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Meta-analysis of randomized clinical evidence of chemotherapy, radiotherapy and endocrine therapy in breast cancer

Μετα-ανάλυση τυχαιοποιημένων κλινικών μελετών χημειοθεραπείας, ακτινοθεραπείας και ορμονοθεραπείας στον καρκίνο του μαστού

by

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Abstract

Background: Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in females worldwide. Despite the growing body of randomized controlled trials (RCTs) regarding the use of various treatment strategies (chemotherapy, endocrine therapy, radiotherapy) in breast cancer, the efficacy of some strategies remains unclear and questionable. A meta-analysis, by combining the results of all available trials that studied the same question, could help to reduce the level of uncertainty and provide reliable conclusions about the role of various therapeutic choices for breast cancer patients.

Objective: The purpose of this thesis was to identify controversial therapeutic strategies in the treatment of breast cancer and to perform, when possible, meta-analyses to find out which of these strategies are valuable in breast cancer therapy. We conducted 7 separate meta-analyses in order to answer to 7 different clinical questions. We choose 7 topics with controversial results in the therapeutic strategy of breast cancer patients, namely the role of bisphosphonates as antitumor therapy and as preventive agents against fractures in adjuvant setting, the risk of osteonecrosis of the jaw (ONJ) with the use of bisphosphonates in adjuvant setting, the use of fulvestrant in advanced breast cancer, the safety of partial breast irradiation (PBI) compared with whole-breast radiotherapy (WBRT), the role of bevacizumab in advanced breast cancer and finally the value of trastuzumab as neoadjuvant therapy in Her2-positive breast cancer patients.

Materials and Methods: In all 7 meta-analyses we used the same basic principles of meta-analysis, with some minor but necessary changes in order to fit our methodology to specific aims of each trial. In general, we conducted systematic reviews of all English and non-English medical literature using MEDLINE, the Cochrane Controlled Trials Register and ISI Web of Knowledge. We set no year restriction. The references of all eligible trials were also searched in order to find any potentially eligible trial that it was not identified by our searching algorithm. Abstracts of major meetings were also searched. Eligible studies were identified according to prespecified criteria for each meta-analysis. Data extraction was conducted independently by two investigators. In case of discrepancy, consensus was reached by involvement of a third investigator. When data on the outcome were not available from trials, we contacted the primary investigators of the eligible trials. Data synthesis was perfomed by choosing the appropriate effect size measure (Odds Ratio, Risk Ratio or Hazard Ratio) for each outcome and by combining the results using fixed- or random-effects models.

Results: Regarding bisphosphonates in adjuvant setting, pooled results showed no statistical significant differences with the use of bisphosphonates in early breast cancer versus non-use for the overall number of deaths (summary OR, 0.708; 95% CI, 0.482–1.041; p-value =0.079), disease recurrences

(summary OR, 0.843; 95% CI, 0.602–1.181; p-value =0.321), and bone metastases (summary OR, 0.925; 95% CI, 0.768–1.114; p-value =0.413). Subgroup analyses for disease recurrences according to the type of bisphosphonate used showed a statistically significant lower risk for disease recurrences with zoledronic acid (6 trials, OR, 0.675; 95% CI, 0.479–0.952; p-value = 0.025). In addition, bisphosphonates did not reduce fracture rate (OR=0.99, 95% CI=0.73–1.34) neither in postmenopausal women (OR=0.82, 95% CI=0.55–1.20) nor in women with breast cancer receiving aromatase inhibitors (OR=0.79, 95% CI=0.53–1.17). Overall, treatment with bisphosphonates was significantly associated with the occurrence of osteonecrosis of the jaw (ONJ) (OR = 3.23, 95% CI = 1.7–8) compared with no use but it was a rare event, occurring in 13 (0.24%) of the 5,312 patients receiving bisphosphonates.

Considering fulvestrant, we found no difference between fulvestrant versus other hormonal agents regarding overall survival (HR: 1.047, 95% CI: 0.688–1.592; p-value = 0.830) and time to tumor progression (HR: 0.994, 95% CI: 0.691–1.431; p-value = 0.975).

Partial breast irradiation (PBI) did not influence survival (OR 0.912, 95% CI, 0.674–1.234, p-value = 0.550) compared with WBRT but it was found to lead to statistically significant higher risk for developing local recurrences (pooled OR 2.150, 95% CI, 1.396–3.312; p-value = 0.001) and axillary recurrences (pooled OR 3.430, 95% CI, 2.058–5.715; p-value < 0.0001).

The combination of bevacizumab and chemotherapy resulted in a statistically significant improvement in progression-free survival compared with chemotherapy alone (HR = 0.70, 95% CI = 0.60–0.82, p-value = $9.3x10^{-6}$), especially when bevacizumab was combined with taxanes. However, the pooled HR for overall survival did not show significant advantage for the use of bevacizumab compared to placebo arm (pooled HR = 0.90, 95% CI 0.80–1.03, p-value = 0.119).

Finally, the use of trastuzumab as neoadjuvant therapy lead to higher absolute pathologic complete response (pCR) rate (38% in trastuzumab arm in comparison with 21% in no trastuzumab arm) (RR 1.85, 95% CI: 1.39-2.46; p-value < 0.001). Two out of 217 (0.9%) patients in the trastuzumab arms presented congestive heart failure compared with none in the chemotherapy alone arms.

Conclusions: The meta-analysis of bisphosphonates in adjuvant breast cancer therapy showed that currently available randomized evidence does not support the hypothesis that using bisphosphonates in adjuvant treatment of early breast cancer alters the natural course of the disease. In addition bisphosphonates do not seem to prevent bone fractures. However, ONJ is a rare event in breast cancer patients treated with adjuvant use of bisphosphonates.

Our meta-analysis of fulvestrant suggests that fulvestrant 250 mg is similar to other hormonal agents with respect to efficacy measures with equal or even better tolerability profile compared with other hormonal agents.

Our meta-analysis of PBI, despite the fact that it is based on limited randomized evidence, suggests that PBI is a safe treatment modality as it does not seem to jeopardize survival compared with standard WBRT. Nevertheless, the issue of locoregional recurrence needs to be further addressed.

The results of the meta-analysis of bevacizumab show that the addition of bevacizumab to chemotherapy offers a statistically significant improvement in progression free survival in patients with metastatic breast cancer but does not benefit overall survival. In addition, clinical significance of this improvement is questionable. As a result, bevacizumab treatment cannot be suggested for treatment of 1st line metastatic breast cancer,

Finally, the meta-analysis of trastuzumab as neoadjuvant treatment underscores the beneficial effects of trastuzumab treatment in neoadjuvant regimens among HER2-positive breast cancer patients in terms of pCR. Of interest, no additional cardiotoxicity was documented in the trastuzumab arms.

Περίληψη

Εισαγωγή: Ο καρκίνος του μαστού είναι ο πιο συχνός τύπος καρκίνου και η κύρια αιτία θανάτου από καρκίνο παγκοσμίως. Παρά το γεγονός οτι ο αριθμός των τυχαιοποιημένων κλινικών μελετών που αφορούν τη χρήση διαφόρων θεραπευτικών μεθόδων (χημειοθεραπεία, ακτινοθεραπεία, ορμονοθεραπεία) στον καρκίνο του μαστού αυξάνεται συνεχώς, η αξία ορισμένων θεραπειών παραμένει υπό αμφισβήτηση. Η μετα-ανάλυση, συνδυάζοντας με στατιστικές μεθόδους τα αποτελέσματα κλινικών μελετών που έχουν μελετήσει το ίδιο κλινικό ερώτημα, μπορούν να βοηθήσουν στη μείωση της αβεβαιότητας και να παρέχουν αξιόπιστα συμπεράσματα για την αξία διαφόρων θεραπευτικών επιλογών στον καρκίνο του μαστού.

Σκοπός: Σκοπός της διατριβής ήταν η διαπίστωση αμφιλεγόμενες θεραπευτικές στρατηγικές στον καρκίνο του μαστού και κατόπιν η εφαρμογή μετα-αναλύσεων ώστε να αξιολογηθεί ποιές από αυτές τις στρατηγικές είναι χρήσιμες στον καρκίνο του μαστού. Προχωρήσαμε σε 7 διαφορετικές μετα-αναλύσεις ώστε να απαντηθούν 7 διαφορετικά κλινικά ερωτήματα. Επιλέξαμε 7 θέματα με αμφιλεγόμενα αποτελέσματα, που περιλαμβάνουν το ρόλο των διφωσφονικών ως αντικαρκινική θεραπεία αλλά και ως θεραπεία προστασίας καταγμάτων όταν χρησιμοποιούνται σαν μεταγχειρητική/προφυλακτική θεραπεία, τον κίνδυνο οστεονέκρωσης κάτων γνάθου με την χρήση διφψσφονικών, τη χρήση του fulvestrant στον προχωρημένο καρκίνο του μαστού, την ασφάλεια της χρήσης μερικής ακτινοβόλησης του μαστού μετά από τμηματεκτομή ή μερική μαστεκτομή αντί της θεραπείας επιλογής που είναι η ολική ακτινοβόληση του μαστού, το ρόλο του bevacizumab σε συνδυασμό με χημειοθεραπεία στον προχωρημένο καρκίνο του μαστου και τέλος την αξία του trastuzumab ως προεγχειρητική θεραπεία σε ασθενείς με Her2-θετική νόσο.

Υλικό και Μέθοδος: Σε όλες τις 7 μετα-αναλύσεις χρησιμοποιήσαμε τις ίδες βασικές αρχές, με κάποιες μικρές διαφοροποιήσεις που ήταν απαραίτητες ώστε να ταιριάζει η μεθοδολογία μας στον ειδικό σκοπό της κάθε μετα-ανάλυσης. Γενικά, προχωρήσαμε σε συστηματική ανασκόπηση της βιβλιογραφίας, χρησιμοποιώντας 3 βάσεις δεδομένων (MEDLINE, the Cochrane Controlled Trials Register, και ISI Web of Knowledge) χωρίς χρονικό ή γλωσσικό περιορισμό. Αναζητήσαμε τυχόν επιπλέον μελέτες μέσω του ελέγχου των βιβλιογραφικών αναφορών των επιλεγμένων μελετών και των περιλήψεων των κύριων διεθνών συνεδρίων Ογκολογίας. Οι μελέτες που ήταν κατάλληλες για τις μετα-αναλύσεις επιλέχθηκαν με βάση προ-αποφασισμένων κριτηρίων. Η εξαγωγή δεδομένων έγινε από 2 ανεξάρτητος ερευνητές, ενώ ένας τρίτος ερευνητής συμμετείχε στη διαδικασία για την επίλυση τυχών ασυμφωνιών. Σε περίπτωση μη διαθέσιμων δεδομένων, επικοικωνούσαμε με τους ερευνητές των πρωτογενών μελετών ώστε να μας τα παρέχουν. Η στατιστική σύνθεση των δεδομένων λάμβανε χώρο μετά από επιλογή του κατάλληλου στατιστικού μέτρου (Odds Ratio, Risk Ratio ή Hazard Ratio) για το κάθε αποτέλεσμα και εφαρμογή του κατάλληλου μετα-αναλυτικού μοντέλου (fixed- ή random-effects models).

Αποτελέσματα: Αναφορικά με τα διφωσφονικά ως προφυλακτική θεραπεία, τα αποτελέσματα των μετα-αναλύσεων δε δείχνουν κάποια στατιστικά σημαντική διαφορά με τη χρήση τους στον αριθμό των θανάτων (OR, 0.708; 95% CI, 0.482–1.041; p-value =0.079), των συνολικών υποτροπών (OR, 0.843; 95% CI, 0.602–1.181; p-value =0.321), και των σκελετικών μεταστάσεων (OR, 0.925; 95% CI, 0.768–1.114; p-value =0.413). Σε ανάλυση υπο-ομάδων με βάση τον τύπο των διφωσφονικών διαπιστώθηκε οτι το zoledronic acid μειώνει τον κίνδυνο για υποτροπή (6 μελέτες, OR, 0.675; 95% CI, 0.479–0.952; p-value = 0.025). Επιπλέον, τα διφωσφονικά δεν μειώνουν τον κίνδυνο για κάταγμα (OR=0.99, 95% CI=0.73–1.34) τόσο στις μετεμμηνοπαυσιακές ασθενείς (OR=0.82, 95% CI=0.55–1.20) όσο και στις ασθενείς που λαμβάνουν αναστολείς της αρωματάσης (OR=0.79, 95% CI=0.53–1.17). Συνολικά, η θεραπεία με διφωσφονικά σχετίζεται σε σημαντικό βαθμό με την εμφάνιση οστεονέκρωσης της γνάθου (OR = 3.23, 95% CI = 1.7–8) αλλά η συγκεκριμένη παρενέργεια είναι ένα σπάνιο συμβάν που συνέβη σε 13 (0.24%) από τις 5,312 ασθενείς που έλαβαν διφωσφονικά.

