (e.g. de-escalated or normal schedule bisphosphonates) or to a control arm which uses standard therapy\textsuperscript{167}. While one such trial has been initiated\textsuperscript{145}, accrual has been below expectation and the study has been suspended. Completion of such studies will help better identify patients’ need for therapy, although funding sources for such studies may be limited.

V: Conclusions

This thesis provides evidence that available biomarkers can be utilized to improve patient selection for targeted drug in terms of improvements in efficacy, toxicity and need for supportive care. Re-biopsy of recurrent tumour was shown to influence physician choice of therapy in advanced breast cancer. A thorough
understanding of uncommon, but potentially serious toxicities of adjuvant endocrine
therapy can allow a more rational choice of these drugs based upon patients’ baseline
health characteristics. Finally, the use of bone resorption biomarkers may have utility in
selecting patients for less frequent administration of supportive care measures such as
administration of bisphosphonates. However, further research is required to enhance
the knowledge of the use of biomarkers in the domains of efficacy, toxicity and
supportive care prior to these predictive factors being used routinely in clinical practice.

The results of this thesis highlight that improvements in the tailored treatment
of breast cancer can be obtained through more rational use of predictive markers. While
novel drug development will remain essential to the aim of improving outcomes for
breast cancer patients, it is hoped that these results will also be of benefit to clinicians
and patients. Ultimately, it is hoped that rational use of predictive biomarkers will
improve patient outcomes at a relatively lower cost than the predominant research
approach of novel drug development.
Candidate’s Role

The candidate (EA) conceived the research questions addressed in this thesis, developed the study protocols with input from thesis committee members (Dr. I. Tannock, Dr. A. Laupacis and Dr. G. Tomlinson) and from Dr. Mark Clemons. The candidate supervised the recruitment of participants, conducted most trial related procedures such as tumour biopsy and performed all data analyses and write-up of the thesis manuscript.
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Appendix A
Patient Satisfaction Questionnaire

Does performing a confirmatory tumour biopsy in patients with metastatic breast cancer alter their management? The DESTINY Trial

Patient initials: ___________________________ DOB: ____-____-____
F. M. L. dd-mon-yyyy
Study #: __________Clinic file #: __________ Hospital file #: __________
Date of assessment: ____-____-____ Investigator: __________
               dd-mon-yyyy

1. Did you experience anxiety before your most recent biopsy?
   a) Yes
   b) No

2. What was the level of pain you experienced with this biopsy?
   a) None
   b) Mild
   c) Moderate
   d) Severe

3. Did you have ongoing pain after the biopsy at the site of biopsy?
   a) Yes
   b) No
   If yes, for how many days?

4. Do you think your treatment was delayed by the time required to undergo the biopsy procedure? If so, by approximately how many days?

5. Having been through this experience, would you recommend having a biopsy to others in your situation?
   a) Yes
   b) No

6. Is there anything your medical team could have done to improve your experience?
Appendix B
Paper 1

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