Σχετικά με το fulvestrant, δε διαπιστώθηκαν διαφορές με τη χρήση του εν συγκρίσει με άλλες ορμονοθεραπείες σε σχέση με την ολική επιβίωση (HR: 1.047, 95% CI: 0.688–1.592; p-value = 0.830) και το χρόνο ως την υποτροπή (HR: 0.994, 95% CI: 0.691–1.431; p-value = 0.975).

Η μερική ακτινοβόληση του μαστού δεν επηρεάζει την επιβίωση των ασθενών με καρκίνο του μαστου (OR 0.912, 95% CI, 0.674–1.234, p-value = 0.550) όταν συγκριθεί με την ολική ακτινοβόληση αλλά οδηγεί σε στατιστικώς σημαντικά μεγαλύτερο κίνδυνο για τοπικές (OR 2.150, 95% CI, 1.396–3.312; p-value = 0.001) και περιοχικές υποτροπες (OR 3.430, 95% CI, 2.058–5.715; p-value < 0.0001).

Ο συνδυασμός bevacizumab και χημειοθεραπείας οδηγεί σε σημαντική βελτίωση του χρόνου ως την υποτροπή σε σύγκριση με μόνο χημειοθεραπεία (HR = 0.70, 95% CI = 0.60–0.82, p-value = 9.3x10-6), ειδικά όταν συνδιάζεται με ταξάνες. Αντιθέτως, δε διαπιστώθηκε καμία βελτίωση στην επιβίωση με την προσθήκη bevacizumab (HR = 0.90, 95% CI 0.80–1.03, p-value = 0.119).

Τέλος, η χρήση trastuzumab ως προεγχηρητική θεραπεία οδηγεί σε αύξηση του αριθμού των παθολογοανατομικών πλώρων υφέσεων (38% στο σκέλος με trastuzumab, 21% στο σκέλος χωρίς trastuzumab) (RR 1.85, 95% CI: 1.39-2.46; p-value < 0.001). Δύο από τους 217 (0.9%) ασθενείς που έλαβαν trastuzumab ανέπτυξαν καρδιακή ανεπάρκεια.

Συμπεράσματα: Η μετα-ανάλυση των διφωσφονικών ως προφυλακτική θεραπεία στον καρκίνο του μαστού έδειξε οτι, με βάση τα υπάρχοντα τυχαιοποιημένα δεδομένα, η υπόθεση οτι τα διφωσφονικά μπορούν να επηρεάσουν τη φυσική ιστορία του καρκίνου του μαστού δεν επιβεβαιώνεται. Επιπλέον, τα διφωσφονικά δε φαίνεται να προλαμβάνουν τα κατάγματα σε αυτούς τους ασθενείς. Σχετικά με τον κίνδυνο οστεονέκρωσης της γνάθου,

φαίνεται να είναι ένα σπάνιο γεγονός σε αυτόν τον τρόπο χρήσης διφωσφονικών.

Η μετα-ανάλυση του fulvestrant προτείνει οτι το fulvestrant σε δόση 250 mg έχει ίδια δραστικότητα με τις υπόλοιπες ορμονοθεραπείες, με παρόμοιο προφίλ παρενεργειών.

Η μετα-ανάλυση της μερικής ακτινοβόλησης του μαστού, παρόλο το γεγονός οτι στηρίζεται σε περιορισμένα τυχαιοποιημένα δεδομένα, προτείνει ότι η μερική ακτινοβόληση είναι μια ασφαλής θεραπευτική επιλογή σε επιλεγμένους ασθενείς καθώς δε φαίνεται να επηρεάζει την επιβίωση σε σύγκριση με την ολική ακτινοβόληση του μαστού, που παραμένει πάντως θεραπεία επιλογής. Όμως. το θέμα των τοπικοπεριοχικών υποτροπών απαιτεί περαιτέρω διερεύνηση.

Τα αποτελέσματα της μετα-ανάλυσης για το bevacizumab δείχνουν οτι η προσθήκη του συγκεκριμένου φαρμάκου στην χημειοθεραπεία σε ασθενείς με προχωρημένο καρκίνο του μαστού προσφέρει μια βελτίωση στον χρόνο ως την υποτροπή αλλά δεν επηρεάζει τη συνολική επιβίωση. Η κλινική αξία της βελτίωσης αυτής είναι αμφιλεγόμενη. Ως εκ τούτου, η θεραπεία με bevacizumab σε αυτούς του ασθενείς δε συνίσταται.

Τέλος, η μετα-ανάλυση του trastuzumab ως προεγχειρητική θεραπεία υπογραμμίζει τη θετική επίδραση του φαρμάκου, σε συνδυασμό με τη χημειοθεραπεία, σε ασθενείς με Her2-θετική νόσο, προσφέροντας σημαντικό όφελος στην πλήρη ύφεση. Είναι άξιο λόγου το γεγονός οτι η προσθήκη του trastuzumab δεν προσθέτει επιπλέον καρδιοτοξικότητα για τους ασθενείς.

Abbreviations

EBM; Evidence-based medicine

RCT; Randomized Controlled Trial

BSE; Breast-self examination

MRI; Magnetic Resonance Imaging

FNA; Fine Needle Aspiration

ER; Estrogen receptor

PgR; Rogesteron receptor

BCT; Breast conservation treatment

ALND; Axillary lymph node dissection

SLNB; Sentinel lymph node biopsy

WBRT; Whole-Breast Radiotherapy

BCS; Breast-Conserving Surgery

Gy; Gray

GnRHAs; Gonadotropin-releasing hormone analogs

AIs; Aromatase inhibitors

TAC; docetaxel-doxorubicin-cyclophosphamide

AC; doxorubicin-cyclophosphamide

TC; docetaxel-cyclophosphamide

FAC; fluorouracil-doxorubicin-cyclophosphamide

FEC; cyclophosphamide-epirubicin-fluorouracil

EC; epirubicin-cyclophosphamide

CMF; cyclophosphamide-methotrexate-fluorouracil

OR; Odds ratio

RR; Relative risk

HR; Hazard ratio

CI; Confidence Interval

IPD; Individual patient data

ONJ; Osteonecrosis of the Jaw

CTIBL; Cancer treatment-induced bone loss

APBI; Accelerated Partial Breast Irradiation

3D; 3-Dimensional

cm; centimeters

mm; millimeters

IORT; Intra-operative Radiation Therapy

kV; KiloVolt

EBRT; External Beam Radiation Therapy

IMRT; Intensity-Modulated Radiation Therapy

VEGF; vascular endothelial growth factor

TTP; Time to progression

EBR; elsewhere breast recurrences

OS; Overall Survival

PFS; Progression-Free Survival

ORR; Objective Response Rate

pCR; pathologic complete response

List of papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

I Mauri D, **Valachis A**, Polyzos NP, Mavroudis D, Georgoulias V, Casazza G.: Does adjuvant bisphosphonate in early breast cancer modify the natural course of the disease? A meta-analysis of randomized controlled trials. J. NCCN 2010; 8: 279-86

II **Valachis A**, Polyzos NP, Georgoulias V, Mavroudis D, Mauri D.: Lack of evidence for fracture prevention in early breast cancer bisphosphonates trials: a meta-analysis. Gynecol Oncol 2010; 117: 139-45

III Mauri D, **Valachis A**, Polyzos IP, Polyzos NP, Kamposioras K, Pesce LL.: Osteonecrosis of the jaw and use of bisphosphonates in adjuvant breast cancer treatment: a meta-analysis. Breast Cancer Res Treat 2009; 116: 433-9

IV **Valachis A**, Mauri D, Polyzos NP, Mavroudis D, Georgoulias V, Casazza G.: Fulvestrant in the treatment of advanced breast cancer: a systematic review and meta-analysis of randomized controlled trials. Crit Rev Oncol Hematol 2010; 73: 220-227

V **Valachis A**, Mauri D, Polyzos NP, Mavroudis D, Georgoulias V, Casazza G: Partial breast irradiation or whole breast radiotherapy for early breast cancer: a meta-analysis of randomized controlled trials. Breast J, 2010;16: 245-51

VI **Valachis A**, Polyzos NP, Patsopoulos NA, Georgoulias V, Mavroudis D, Mauri D.: Bevacizumab in metastatic breast cancer: a meta-analysis of randomized controlled trials. Breast Cancer Res Treat,. 2010; 122: 1-7

VII **Valachis A**, Mauri D, Polyzos NP, Chloverakis G, Mavroudis D, Georgoulias V.: Trastuzumab combined to neoadjuvant chemotherapy in patients with HER2-positive breast cancer: a systematic review and meta-analysis. The Breast, 2011: 20; 485-490

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1. General Introduction

During the last decades clinicians are confronted by an enormous body of medical information, much of it invalid or irrelevant to clinical practice. This phenomenon has led to the need of prioritizing information so as to provide the best possible care for patients. Evidence-based medicine (EBM) is the process of systematically reviewing, appraising and using clinical research findings to aid the delivery of optimum clinical care to patients [1].

Evidence-based medicine seeks to prioritize information in a hierarchy of evidence by study design from the most biased to the least biased [2,3]. Randomized controlled trials (RCTs) represent the cornerstone of EBM and offer a high level of evidence (level I of evidence). Unfortunately, the results of RCTs can be inconclusive and misleading due to deficiencies in the study design, the small sample size and, consequently, the unavailability to show statistically significant differences in outcomes; moreover, the report of conflicting results among RCTs that address the same clinical question which could be due to the enrollement in the trials of heterogeneous patient populations further confound the answers to the clinical questions.

Meta-analysis offers a quantitative synthesis of previously conducted studies. By pooling the quantitative results from all properly RCTs in a statistically valid fashion, it is possible to increase the power of the study and arrive at a more precise estimate of treatment effect. As a result, meta-analyses give the opportunity to address the above mentioned limitations and biases of RCTs. They are also considered level I evidence if the study is limited to randomized controlled trials.

Despite the growing body of RCTs regarding the use of various treatment strategies (chemotherapy, endocrine therapy, radiotherapy) in breast cancer, the efficacy of some strategies remains unclear and questionable. Meta-analyses could help to reduce the level of uncertainty and provide reliable conclusions about the role of various therapeutic choices for breast cancer patients.

2. General Part

2.1. Breast Cancer

2.1.1 Breast Cancer Epidemiology

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in females worldwide, accounting for 23% (1.38 million) of the total new cancer cases and 14% (458,400) of the total cancer deaths in 2008 [4]. About half the breast cancer cases and 60% of the deaths are estimated to occur in economically developing countries [4].

The well-documented geographical variation in breast cancer incidence worldwide, with higher incidence in Northern Europe, Australia/New Zealand, and North America; intermediate in South America, the Caribbean, and Northern Africa; and low in sub-Saharan Africa and Asia [4,5] can be explained by differences in known risk factors of the disease between those populations including reproductive and hormonal factors as long as socio-economic factors, alcohol consumption and the availability of early detection services [5].

In many Western countries, a large increase in breast cancer incidence was observed between 1964 and 2003 which, probably, could reflect the dramatic changes in exposures to reproductive and nutrition-related determinants that occurred during this period in those countries [5-7] as well as the increasing diagnostic activity, due to mammography screening and increasing individual awareness of the disease [5,8]. However, the prevalence of the disease in some Western countries has been decreased from the beginning of millennium partly due to the lower use of combined postmenopausal hormone therapy [9, 10].

In most Western countries, the breast cancer mortality has been decreased in recent years; this could be attributed to the early implementation of organized mammography screening and to a high and relatively uniform standard of living, diagnosis and treatment [8,11]. This declining trend is predicted to continue in Europe in 2011 [12].

2.1.2 Breast cancer diagnosis

Breast cancer, at least in its early stage, does not cause any specific symptoms. Mastalgia and nipple discharge are rarely associated with breast

cancer. In locally advanced disease a clinical appearance with warm and thickened skin with ulceration and / or a peau d'orange (skin of an orange) appearance and retraction of the nipple may be presented. This clinical presentation can be confused with inflammatory breast cancer [13].

Breast self-examination (BSE) is considered to be an important method for breast cancer diagnosis and it was recommended by the most screening organizations as a routine screening modality before 2000 [14]. However, in two randomized trials [15] BSE failed to reduce breast cancer mortality and, in contrast, it was associated with unnecessary diagnostic procedures, due to false-positive results, as well as psychological consequences such as anxiety, worry and depression. Nevertheless, since a large amount of breast cancers are found by women themselves [16,17], self examination may optimize the chances of a woman to find a change from normal.

The most common route for identification of breast cancer is through screening. Mammography screening is sensitive (77-95%), specific (94-97%), and acceptable to most women [14]. Randomized trials of screening mammography demonstrate reduced mortality with screening [18,19].

Magnetic resonance imaging (MRI) of the breast is not indicated as a routine procedure, but may be considered in cases involving diagnostic problems due to several reasons such as: dense breast tissue especially in young women, in cases of familial breast cancer associated with BRCA mutations or women with greater than 20% lifetime risk of developing breast cancer as defined by risk prediction models based on family history of breast or ovarian cancer, in patients with silicone gel implants, positive axillary lymph node status with occult primary tumor in the breast, in cases of suspected multiple tumor foci and especially in patients with lobular carcinomas [20,21].

In case of symptoms and / or a palpable finding on BSE the initiation of a triple assessment includking clinical examination, imaging and sampling of lesion for cytological/histological assessment provides the most accurate diagnostic algorithm [22]. Once a suspicious abnormality is detected on screening or imaging, a confirmatory cytological/histological diagnosis is essential.

There are several breast biopsy techniques in current use ranging from minimal invasive percutaneous needle biopsies (fine-needle aspiration [FNA], core biopsy, vacuum-assisted breast biopsy) with or without image-guidance (including ultrasound, mammography, MRI) to excisional biopsies. The type of technique performed in each case is based on the characteristics of the lesion (e.g., palpable versus nonpalpable; solid mass versus microcalcifications), as well as patient's and physician's preferences [23].

FNA refers to the use of a needle to obtain samples from a solid mass for cytology. Drawbacks of FNA are that is limited to cytologic but not histologic examination and that it yields a higher false-negative rate [24]. Ultrasound or stereotactic guidance is used to assist in collecting an FNA from a non-palpable lump.

Ultrasound-guided core needle biopsy is the modality of choice for most patients when a suspicious abnormality is visible on ultrasound [25]. This technique offers a high diagnostic accuracy rate since it provides sufficient material for histological assessment [26]. In some cases, the severity of disease can be underestimated by core-biopsy. In an attempt to reduce this under-estimation rate, a vacuum-assisted breast biopsy was developed in which vacuum suction is used to retrieve larger amount of tissue [27].

In case of breast masses visible only in mammography, a stereotactic core biopsy is performed [28]. In this technique, the target is located with the use of digital mammography. Recently, MRI-guided core biopsy is also available in case of masses visible only in MRI [29].

Excisional biopsy is done when needle biopsies are negative but the mass is clinically suspected of malignancy. Excision may be the initial procedure of choice if the probability of malignancy is high. If the mass is not palpable, wire localization of the mass can be done before the biopsy.

2.1.3 Staging of breast cancer

Once breast cancer diagnosis is established, it is essential to accurately define the initial extent of disease since breast cancer stage affects subsequent treatment decisions.

Breast cancer is classified according to the American Joint Committee on Cancer and the International Union for Cancer Control

(AJCC-UICC) TNM breast cancer staging system [30]. The TNM staging system was designed to be a useful instrument in determining the prognosis of cancer patients and in planning their treatment. The system is derived from tumour size (T), lymph node status (N) and distant metastasis (M). Clinical stage is based on all information, including physical examination and imaging before surgery. Pathological staging (pTNM) adds additional information gained by examination of the tumour microscopically by a pathologist.

1. Primary tumour (T); Tx, primary tumour cannot be assessed; T0, no evidence of primary tumour; Tis, carcinoma in situ or Paget disease of the nipple; T1, tumour 20 mm or less; T2, tumour more than 20 mm but nor more than 50 mm; T3, tumour more than 50 mm; T4, tumour of any size with direct extension to chest wall or skin, or inflammatory breast cancer.

2. Regional lymph nodes N0; no node metastasis (includes cases with only isolated tumour cells, or small clusters of cells, not more than 0.2 mm); N1mi, micrometastasis (larger than 0.2 mm, but none larger than 2 mm); N1, metastasis in 1-3 ipsilateral axillary node(s) and/or in ipsilateral internal mammary nodes with microscopic metastasis detected by sentinel lymph node dissection but not clinically apparent; N2 metastasis in 4-9 ipsilateral axillary lymph nodes or in clinically apparent internal mammary lymph node(s); N3, metastasis in 10 or more ipsilateral axillary lymph nodes, or in infra- or supraclavicular lymph nodes, or in both ipsilateral axillary lymph nodes and clinically apparent ipsilateral internal mammary lymph nodes. 13.

3. Distant metastasis (M); M0, no distant metastasis; M1, presence of distant metastasis

2.1.4 Prognostic and predictive factors

A prognostic factor is defined as any indicator that correlates with the prognosis of disease. It is well documented that many patients with early breast cancer benefit from postoperative adjuvant therapy, a treatment strategy with view to eradicate distant micrometastatic deposits. The most important clinical challenge is to identify those patients and to avoid over-treatment and as a result of treatment-related side effects in those who are not going to benefit from adjuvant therapy. The use of established prognostic

factors is therefore essential for the appropriate selection of adjuvant therapy in breast cancer patients.

In addition, predictive factors, which are defined as factors that predict the responsiveness at a specific cancer therapy, may identify the appropriate therapy for an individual patient.

There are several clinicopathological factors that are associated with breast cancer prognosis and treatment response including lymph node involvement, tumor size, grading, Ki-67 expression, ER and PR status, and Her2 status. Their determination is a critical element for the treatment decision making [31].

Considering these factors in combination is of greater clinical value than viewing each in isolation, and the combined approach constitutes the basis of a number of schema used to group patients into various risk categories such as the St Gallen criteria [32], the NIH consensus criteria [33], the Nottingham Prognostic Index [34] and Adjuvant!Online (www.adjuvantonline.com)

Lymph node involvement is the most important prognostic factor for survival in breast cancer patients and, consequently, information about it provides both staging information and guidance regarding treatment options [35].

Tumor size is associated not only with lymph node metastasis but also with distant recurrence. Indeed, larger tumors increase the risk for recurrences and worse survival [36,37].

In addition, the traditional pathologic subtyping and grading of breast cancer have also prognostic significance. Certain subtypes such as tubular, mucinous, and medullary have a more favorable prognosis than other types [38-40]. Tumor grade, as determined by the Nottingham (Elston-Ellis) modification of the Scarff-Bloom-Richardson grading system, also known as the Nottingham Grading System (NGS) [41], is another prognostic factor. According to a grading system which based on certain morphological features (tubule formation, nuclear pleomorphism, and mitotic count) breast cancer can be classified as well differentiated (grade 1), moderately differentiated (grade 2) and poorly differentiated (grade 3). Patients with breast cancer classified as grade 3 have worse outcome compared with those with grade 1 and 2 breast cancer [42].

In the last decades, various methods of measuring the proliferative rate of tumors have been evaluated in an attempt to correlate them with prognosis. The only proliferative biomarker that has found its role as prognostic factor in breast cancer is Ki-67 expression. High Ki-67 expression in patients with early breast cancer confers worse prognosis [43].

ER and PgR status are both prognostic and predictive factors. Their prognostic significance is difficult to evaluate because it is covered by their strong predictive role. The presence of ER and / or PgR receptors is strongly associated with better response to adjuvant endocrine therapy [44]. It has further been conclusively demonstrated that PgR status is independently associated with disease-free and overall survival; indeed, patients with ER-positive/PgR-positive tumors have a better prognosis than patients with ER-positive/PgR-negative tumors, who in turn have a better prognosis than patients with ER-negative/ PgR-negative tumors [45].

The c-erbB-2 (HER2/neu) proto-oncogene, which encodes for the transmembrane glycoprotein p185HER2 with intrinsic tyrosine kinase activity is homologous to the epidermal growth factor receptor 2 [46]; HER2 was first identified to be an indicator of patient's prognosis. In cases of HER2 overexpression, breast cancer patients were more likely to suffer from relapse and shorter overall survival [47,48]. Regarding the role of HER2 overexpression as predictive factor, there is some evidence that its overexpression is associated with better clinical responsiveness to anthracycline-containing chemotherapy [49] and to the addition of paclitaxel after adjuvant treatment with doxorubicin [50]. The revolution, however, in the use of HER2 overexpression as predictive biomarker took place after the development of Trastuzumab, a monoclonal antibody directed against the extracellular domain of the HER2 molecule, has altered the natural history of HER2 positive breast cancer [51]. Recently, Lapatinib, a new tyrosine kinase dual inhibitor of EGFR and HER2, showed clinical efficacy in metastatic HER2 positive breast cancer and has been approved for clinical use [52]. Currently, other HEr2-targeting agents have been tested (i.e. Pertuzumab, TDM-1). In all those targeted agents, HER2 status is the absolute predictive biomarker.

Despite the clinical usefulness of the above mentioned clinicopathological characteristics as prognostic and predictive factors, such factors have only limited ability to predict individual patient outcomes. Indeed, patients with same clinicopathological characteristics may have

completely different clinical courses. The understanding of human genome and the development of high-throughput technologies have led to the idea of analyzing breast cancer in a molecular basis by using molecular signatures.

The performance of gene expression profiling studies, using DNA microarrays, in breast cancer indicated the existence of at least 4 molecularly distinct neoplastic subtypes, which appear to originate from different cell types: the **basal-like** subtype, which is predominantly ER-negative, PgR-negative and Her2-negative (often referred to as triple negative); the **Her2-like subtype**, characterized by the increased expression of several genes of the *Her2* amplicon at 17q22.24 including *ERBB2* and *GRB7* and, at least, two **luminal-like** subtypes, predominately ER-positive, the **luminal A and B** [53,54]. Importantly, the newly defined molecular subgroups have distinct clinical outcomes and responses to therapy. Indeed, the low-grade and low-proliferation luminal A tumours are sensitive to endocrine therapy and have a more favourable prognosis than the ER-negative and high-grade basal-like tumours despite the fact that the ER-negative and high-grade basal-like tumours that are endocrine unresponsive respond better to chemotherapy [55].

Based on these results, two commercially available genomic tests based on genomic profiling have been developed. These include the 70-gene expression signature as used in the MammaPrint® (Agendia, Amsterdam, the Netherlands) assay [56], and the 21-gene profile used in the Oncotype Dx® (Genomic Health, Redwood City, CA, USA) assay [57]. The aim of these assays is to provide better prognostic information compared with traditional prognostic indicators so as to make the clinical decision making more accurate and individualized. The St Gallen International Expert Consensus [31] have concluded that the 21-gene signature (Oncotype DX) may be used where available to predict chemotherapy responsiveness in an endocrine responsive cohort where uncertainty remains after consideration of other tests [58,59]. Conversely, the chemopredictive properties of the 70-gene signature (MammaPrint) were not yet sufficiently established. Two ongoing large prospective trials [MINDACT (Microarray In Node negative Disease may Avoid ChemoTherapy), TAILORx (Trial Assigning IndividuaLized Options for Treatment (Rx)] are going to assess the abilities of molecularly based assays to determine best adjuvant treatment for specific subsets of breast cancer patients.

2.1.5 Treatment strategies

The treatment decision of breast cancer is based on the stage of the disease, the functional status and comorbid condition of the patient as long as the patient expectation that may impact treatment decisions.

The treatment strategies for early breast cancer can be divided into 4 categories: surgery, postoperative radiotherapy, postoperative / adjuvant chemotherapy and adjuvant endocrine therapy. Multidisciplinary treatment planning involving at least a breast surgeon, radiologist, pathologist, and medical and radiation oncologists should be used to integrate local and systemic therapies and their sequence [60].

The cornerstone of early breast cancer therapy is surgery. The major change in the surgical treatment of breast cancer was the effort to reduce surgical extent from mastectomy to breast conservation treatment (BCT). BCT refers to surgical removal of the tumor without removing excessive amounts of normal breast tissue. The aim of BCT was to provide a cancer operation equivalent to mastectomy and a cosmetically acceptable breast, with a low rate of recurrence in the treated breast. Indeed, a number of randomized trials documented that mastectomy with axillary lymph node dissection (ALND) is equivalent to BCT with lumpectomy, ALND and postoperative whole-breast radiotherapy [61,62]. Breast conserving surgery requires the complete excision of the tumour with clear margins and an acceptable cosmetic result following excision and radiotherapy.

Although BCT has become the gold standard for patients with early breast cancer, mastectomy remains an option and it is necessary in at least one-third of those women because of tumor size (e.g. >4cm diameter), or tumor multifocality / multicentricity, central tumor site within the breast and prior radiation to the chest wall or breast [63]. The European treatment guidelines recommend that breast reconstruction should be available to those women requiring mastectomy [64] since reconstruction does not appear to be associated with an increase in the rate of local cancer recurrence or worse survival [65,66], and can yield psychological benefit [67,68]. Reconstruction can be performed either immediately following mastectomy and under the same anesthesia or in a delay fashion following mastectomy.

Regarding axillary staging, sentinel lymph node biopsy (SLNB) has replaced ALND as the standard of care, unless axillary node involvement is

suspected clinically or on ultrasound. SLNB offers the same efficacy and accuracy in staging as ALND with lower morbidity [69,70]. Large randomized studies have confirmed that patients with negative sentinel node and no additional ALND have at least equivalent survival rates with node-negative patients who performed ALND [71-73]. Thus, the omission of ALND in patients with negative SLNB seems completely justified.

The presence of macrometastatic spread in the SLNB traditionally mandates conventional ALND. However, a recent randomized trial supported the safety of omitting ALND even in patients with a clinically node negative axilla but pathological macrometastatic involvement of one or two sentinel nodes in the context of breast-conserving surgery with tangential field radiation therapy [74]. Recently, the St Gallen International Expert Panel accepted the option of omitting axillary dissection for macrometastases in the context of lumpectomy and radiation therapy for patients with clinically node negative disease and 1–2 positive sentinel lymph nodes. However, in patients undergoing mastectomy, those who will not receive whole-breast tangential field radiation therapy, those with involvement of more than two sentinel nodes, and patients receiving neoadjuvant therapy, axillary dissection should remain standard of care [31]. This trend of minimizing the extent of surgical intervention in the axilla is similar to the trend observed in breast cancer surgery and led to the use of BCT instead of mastectomy.

Recently, due to the advances of pathologic examination techniques and the use of immunohistochemistry for the examination of sentinel nodes, identification of sentinel nodes with low-volume metastatic disease (micrometastasis and isolated tumor cells) has been appeared These terms are defined by the American Joint Committee on Cancer (AJCC) as follows: micrometastases as clusters of cancer cells greater than 0.2 mm but not larger than 2.0 mm and isolated tumor cells as individual cancer cells or cell clusters no larger than 0.2 mm [30]. The optimal management of micrometastatis or isolated tumor cells in the sentinel node is the subject of ongoing active research. In general, there was a trend for management of micrometastasis as macrometastasis (ALND) while patients with isolated tumor cells were treated with no further axillary node dissection [75]. Recently, two large prospective studies, which failed to demonstrate clinically significant association between micrometastasis in sentinel node and overall survival [76,77], have altered the perceptions about the

usefulness of immunihistochemistry in the evaluation of sentinel nodes. Indeed, the St Gallen International Expert Panel proposed that isolated tumor cells, and even metastases up to 2 mm (micrometastases) in a single sentinel node, should not be constitute an indication for axillary dissection regardless of the type of breast surgery carried out [31].

Considering the absence of data demonstrating superior survival with ALND or SLNB, these procedures may be considered optional in patients with co-morbidities, elderly patients or patients for whom the selection of adjuvant therapy is unlikely to be affected by the results of this procedure [78]

Postoperative whole-breast radiation therapy (WBRT) after breast-conserving surgery (BCS) is known to reduce the rate at which the disease recurs in about 50% and the breast cancer death rate by about a sixth. [79]. Thus, WBRT is strongly recommended after BCS. However, in certain cases with patients >70 years of age who have endocrine-responsive invasive breast cancer with maximum stage pT1N0 and clear margins, it may be possible to omit radiation therapy without compromising survival [80,81].

Postmastectomy radiotherapy (to the ipsilateral chest wall, mastectomy scar and drain sites) in node negative breast cancer could be considered an option in case of large tumors (> 5 cm) with close or positive margins [82].

Regional lymph node irradiation is strongly recommended for patients with 4 or more axillary lymph nodes involved regardless the type of surgery [60,78]. Radiation should be delivered to the chest wall (in case of mastectomy) or remaining breast tissue (in case of BCS), plus axillary, supraclavicular and infraclavicular lymph nodes; inclusion of internal mammary lymph nodes in the radiation field can be considered but should be included in the target volume in cases of metastatic spread to this area.

Unclear recommendations exist about the role of radiotherapy in patients with 1-3 positive lymph nodes [60,78]. Radiotherapy may be considered as a treatment option in those patients regardless type of surgery. There is evidence that radiotherapy may have beneficial effect on survival both after mastectomy [83] and after BCS [84] in patients with 1-3 positive lymph nodes.

Adjuvant doses typically used for local and/or regional irradiation are 45 to 50 Gray (Gy), in daily, Monday-to-Friday fractions of 1,8-2,0 Gy, over a 5-week period. A boost to the tumor bed is recommended in patients at higher risk for local failure (age < 50 years old, close margins, multifocality) with an additional 10 to 16 Gy / 2 Gy fraction over 1 to 2 weeks.

Adjuvant systemic therapy refers to the administration of chemotherapy, hormone therapy and/or trastuzumab (a monoclonal antibody directed against HER2) following primary surgery for early breast cancer. The purpose is to eliminate or delay the subsequent appearance of clinically occult micrometastases, thought to account for distant treatment failures in women undergoing local therapy alone. Treatment is recommended if a relevant reduction of the estimated risk of recurrence and death can be expected with an acceptable level of treatment-related adverse effects. A number of prognostic factors and prognostic tools, as those described above, have been used in order to estimate the absolute benefit expected from systemic adjuvant therapy.

Patients with invasive breast cancer that are ER- and / or PgR-positive should be considered for adjuvant endocrine therapy regardless of patient age, lymph node status or adjuvant chemotherapy. Possible exception from this recommendation is those patients with favorable prognostic features and tumor size less than 1 cm [78].

In premenopausal patients tamoxifen alone (20 mg daily for 5 years) decreases the risk for disease recurrence and death [85]. In patients receiving tamoxifen and adjuvant chemotherapy, tamoxifen should be administered after chemotherapy [86]. At present, the role of ovarian suppression in addition to tamoxifen for premenopausal patients is not known. Ovarian suppression may be achieved either with bilateral oophorectomy which leads to irreversible ablation of ovarian function or with the use of Gonadotropin-releasing hormone analogs (GnRHAs) which lead to reversible ovarian suppression. There is data supporting the usefulness of ovarian function ablation [87] but it has not gained acceptance among oncologists as standard of care [31].

In postmenopausal women, aromatase inhibitors (AIs) should be used during the course of adjuvant treatment to lower recurrence risk, either as primary therapy or after 2 to 3 years of tamoxifen [88]. In the sequential

setting, patients should receive an AI after 2 or 3 years of tamoxifen for a total of 5 years of adjuvant endocrine therapy. Patients initially treated with an AI but who discontinue treatment before 5 years of therapy should consider incorporating tamoxifen for a total of 5 years of adjuvant endocrine therapy [89]. For patients who have completed 5 years of tamoxifen the addition of an AI for a further period of 2–5 years is recommended especially for patients with node-positive disease [90-92]. The extended adjuvant endocrine therapy strategy seems to offer benefit in reducing the recurrence rate but none of the studies revealed any survival benefit. In postmenopausal patients, 5 years of tamoxifen alone is still a viable option for certain patients at very low risk of recurrence. The total duration of adjuvant endocrine therapy should not exceed 5-10 years while there is no data for the use of AIs for more than 5 years [60].

Chemotherapy as adjuvant therapy is recommended in patients with node positive disease, with unfavorable prognostic factors, Her2-overexpression or with tumors unresponsive to hormone manipulations. Several chemotherapy regimens may be considered in the adjuvantr setting such as docetaxel-doxorubicin-cyclophosphamide (TAC), doxorubicin-cyclophosphamide (AC), dose-dense AC with paclitaxel, AC followed by weekly paclitaxel, docetaxel-cyclophosphamide (TC), fluorouracil-doxorubicin-cyclophosphamide (FAC), cyclophosphamide-epirubicin-fluorouracil (FEC), epirubicin-cyclophosphamide (EC), AC with sequential docetaxel, FEC followed by docetaxel or weekly paclitaxel, and cyclophosphamide-methotrexate-fluorouracil (CMF) [78]. At present, the use of anthracyclines may be recommended for most patients [60]. In addition, the combination of anthracyclines and taxanes seems to offer some benefit especially when taxanes follow anthracyclines in a sequential setting [93].

Recently, the integration of capecitabine into standard adjuvant chemotherapy with anthracyclines and taxanes was found to reduced breast cancer recurrence [94] and improve overall survival [95] especially in patients with high-risk breast cancer (triple negative [96] or high Ki-67 expression [97].

Patients with breast cancers that overexpress Her2 or have Her2 gene amplification benefit from adjuvant treatment with trastuzumab [98]. It seems that concomitant adjuvant trastuzumab therapy with taxanes gives a significant and greater benefit than sequential administration in both disease-

free and overall survival [99]. However, the concurrent administration of anthracyclines and trastuzumab should be avoided due to the higher risk for cardiotoxic effect [100]. While randomized trials have excluded patients with small primaries of <1 cm, overexpression of Her2 confers a poorer prognosis even in these small tumors [101], and the use of trastuzumab should be discussed with women with small, node-negative breast cancers. The optimum duration of adjuvant trastuzumab has not yet been established, but for the time being 1 year is recommended [60].

Preoperative (neoadjuvant) chemotherapy is a treatment option for women with locally advanced breast cancer including inflammatory breast cancer and for large operable tumors for reducing tumor size in order possibly to perform BCS [60,78]. The survival benefit gained from chemotherapy in breast cancer patients has been found to be equal regardless whether chemotherapy is administered preoperatively or postoperatively [102]. The chemotheraupetic regimens recommended in adjuvant setting can be used in neoadjuvant setting as well [78]. Primary systemic therapy should be followed by both surgery and radiation therapy according to the principles outlined above. In postmenopausal women with highly endocrine-responsive disease, neoadjuvant endocrine therapy with aromatase inhibitors for a minimum of 4–8 months is a valid option [103,104]. If the tumor responds preoperatively, BCS can be performed. With multifocal disease or in the case that the tumor shrinkage is limited, mastectomy will still be required.

In metastatic disease, the management should involve all appropriate specialties in a multi-/interdisciplinary team (medical, radiation, surgical and imaging oncologists, palliative care specialist, psychosocial support), and patients should be offered personalized appropriate psychosocial, supportive and symptom-related interventions as a routine [60]. The treatment goal is to prolong survival and improve quality of life. Thus, the use of treatments with minimal toxicity is preferred.

A growing body of evidence supports the need for biopsy of metastatic lesions and re-assessment of biological and phenotypic characteristics of the tumor because of the risk for discordance in estrogen receptor / Her2 status between primary and metastatic tumor [105,106]. Biopsy of metastases should be considered in all patients, when safe and easy to carry out, since it is likely to impact treatment choice and, consequently, clinical outcome [107,108].

Women with metastatic disease characterized by tumors that are ER- and / or PgR- positive are candidates for initial endocrine therapy. Limited studies document a progression-free survival benefit by adding trastuzumab or lapatinib to AIs in postmenopausal women with Her2 positive disease [109,110]. Women with metastatic breast cancer who respond to endocrine therapy should receive additional endocrine therapy at the time of disease progression [78]. Patients with clear evidence of endocrine resistance should be offered chemotherapy.

Women with hormone-receptor negative tumors, symptomatic visceral metastases or endocrine resistance are candidates for cytotoxic treatment [60,78]. The only standard is the use of a taxane-based regimen as first-line therapy in patients progressing after adjuvant anthracycline-based chemotherapy [60]. A variety of chemotherapeutic regimens have a proven efficacy in metastatic breast cancer. The selection of the best agent/regimen should be individualized. For the majority of patients, overall survival outcomes from the sequential use of single cytotoxic drugs is equivalent to that of combination chemotherapy, with less associated toxicity and better quality of life. Therefore, in the absence of the need for a rapid and significant response for symptom control or life-threatening disease, preference should be given to the sequential use of a sequential single cytotoxic agent approach [60].

In patients with metastatic breast cancer and Her2-positive disease, trastuzumab is highly recommended [60,78]. After progression on first-line trastuzumab-containing treatment, the continuation of trastuzumab with a different chemotherapeutic regimen is recommended. Another valid option after trastuzumab progression can be the combination of lapatinib-capecitabine [111].

In patients with bone metastases, bisphosphonates are recommended since their use is associated with fewer skeletal-related events, pathologic fractures and less need for radiation therapy or surgery to treat bone pain [112,113]. A recent study demonstrated superior activity in terms of skeletal-related events and a favorable toxicity profile of the RANK-ligand antibody denosumab in breast cancer-related bone disease [114]. Consequently, both bisphosphonates and denosumab are considered drug of choice in patients with breast cancer and bone metastases.

2.1.6 Follow-up after breast cancer treatment

The purpose of follow-up is to detect recurrences at an early stage so as to begin treatment for any relapse quickly.

A meta-analysis of randomized trials compared follow-up based on clinical visits and mammography with a more intensive scheme including radiological and laboratory tests could not reveal any differences in terms of overall or disease-free survival between the 2 follow-up methods [115]. Consequently, follow-up programs based on regular physical examinations and yearly mammography alone seems to be as effective as more intensive approaches. Other laboratory or imaging tests are indicated in case of suspected relapse.

Whatever the follow-up protocol and the frequency of visits, every visit should include history taking, eliciting of symptoms and physical examination tests so as to assess not only the risk for recurrence but also the therapy-related complications and the need for psychosocial support in order to enhance returning to normal life after breast cancer [60].

2.2 Meta-analysis

2.2.1 General Information

Meta-analysis has been defined as "the statistical analysis of a large collection of analysis results from individual studies for the purpose of integrating the findings." [116]. Since their invention and subsequent application in the medical literature in the early 20th century [117], meta-analyses have continuously evolved. In fact, a simple Medline search, limited in published studies before 2000, with the keywords "cancer" and "meta-analysis" revealed 1060 articles, while the same searching algorithm for the years 2001-2005 and 2006-2010 revealed 1143 and 2865 articles, respectively. It is, therefore, obvious that the number of meta-analyses in the cancer field has increased substantially.

A meta-analysis, by combining the results of all available trials that studied the same question, gives the opportunity to address certain biases and limitations that may exist in RCTs. Meta-analysis is part of a more general process known as systematic review, which involves identification of all the relevant papers, analysis of their quality and, a description of the trials. If the trials are similar enough and if the quality and the quantity of the

data are adequate, a quantitative synthesis of these data, ie a meta-analysis, can be carried out as the last step of a systematic review.

When conducted with appropriate statistical techniques and with high-quality data, findings from meta-analyses are considered to be the highest level of evidence (level 1a evidence) [118].

The importance of meta-analyses in health care is shown by the fact that clinicians read them to keep up to date with their field [119,120] and they are often used as a starting point for developing clinical practice guidelines.

2.2.2 Steps in Performing a Meta-Analysis

The design of a high-quality meta-analysis requires the same careful planning and should meet the standards one would expect in the design of a high-quality RCT.

As will all research, the quality of meta-analysis is an important consideration for the interpretation of the results. It has been found that the quality of reporting of systematic reviews and meta-analyses is not optimal [121-124]. In 1996, to address the suboptimal reporting of meta-analyses, an international group developed a guidance called the QUOROM Statement (QUality Of Reporting Of Meta-analyses), much like as the CONSORT guidelines for reporting RCTs in the peer-reviewed literature [125]. This guidance is focused on the reporting of meta-analyses of randomized, controlled trials [126]. A revision of these guidelines, renamed PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) has been recently published [127]. The PRISMA Statement consists of a 27-item checklist with aim to help authors to improve the quality of reporting of systematic reviews and meta-analyses.

The first step of any meta-analysis is to identify the clinical question. The question should contain the disease or condition of interest, the population of interest, the specific treatments or exposures being studied and the outcome measurements (efficacy, adverse reactions or both) being studied [128]. After a question has been identified, the protocol should be written specifying the aim of the study, the inclusion and exclusion criteria for the trials, the planned analyses and the statistical methods that will be used [129].

The next step is to identify and select the trials that are potentially eligible to be included in the meta-analysis. Inclusion and exclusion criteria for studies are as necessary in a meta-analysis as they are in clinical studies to safeguard against selection bias. These criteria need to be specified in the meta-analysis protocol, just as inclusion / exclusion criteria are specified in a clinical protocol [128].

Ideally, all trials on the question under study should be included in a meta-analysis, whether or not they are published. In order to achieve this goal, a comprehensive searching procedure is mandatory. A structured plan is necessary to manage the frequently large number of papers. Most meta-analyses begin with an electronic search to more than one scientific database (Medline, Embase, Cochrane Clinical Trial Registry, ISI Web of knowledge). Use of computer searches to select the trials is insufficient because, in the past, indexing of trial publications was inadequate [130]. As a result, computer searches should be supplemented by screening for references in published trials and reviews, manual searching of proceedings from major conference in the field, and by personal contacts with investigators and with drug companies. This comprehensive searching approach is trying to ensure that even the so-called "grey literature" (i.e. literature that has not been formally published) will be included in the meta-analysis. It has been found that published trials tend to be larger and show an overall greater treatment effect than grey trials [131]. The publication bias refers to a well-established phenomenon in medical literature in which trials showing statistically significant effects are more likely to be published [132]. Clearly, the exclusion of negative trials from the meta-analysis will substantially bias the results so that the treatment will appear more effective than it actually is [133]. Thus, the inclusion of both published and unpublished trials is essential in order to overcome this potential bias.

Two reviewers using defined criteria to independently search and select studies can improve reliability of the selection process. A flow-chart diagram, which summarizes the results of the searching process, is necessary according to PRISMA statement.

After the identification of potentially eligible trials and the selection of trials that will be included in the meta-analysis, based on the pre-specified inclusion / exclusion criteria, verification of the quality of each trial is essential because it is a component of the quality of the meta-analysis as a whole [129]. Recently, Cochrane Collaboration's tool for assessing risk of

bias has been developed to assess the quality of RCTs included in a meta-analysis [134]. This tool is taking into account the description of randomization model and allocation concealment, the blinding of participants and during the outcome assessment, the presence and handling of incomplete outcome data, the reporting of selective outcome and the presence of other potential biases. The assessment tool can be used in several ways: as a cutoff, which means that the meta-analysis is restricted only to studies at low risk of bias; as a stratifying method, in which meta-analyses are presented stratified according to risk of bias; or as a descriptive characteristic of the study, used in explaining study variability and heterogeneity. The first two methods are preferable because the third one poses the risk that bias is downplayed in the discussion and conclusions of a review, so that decisions continue to be based, at least in part, on flawed evidence.

The next step includes the data abstraction. Data should be abstracted onto structured forms designed to capture relevant information in a concise, focused fashion. The protocol should specify the items, the information to be collected for each item and the format for collecting the items [128]. The data abstraction form should be headed with a study number with the name of the study, the publication or source of data, the name and affiliation of the investigators. There should be descriptions of the study groups, including number of groups, size of group, age, gender distribution, diagnoses, clinicopathological features, treatments (including placebo), other treatment or descriptive variables and length of treatment. The summary of the results can be quite extensive, including descriptive statistics for all groups and all outcome measures. Two investigators should independently perform data abstraction for all the included studies. If consensus is not achieved, a third investigator can be consulted. In case of missing information from primary studies, principal investigators should be contacted in order to retrieve, if available, the missing data.

Data analysis is the last step of a meta-analysis. The analysis must include all eligible trials, published or not, and must be based on the intention-to-treat principle. This means that all patients in the treatment group defined by randomization must be included, whether or not they actually received the assigned treatment or whether they were excluded from the analysis done by the investigator. Data synthesis follows a 2-stage approach; in the first stage, effect size measures, together with their

confidence interval, are generated for each trial and in the second stage, these statistics are summarized in a final estimate of the treatment effect as a weighted average of the treatment effects estimated in the individual trials. The data from each individual study are weighted such that studies that have less variance (spread of data) or a larger sample size contribute more heavily to the overall estimate of effect.

There are effect size measures for dichotomous and continuous variables. Dichotomous (binary) outcomes are, for example, death yes / no, relapse yes /no or therapy response yes / no, yielding such effect sizes as odds ratio, relative risk or risk difference [135]. "Odds" is defined as the ratio of events to nonevents, and the OR is defined as the odds in 1 group (eg, treatment group) divided by the odds in a second group (eg, control group). An OR greater than 1 means that the event is more likely in the treatment group, and therefore, the treatment group is favored if the "event" is desirable. "Risk" is defined as the number of patients with an event divided by the total number of patients, and the risk ratio is defined as the risk in 1 group (eg, treatment group) divided by the risk in a second group (eg, control group). An RR less than 1 favors the treatment group if the "event" is not desirable. The risk difference is defined as the risk in the treatment group minus the risk in the control group, which quantifies the absolute change in risk due to the treatment.

For continuous variables, measurements should be made on the same scale and the mean difference between the treatment and control groups is used.

In case of time-to-event outcomes (disease-free survival, overall survival), a Hazard Ratio (HR) for each trial is estimated. HR less than 1 indicates that the condition or event is less likely in treatment group when compared with control group.

In combining single study results, two models can be approached: the fixed effects and the random effects model. In the fixed-effect model it is assumed that the true effect is the same or similar for each trial and that any variation is due solely to the play of chance. In contrast, the random effects model assumes that population effect sizes vary from study to study. Whenever there is significant heterogeneity between trials, it can be taken into account by use of a random-effect model, developed by DerSimonian and Laird, to estimate and test the overall treatment effect [136]. It leads to a

larger Confidence Intervals (CI), and a less powerful test than the fixed-effect model when there is heterogeneity, because the variance of the overall effect is inflated to account for the variation between trials [137].

An important issue that should be assessed in any meta-analysis is the between-study heterogeneity. There are two possible sources of heterogeneity: i) the first is clinical heterogeneity and results from differences in the patients, interventions, and outcomes assessed in each study. Assessment of clinical heterogeneity requires critical appraisal and clinical sensibility, but not a statistical test; ii) the second is methodological, which refers to differences in treatment effects between studies that result from differences in methodological safeguards against bias. Before the data analysis, the presence of heterogeneity should be determined. The first method to determine methodological heterogeneity is by looking at a forest plot to see how well the confidence intervals overlap. If the confidence intervals of two studies don't overlap at all, there is likely to be more variation between the study results than what you would expect by chance (unless there are lots of studies), and you should suspect heterogeneity. The second method is by performing statistical tests. A test which was commonly used is Cochrane's Q, a statistic based on the chi-square test [138]. The $\chi 2$ test determines whether there is greater spread of results between the studies than is due to chance (hence, heterogeneity is present) and a value less than 0.10 usually suggests this. Unfortunately, this test is thought to have low power; that is, it may sometimes fail to detect heterogeneity when it is present. To try to overcome this, a second test, the I^2 statistic, was developed [139]. This test seems attractive because it scores heterogeneity between 0% and 100%. Further, a rule of thumb was proposed, with 25% corresponding to low heterogeneity, 50% to moderate and 75% to high.

Several approaches have been proposed when heterogeneity is observed. If the results of the studies differ greatly then it may not be appropriate to combine the results at all. In this case, a critical appraisal of the literature should be preferred instead of meta-analysis. However, it is unclear how to ascertain the inappropriateness of performing meta-analysis. Another approach is to perform a meta-analysis by using the random-effect model, as described above. An alternative approach is to try to explore the between-study heterogeneity. This is possible with subgroup analysis and with meta-regression analysis. Subgroup analyses are meta-analyses on individual clinical subgroups that determine the specific effect for those

patients. Ideally, subgroup analyses should be limited in number and should be specified a priori [140]. By this technique, the reviewers could test whether grouping the studies according to certain characteristics reduces heterogeneity within each subgroup. If so, then this result indicates that the characteristic that defined the subgroup is related to treatment effect and that the pooled estimate within the subgroup may represent studies in this subgroup more sensibly. Meta-regression is a technique which allows researchers to explore which types of patient-specific factors or study design factors contribute to the heterogeneity. [141].

An easy way to report the combined data from meta-analyses is by using forest plots. The plot allows readers to see the information from the individual studies that went into the meta-analysis at a glance. [142]. Forest plots use boxes and "whiskers" (horizontal lines indicating the spread of the 95% confidence interval) to represent individual trials. On close inspection, the reader will note that the size of the boxes varies among the different studies represented. In fact, box size correlates directly with the sample size (number of patients enrolled) of an RCT. As mentioned above, the whiskers represent the 95% CIs. These 95% CIs comprise the range of values for which you can be 95% confident that the true value is included, and help provide the reader with an appreciation of the reliability of the results. The wider the 95% CIs, the higher the uncertainty that the reported results are accurate In addition to providing information on the reliability of the results, the whiskers of a 95% CI can inform the reader as to whether the study was statistically significant. If the CI crosses the vertical line of no effect (0 for a difference between two groups and 1 for a ratio of two groups) then that trial result, taken individually, is *not* statistically significant. Conversely, if the CI does not cross the vertical line of no effect, the result *is* statistically significant. The overall result (summary effect) is represented by the diamond shape [143].

Several meta-analysis software and general statistical packages have been developed during the last decades and they are currently being used in the meta-analyses. Each available program has advantages and limitations that the user should be aware of in order to choose the most appropriate software depending on the user's needs and preferences [144].

2.2.3 Advantages and Potential biases in meta-analysis

The primary advantage of meta-analyses over RCTs is the ability to increase the statistical power of the study. The power of a study is defined as the probability of finding statistically significant result (i.e., rejecting the null hypothesis) in a study when a true difference exists between or among the groups of subjects being compared. RCTs frequently prove to be underpowered due to the small sample size or the limited number of observed events. This can lead to a statistically negative result which is unclear whether it truly reflects the fact that there is no difference between treatments, or whether the sample size was simply too small to demonstrate that a detected difference was significant. As mentioned earlier, meta-analyses may overcome this limitation by combining different RCTs, thereby increasing overall sample size and as a result power of the study.

Because of its power, meta-analysis is a good tool for studying interactions between subgroups of patients (depending on clinical or pathological characteristics) and treatment effect. Indeed, the larger the sample size available, the more precise the estimate of the effect, and the hypothesis of subgroup effects can be more reliably investigated. The results of such subgroup analyses should, however, being considered as tool for generating hypotheses requiring confirmation in a new randomized trial.

Furthermore, meta-analysis is an extremely useful method for reconciling of contradictory results among various RCTs in a given area of interest.

On the contrary, several drawbacks regarding the methodology of meta-analysis do exist and numerous criticisms about the validity of meta-analysis have been raised.

First, the meta-analytical combination of individual studies has been criticized as comparing “apples with oranges”; for instance, studies with different dose schedules, follow-up, types of participants, or modes of treatment. If studies are clinically (or methodologically) too diverse, the results of a meta-analysis may be meaningless. Clear inclusion criteria, the graphical presentation of the results in forest plots and heterogeneity statistics, as mentioned above, are an aid in keeping the apples separate from the oranges.

Second, the "Garbage in-garbage out" phenomenon refers to the fact that if a meta-analysis includes many low-quality RCTs, then fundamental errors in the primary studies will be carried over to the meta-analysis. Unfortunately, it is true that no amount of statistical technique can improve the fundamental quality of the data being combined for the meta-analysis. There is a series of biases that threaten the validity of clinical trials. These relate to systematic differences in the patients' characteristics at baseline (selection bias), unequal provision of care apart from the treatment under evaluation (performance bias), biased assessment of outcomes (detection bias), and bias caused by exclusion of patients after they have been allocated to treatment groups (attrition bias) [145]. The use, however, of tools for assessing the risk of bias in primary studies is a valid way to overcome this phenomenon.

Publication bias is a well-known potential threat in meta-analyses. It refers to the selective publication of research findings based on the magnitude, direction, or statistical significance of the study results [146]. The most common expression of publication bias is the fact that positive trials (studies that produce a statistically significant result) are much more likely to be published than so-called negative trials (studies producing no statistically significant association), or trials that produce equivocal results [147].

There are several facets of publication bias [148]. Language bias may exist when literature searches fail to include foreign studies, because significant results are more likely to be published in English [149]. Database bias refers to the exclusion of relevant studies that merit inclusion in meta-analysis due to their publication in journals not indexed in one of the major databases [148]. Citation bias refers to the fact that studies with significant findings tend to be cited more frequently, and those with negative or nonsignificant findings are less likely to be cited. Finally, multiple publication bias can occur if several publications are generated from a multicenter trial or a large trial reporting on a variety of outcomes. If the same set of patients is included twice in the meta-analysis, the treatment effect can be overestimated [148].

All these biases cause an unrepresentative sample of primary studies which may lead to overestimating the effect of the intervention being examined in the meta-analysis. In any meta-analysis, it is therefore important to assess publication bias. The simplest and most commonly used method to

detect such bias is the funnel plot [150]. In a funnel plot, the treatment effect of each individual study is plotted against some measure of its size, like the standard error or the overall sample size. In the absence of publication bias, these plots should be symmetrically shaped like a funnel, because the estimate of the effect of a treatment has a larger variability in smaller studies compared with the variability in larger studies. Since smaller and negative studies are less likely to be published, trials in the bottom left-hand corner of the plot are often absent, creating a degree of asymmetry in the funnel.

A comprehensive searching of the literature, without language restriction, could help to overcome publication bias. Furthermore, the requirement that any RCT should be registered to an electronic database (www.clinicaltrials.gov) before patient accrual is another step towards minimizing risk for publication bias since it is mandatory for all studies to be published irrespectively study outcome [151].

Finally, between-study heterogeneity is a serious drawback in meta-analysis. The assessment, investigation and dealing of heterogeneity have been described above.

2.2.4 Future in meta-analysis research

The field of meta-analysis is in a period of rapid development, with theoretical and methodological advances.

Newer meta-analytical techniques have been developed. Individual patient data (IPD) meta-analysis involves the re-analysis of the actual raw data from all relevant RCTs. The researcher conducting the individual patient-level data study collects the data and re-analyzes it. When literature and IPD meta-analyses have been conducted on the same question, the literature-based method over-estimated the benefit associated with the experimental treatment in most cases. IPD meta-analysis offers advantages and, when feasible, should be considered the best opportunity to summarize the results of multiple studies [152]. Multiple-treatment meta-analysis is a meta-analytical technique that gives the opportunity to rank the effectiveness of many treatments in the same disease, even if no head-to-head comparison among all the treatments has been performed [153]. In addition, a prospective meta-analysis can be conducted if one designs a set of parallel randomized trials, comparing the same two treatments on similar populations, registering the same minimum dataset, and planning a pooled

analysis of the main outcome, eg overall survival or disease-free survival [154].

Finally, an important step for the improvement of quality and increasing confidentiality of systematic reviews and meta-analysis was the establishing of PROSPERO, an international database of prospectively registered systematic reviews in health and social care (www.crd.york.ac.uk/prospero).

3. Specific Part

3.1 Bisphosphonates in adjuvant setting: rationale and risk for Osteonecrosis of the Jaw (ONJ) (Paper I-III)

Female patients receiving adjuvant treatment for early breast cancer are at significant risk of cancer treatment-induced bone loss (CTIBL), including osteopenia and osteoporosis. Cytotoxic chemotherapy results in chemotherapy-induced menopause, which is associated with significant bone loss [155-157], whereas hormonal therapies with AIs can lead to bone metabolism changes including increased rate of bone loss which is, at least, double those reported during early menopause [158,159]. Patients with CTIBL, are at high risk for fractures, which are associated with chronic pain, loss of mobility and even shorter survival [160].

Bisphosphonates are antiresorptive agents that inhibit osteoclast function and they are widely used in benign and malignant bone diseases [161]. Bisphosphonates have an established role in treating patients with bone metastases from breast cancer for preventing skeletal-related events [162].

Evidence from clinical trials supports the efficacy of bisphosphonates for preventing bone loss in patients with early breast cancer during therapy with AIs or in patients with chemotherapy-induced ovarian failure [163]. Current guidelines recommend that patients at risk of developing chemotherapy- or hormone-induced osteopenia or osteoporosis should be considered for preventative bisphosphonates therapy [164]. However, the value of bisphosphonates in the prevention of fractures in average-risk early breast cancer patients is still under investigation.

Except from the potential role of bisphosphonates as preventive agents against bone loss, a growing body of evidence from preclinical studies has shown that bisphosphonates may have antitumor activity through preventing tumor cell adhesion to bone [165,166], inducing tumor cell apoptosis [167], and inhibiting angiogenesis [168]. Furthermore, zoledronic acid has been found to alter the adhesion of breast cancer cells to mesenchymal cells which can theoretically reduce the metastatic potential of cancer cells [169].

Considering the potential dual role of bisphosphonates in breast cancer patients, both as antitumor treatment and as preventive therapy against bone loss, many randomized trials have been conducted examining the role of bisphosphonates in adjuvant setting with conflicting results.

The use of bisphosphonates has been associated with an uncommon but serious side effect, the osteonecrosis of the jaw (ONJ). The incidence of ONJ varies with length of exposure, being 5% among patients treated for 4–12 months, and rising to 7.7% after treatment for 37–48 months [170]. This complication has drawn the attention of the medical community due to the subsequent significant morbidity, ranging from discomfort to significant loss of bone and function, and the fact that bisphosphonates have become commonly prescribed drugs. Considering the long life expectancy of patient with primary breast cancer, ONJ complication may jeopardize the quality of life of these patients for all their life. Fortunately, dose intensity of bisphosphonates regimens used in the treatment of primary breast cancer, is notably lower than those used for metastatic disease, but no study to date was powered enough to estimate the incidence of this complication in adjuvant breast cancer setting.

3.2 Fulvestrant: mechanism of action and role in advanced breast cancer (Paper IV)

Hormone therapy is generally considered as treatment of choice for postmenopausal patients with newly diagnosed advanced breast cancer if the patient's tumor is hormone responsive [60]. Currently, standard therapeutic options in breast cancer are the selective oestrogen receptor modulator tamoxifen, the third generation non-steroidal AIs, anastrozole and letrozole and the steroidal AI exemestane.

While tamoxifen was the cornerstone of hormonal therapy for many years, several randomized trials [171-173] and a meta-analysis [174] demonstrated equivalence or superiority in terms of progression-free survival and overall survival for the AIs compared to tamoxifen.

Despite the advances in hormonal therapy, the majority of patients with advanced breast cancer will experience a disease progression during endocrine therapy. It is therefore becoming extremely important to identify and evaluate new hormonal agents that are effective after disease progression.

Fulvestrant is a steroidal analog of 17β-estradiol, which competitively binds to the ER with high affinity [175]. Unlike tamoxifen, fulvestrant has no ostrogen agonist effect and it is considered a pure estrogen antagonist. Fulvestrant also has a novel mode of action since it binds, blocks and accelerates degradation of oestrogen and progesterone receptor protein, leading to an inhibition of oestrogen signalling [176]. Due to its unique action in downregulation of the ER, fulvestrant lacks of cross-resistance with other antiestrogenes. Indeed, a proposed mechanism of endocrine resistance includes the activation of ER, in the absence of estrogen, from epidermal growth factors, resulting in "cross-talk" or activation of other pathways which promote cell growth [177,178]. Fulvestrant may, at least partially, circumvent this mechanism of resistance by degradation of ER. Early preclinical data demonstrated both the superior inhibition of tumor cell growth with fulvestrant compared with tamoxifen [179] and its activity after tamoxifen resistance [180]. Fulventrant's unique mechanism of action offers another theoretical advantage since the combination with other endocrine therapies may result in a synergistic effect, a concept that is supported by preclinical data [181].

Based on the theoretical advantages of fulvestrant and the promising preclinical studies, several clinical trials were designed to evaluate fulvestrant in postmenopausal women with advance breast cancer.

3.3 Partial breast irradiation: rationale and available techniques (Paper V)

In the past few decades, a major shift in the local management of breast cancer has occurred: mastectomy was replaced by BCS followed by post-operative WBRT [61,62]. A total dose of 45-50 Gy is delivered to the entire breast over 5 to 6 weeks (1.8 to 2 Gy per fraction). In some patients, a boost dose of 10-16 Gy to the tumor bed is added. Solid evidence from randomized trials supports that the combined treatment has equivalent results to mastectomy in terms of local control and survival rates [61,62]. This is a paradigm of a changing standard of care in favor of a new treatment that provides the same clinical results in terms of cure rates, decreased side effects, organ sparing and the prospect of a better quality of life.

Over the past several years, there has been growing interest in the use of accelerated partial-breast irradiation (APBI) as an alternative to WBRT. APBI consists of the irradiation of a limited volume of mammary

gland immediately surrounding the tumour bed offering decreased overall treatment time and several theoretical advantages over WBRT.

Indeed, it has been reported that many women who are candidates for BCS followed by WBRT either elects to have a mastectomy [182] or complete their local treatment with lumpectomy alone [183]. The limited use of WBRT could be explained by the fact that some women cannot or will not undergo the usual 6-7 week course of radiotherapy due to limited financial means, and/or long travel distances to the radiation facilities, and/or lack of time and/or due to poor ambulatory status of the patient [184,185]. Moreover, in countries with limited radiotherapy facilities, patients treated with BCS may wait for a long time before beginning radiotherapy. Consequently, there is a delay in the initiation of postoperative radiotherapy, which may affect not only local control [186] but also overall survival [187,188]. APBI offers increased convenience due to a shorter duration of radiation therapy (5-7 days versus 6 weeks). This significant shortening of the treatment time is extremely important in order to overcome the above mentioned socioeconomic and radiation therapy facilities-related barriers that negatively affect patient compliance with radiotherapy.

Along with the practical advantages, APBI offers some theoretical advantages as well, compared with WBRT. The stronger case for APBI has come from clinical [189] and pathological [190] observations of long-term studies reporting that the majority of breast tumour recurrences occur in proximity to the lumpectomy cavity. In addition, breast cancer relapses outside of the original tumour bed appear to occur with the same frequency following lumpectomy regardless of whether or not adjuvant WBRT is delivered [191]. Consequently, WBRT may not be necessary since most ipsilateral breast tumour relapses occur in the vicinity of the primary tumour and radiotherapy does not seem to prevent other quadrant relapses.

An additional theoretical advantage of APBI is a decreased dose to normal tissue. With a smaller target volume, it may be expected that adjacent organs such as the heart and lungs will receive less radiation.

APBI can be carried out with four principally different techniques: interstitial brachytherapy with multiple catheters, intracavitary brachytherapy, intraoperative radiotherapy and external 3D conformal radiotherapy. Each of these techniques has unique advantages and limitations and is in a different stage of development and acceptance.

Multi-catheter interstitial brachytherapy is the APBI technique that has been utilized the longest and has the most extensive follow-up. In this approach, catheters are placed at 1–1.5 cm intervals through the breast tissue surrounding the lumpectomy cavity. The number of catheters is determined by the shape and the size of the target. Interstitial brachytherapy has been used with all possible dose rates, including low- and high-dose rates. This technique permits individual conformation of the irradiated volume precisely adapted to the anatomical conditions. However, this approach results in significantly heterogeneous delivered dose, the target coverage might be inferior to other PBI techniques and the skin dose might be increased [192].

Intracavitary brachytherapy has been developed to be a less operator-dependent procedure compared with interstitial brachytherapy. The MammoSite is a balloon catheter, which consists of a double-lumen catheter with an inflatable balloon at the distal tip. The balloon is inserted in the lumpectomy cavity, either during or following breast-conserving treatment, and is then filled with saline and contrast material such that the surrounding tissue is stretched tightly around it. Treatment is delivered immediately in the lumpectomy cavity using a high-dose source, which is inserted into the centre of the balloon. The method is simple, with a short learning curve. Conversely, the limitations of the technique are that the target volume is standardized without allowing individual conformation and the therapeutic range is only 10 mm. In addition, the distance between the balloon and the skin appears to be the most important factor for achieving optimal cosmetic results. Moreover, catheters can be a source of discomfort and potentially promote bleeding, infections and late damage such as fibrosis and telangiectasia [193].

Intra-operative radiation therapy (IORT) refers to the delivery of a single fractionated dose of irradiation directly to the tumor bed during surgery. Two modalities of IORT have been described using either electron, as developed in Milan [194], or photon beams (based on ‘soft’ X-rays of 50kV) developed by the University College of London [195]. The potential advantages of IORT include delivering of the radiation before tumor cells have a chance to proliferate. Furthermore, tissues under surgical intervention have a rich vascularization, with aerobic metabolism, which makes them more sensitive to the action of the radiation (oxygen effect). Also, the radiation is delivered under direct visualization at the time of surgery. IORT has the potential for accurate dose delivery: by permitting delivery of the

radiation dose directly to the surgical margins, IORT eliminates the risk of geographical miss in which the prescribed radiation dose is inaccurately and incompletely delivered to the tumor bed. As this irradiation is performed during the same surgical procedure, there is no need for future hospitalization and transportation of patients. The major flaw of this technique is the lack of definite pathological data regarding resection margins, histological features and axillary nodal status at the time of radiation therapy [193].

Three-dimensional (3-D) conformal external beam radiation therapy (EBRT) is a type of computerized radiation that uses computer-generated images to show the size, localization, and shape of the tumor. Some beams may be filtered to adjust the intensity of radiation delivered (intensity-modulated radiation therapy (IMRT)). This adjustment allows concentration of the radiation in the region of the cancer, and minimizes the dose to the surrounding normal organs. This technique has some potential advantages over the other techniques [196] since it is a non-invasive technique, the learning curve for radiation oncologists is smaller and, it is intrinsically more likely to generate better dose homogeneity and, thus, resulting to a better cosmetic outcome. Despite the above appeal of EBRT APBI, many issues and questions remain unanswered. These include breathing motion which could affect the dose delivering, identification and contouring of the lumpectomy cavity and the fractionation scheme adopted.

Several phase II trials which supported the efficacy of APBI demonstrate comparable long-term local and regional control rates and significantly better cosmetic results when compared to the corresponding results of conventional WBRT. However, a small number of phase III trials comparing standard WBRT versus limited field irradiation have been already published.

3.4 Bevacizumab in breast cancer: mechanism of action and role in metastatic breast cancer (Paper VI)

The angiogenesis is an important developmental mechanism implicated in several physiological and pathological processes. Amongst the many factors implicated in angiogenesis most attention is catalyzed, during these years, by VEGF (vascular endothelial growth factor) system.

VEGF expression is increased in many tumor types including breast cancer [197]. In breast cancer, this increase is associated with poor clinical outcome, including decreased disease-free and overall survival [198,199].

The VEGF-overexpression leads to several activities that promote the tumor growth including the growth of structurally chaotic and functional aberrant vessels into tumor burden [200], the raising of intratumoral pressure that prevents the penetration of chemotherapeutic agents into the tumor [201], and the prevention of the apoptosis of immature pericyte on vessels critical for tumor nutrition [202]. Moreover, overexpression of VEGF has been shown not only to enhance estrogen-dependent tumor growth, but also to have an estrogen-independent effect through an autocrine mechanism of growth stimulation [203].

These observations, along with the limited role that VEGF plays in healthy adults, suggest that inhibition of VEGF could be a rational therapeutic approach for the treatment of breast cancer.

The first VEGF antagonist was the monoclonal antibody bevacizumab (Avastin, Genentech, USA) which was approved by FDA for the treatment of metastatic colorectal patients (February 2004) and, after few months, worldwide with the same indication. Bevacizumab is a humanized mouse monoclonal antibody (93% human) that is composed of the mouse VEGF-binding site joined to a human IgG framework. Bevacizumab recognizes all isoforms of VEGF-A preventing the binding of the ligand to the receptor and, thus, leading to inhibition of angiogenesis and tumour growth. In vitro bevacizumab inhibits VEGF-induced endothelial cell proliferation and migration. In addition to anti-angiogenic effects, VEGF targeting may produce clinical benefit through other mechanisms, including direct action against tumor cells [204]. In preclinical studies, the anti-tumour activity of bevacizumab seems to be enhanced by the combination with cytotoxic chemotherapy [205]. In human breast cancer xenograft models either the A4.6.1, the murine precursor of bevacizumab or bevacizumab has demonstrated a certain activity in inhibiting angiogenesis and tumour growth and spreading, given alone or in combination with chemotherapeutics and other targeted agents (trastuzumab) [206].

After demonstrating anti-angiogenic and anti-tumour activity in preclinical and animal models, bevacizumab produced anti-tumour activity in a number of frequently occurring tumour types in prospective phase II and

in phase III randomized clinical trials. Consequently, bevacizumab obtained regulatory approval for the treatment in first-line metastatic colorectal cancer, non-small cell lung cancer and renal cell carcinoma [207].

In metastatic breast cancer patients, randomized controlled trials evaluated the combination of bevacizumab with chemotherapy in the first-line setting and the results showed improvements in tumor response rate and progression-free survival (PFS). However, none of the trials showed significant survival benefit with the use of bevacizumab partially because all those trials were designed to detect differences regarding progression-free survival events.

3.5 Trastuzumab in breast cancer: mechanism of action and role as neoadjuvant therapy (Paper VII)

Neoadjuvant therapy is the standard of care in patients with locally advanced breast cancer [60,78]. The survival benefit gained from chemotherapy in breast cancer patients has been found to be equal regardless whether chemotherapy is administered preoperatively or postoperatively [102]. Neoadjuvant chemotherapy offers some attractive benefits since it can downstage the primary tumor in most women allowing higher breast-conserving surgery rates or improving respectability [208] and it can also provide an in vivo assessment of tumor response to chemotherapy since pathologic complete response (pCR) to neoadjuvant treatment could be a reliable prognostic factor [209].

Approximately 20% of breast cancer tumors show overexpression of human epidermal growth factor receptor 2 (HER2). The HER-2/neu gene encodes a 185-kd transmembrane glycoprotein (p185HER–2/neu) that is a member of the family of epidermal growth-factor receptors with intrinsic tyrosine kinase activity. Overexpression of p185/HER2/c-neu in patients with primary breast cancer is associated with a number of adverse prognostic factors, including advanced stage axillary lymph node involvement, absence of estrogen and progesterone receptors, increased S-phase fraction, and high nuclear grade [47,48]. As a result, patients with HER2 overexpression have been found to have poor disease-free survival and overall survival [47,48].

Based on the association between poor prognosis and HER-2 overexpression, antibodies, specifically targeting HER-2, were developed and evaluated for their therapeutic efficacy. Trastuzumab (Herceptin;

Genentech, South San Francisco, CA) is a recombinant, DNA-derived, humanized, monoclonal antibody that selectively binds with high affinity to the extracellular domain of HER-2. Although it is not fully understood how trastuzumab inhibits HER2 activity, some studies have suggested that the drug might promote internalization and degradation of HER2 [210,211]. Another potential mechanism of action could be the induction of an immune system-mediated antitumor response. Indeed, data from several in vivo experiments have indicated that trastuzumab is capable of mediating the induction of immune responses such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity [212].

The administration of trastuzumab has improved the survival of HER2-positive breast cancer both in the adjuvant [213] and the metastatic settings [214]. Recent data showed that women with HER2/neu-positive disease who received trastuzumab with systemic therapy may have either comparable or even better prognosis compared with women with HER2/neu-negative disease [51].

Considering the benefits from neoadjuvant chemotherapy and the well-documented effect of trastuzumab in HER2 positive breast cancer in the adjuvant and the metastatic settings, the addition of trastuzumab to neoadjuvant treatment appears to be appealing since it might also be associated with better overall responses and survival outcomes. Furthermore, preclinical data indicate that the use of trastuzumab prior to surgery might be of great benefit by limiting the proliferation and improving the control of residual tumor.

4. Aims of the study (specific aims)

Paper I

We performed a meta-analysis of randomized controlled trials to address whether the use of bisphosphonates in the adjuvant setting of breast cancer might have any effect on the natural course of the disease. Most specifically, we investigated for any beneficial effects on overall survival, prevention of disease recurrences, and occurrence of bone metastases.

Paper II

We conducted a systematic review and meta-analysis in order to examine the efficacy of bisphosphonates in preventing fractures among patients with early breast cancer.

Paper III

We planned a meta-analysis, in order to estimate the cumulative evidence for bisphosphonates to induce jaw osteonecrosis in primary breast cancer setting. Secondary outcome was the subgroup analysis by the use of each bisphosphonate agent.

Paper IV

We conducted a systematic review of the literature and meta-analysis in order to compare both the efficacy and tolerability of the new agent fulvestrant versus other hormonal agents, such as tamoxifen and aromatase inhibitors, which are currently standard treatments for patients with advanced breast cancer.

Paper V

Due to the lack of sufficiently powered results from the existing phase III trials, we conducted a systematic review and meta-analysis to evaluate the current randomized evidence for the role of APBI in breast cancer treatment.

Paper VI

We conducted a meta-analysis to synthesize available evidence for use of bevacizumab in metastatic breast cancer patients in terms of overall survival, progression free survival and response rate.

Paper VII

We conducted a meta-analysis of randomized trials which evaluated the efficacy of incorporating trastuzumab into neoadjuvant chemotherapy for HER-2 positive breast cancer. We aimed to determine whether this approach improves pathologic complete response with acceptable toxicity.

5. Materials and Methods

5.1 Paper I-III

Identification of randomized trials

We conducted a systematic review of all English and non-English medical literature using MEDLINE, the Cochrane Controlled Trials Register and ISI Web of Knowledge. We set no year or country restriction. The searching algorithm used in electronic database search was as follows: (early OR adjuvant) AND (breast OR mammary) AND (tumour OR malign* OR carcinoma* OR cancer) AND (biphosphonates OR bisphosphonates OR clodronate OR pamidronate OR zoledronic acid OR ibandronate). The latest search was done on January 2009.

The reference lists of all studies included in the meta-analysis were examined for other relevant articles missed by the electronic searches.

Abstracts from major scientific meetings were electronically searched (American Society of Clinical Oncology annual Meeting, San Antonio Breast Cancer Symposium, the European Cancer Conference).

Eligibility criteria

Eligibility and exclusion criteria were pre-specified. We included all phase III and randomized controlled trials that were published as full articles in peer-reviewed journals or that were presented at the aforementioned meetings up to January 2009 in which patients with primary breast cancer were randomized to receive any bisphosphonate in the adjuvant setting versus a control group receiving no treatment or placebo. We also included trials that randomized breast cancer patients to receive either upfront or delayed bisphosphonates. Trials fulfilling the above inclusion criteria were considered eligible irrespectively of the study sample size, the type and the dosage of bisphosphonates used. Nonrandomized studies were considered ineligible.

When multiple records were related to the same study, end point data was extracted from the report with the longest follow-up (largest

number of events) to avoid duplication of information in the meta-analysis calculations.

Data extraction

Data extraction was conducted independently by two authors, and consensus was achieved for all data. When data on the outcome were not available from trials, we contacted the primary investigators of the eligible trials.

From each eligible trial, we recorded authors' names, journal and year of publication, country of origin, inclusive dates of patient enrolment, number of centers involved and study design items (including whether there was a description of the mode of randomization, allocation concealment, and number of withdrawals per arm, blinding and whether any planned or unplanned interim analyses had been performed). Additionally, we recorded the following items for both arms of each eligible trial: number of patients randomly assigned to treatment and analyzed per arm, age, tumour stage, menopausal status, exact regimen, schedule and dosing scheme of bisphosphonate used and any additional breast cancer treatment given.

Outcomes

For Paper I, primary outcome was to evaluate whether the adjuvant use of bisphosphonates in breast cancer might have any effect on overall survival, disease recurrences, and occurrence of bone metastases compared with non-use. Furthermore, we pooled estimates for distant metastases, visceral recurrences, and occurrence rate of locoregional relapses. Considering that treatment outcomes may vary among different types of bisphosphonates, the investigators performed subgroup analyses for deaths, disease recurrences, and bone metastases according to the bisphosphonate used (zoledronic acid/clodronate/pamidronate/risedronate).

For Paper II, the primary outcome was to evaluate the fracture rate in breast cancer patients receiving adjuvant bisphosphonates compared with patients receiving no treatment or placebo. Furthermore, we estimated the efficacy of bisphosphonates in reducing the fracture rate in patients at increased fracture risk: (a) postmenopausal patients and (b) patients receiving AIs.

Since our study analyzed the bone fracture occurrence in an ITT setting (intent to treat), only studies reporting the overall fracture rate (any cause) were included in our analysis. Since beneficial effects of bisphosphonates among metastatic patient with bone secondarisms had been clearly reported in literature, studies underreporting their fracture data and limiting their report only for patients who will develop bone secondarisms were excluded from analysis. This decision stems from the fact that in adjuvant setting only a minority of the patients become metastatic. Thereafter, the beneficial effects of bisphosphonates among patients who will develop bone metastasis might be heavily counterbalanced by potential detrimental effects of bisphosphonates on physiological bone metabolisms of "healthy" patients who want developed bone metastasis. Conversely, only a solid ITT analysis might have sense and drive to firm conclusions.

For Paper III, primary outcome was to estimate and compare the incidence of patients with osteonecrosis of the jaw in patients with breast cancer receiving adjuvant bisphosphonates compared with those without adjuvant bisphosphonates. Secondary outcome was the subgroup analysis of primary outcome according to the kind of bisphosphonate agent used.

Statistical analysis

For Paper I, the number of events (deaths, disease recurrences, and bone metastases) and the number of non-events in treated and control groups were retrieved from each primary study and 2x2 tables were constructed. Odds ratios (OR) of events for treated patients with respect to those who were not and the 95% CIs were calculated. X2 test was used to assess heterogeneity between studies (significance level set at 0.1). In the absence of heterogeneity, pooled estimates of ORs with their 95% CIs were calculated using the Mantel-Haenszel method. In the presence of heterogeneity, the DerSimonian and Laird random effects method was used to pool primary studies estimates [136]. Statistical software STATA 8.0 (Stata Corporation, College Station, Texas) was used for statistical analysis.

For Paper II, 2X2 tables were constructed and odds ratio (OR) was calculated for each primary study to estimate the relative risk of fractures in patients with breast cancer receiving adjuvant bisphosphonates compared with those receiving no treatment or placebo. For each eligible study group, we estimated the odds ratio for bone fractures between the groups in comparison and the 95% confidence interval (CI). Studies with zero events

in both groups (treatment and no treatment) were excluded from analysis. Between-study heterogeneity for the odds ratio was evaluated using the Q statistic. We then synthesized the data across studies using fixed effects (Mantel–Haenszel) or random effects (DerSimonian and Laird) modeling [136]. Analyses were performed in STATA SE 10.0 (Stata Corp, College Station, TX). All P-values are two-tailed.

Pre-specified subgroup analyses were performed in order to assess the effect of treatment in groups of patients with accelerated bone loss and increased fracture risk. We estimated the effect of treatment in postmenopausal patients (a trial was considered eligible if ≥75% of enrolled patients in both arms was postmenopausal) and in patients receiving AIs (a trial was considered eligible if ≥75% of enrolled patients in both arms received an AI).

For Paper III, the number of patients with osteonecrosis of the jaw in treated and control groups were retrieved from each primary study and 2X2 tables were constructed. Because of the large number of empty cells found and the fact that the control arm had a total of one event, this problem presents itself as extremely sparse. The effect of such dataset is to render the effect of any kind of continuity correction or in general any compensation for the failure of the various approximations (e.g., normality assumption) used to compute estimates and variances relatively large.

We thereafter performed a meta-analytical approach for rare events. Sweeting approach was used for the analysis of sparse data [215]. We discarded inverse variance methods and fixed effect simple Bayesian methods, since they are very likely to be biased, unstable or both for this type of data [215]. Since the studies analyzed were very well balanced, Mantel-Haenszel (M-H) pooled odds ratio using a small continuity correction and Peto's should be the least biased, and were therefore used in the analyses [215]. Multiple approaches were used to provide a sensitivity analysis, i.e., to give a better idea of how the results depend upon the estimation method chosen. Q statistic was used to evaluate test heterogeneity.

5.2 Paper IV

Identification of randomized trials

We searched MEDLINE, ISI Web of Science and the Cochrane Central Register of Controlled Trials, without year and language restriction. We used (fulvestrant OR faslodex) AND (breast OR mammary) AND (tumour OR malign* OR carcinom*ORcancer) as searching algorithm. The last search was updated in August 2008. On the basis of the title and abstract, we downloaded or requested full articles. Reference lists in these trials were checked to identify any other published or unpublished data.

We hand-searched the references of review articles and evaluated symposia proceedings, poster presentations, and abstracts from major cancer meetings (including American Society of Clinical Oncology Annual meetings, San Antonio Breast Cancer Symposium). Cross searches were performed in MEDLINE using the names of investigators who were lead authors in at least one eligible trial.

Eligibility criteria

Abstracts, full articles, and the grey literature that passed the primary screening were retrieved and scrutinized. For inclusion, an article had to be a randomized controlled trial. We considered eligible all randomized controlled trials comparing fulvestrant versus any other hormonal therapy in patients with advanced breast cancer. If multiple publications of the same trial were retrieved or if there was a case mix between publications, only the most recent publication was included.

We excluded single arm studies and dose-escalation studies. Non-randomized studies were also excluded, as were case reports, letters, editorials, commentaries, reviews, and abstracts with insufficient details to meet the inclusion criteria.

Data extraction and outcomes

From each eligible trial, we recorded the following items for both arms: authors' names; journal and year of publication; years of patient enrolment; number of patients randomly assigned and analyzed per arm, age, BMI, site of measurable lesions, tumor stage, and menopausal status; hormonal receptor status; the exact regimens used and their dose and

schedule; the line of treatment; any additional treatments given to both arms. We recorded methodological quality items including whether there was a description of the mode of randomization, allocation concealment subject withdrawals, whether a description of the reason for withdrawal was reported and whether any planned or unplanned interim analyses had been performed. We also recorded whether all randomized women were included in the analysis according to intention-to-treat principle.

The primary outcomes of our study were to compare overall survival, time to tumor progression (TTP), objective response and clinical benefit in patients receiving fulvestrant versus other hormonal agents (tamoxifen, exemestane, and anastrozole).

Objective response was defined as the proportion of patients with complete response or partial response after treatment, and the clinical benefit as the proportion of patients with an objective response or stable disease lasting ≥24 weeks.

The secondary outcome was to evaluate the safety of fulvestrant versus other hormonal agents comparing the number of adverse events in each arm. We analyzed only adverse events reported in three or more eligible trials.

Statistical analysis

For the meta-analysis of overall survival and time to progression hazard ratios (HRs) were extracted from each study. For the meta-analysis of clinical benefit, objective response and all secondary outcomes, 2X2 tables were constructed and odds ratios (ORs) were calculated for each primary study. Heterogeneity of estimates between primary studies was statistically evaluated (chi-square test statistic, with a significance level of 0.1). In case of homogeneity of the estimates of effects between studies, pooled estimates (with 95% confidence intervals) were calculated according to Peto method (for log transformed HRs) or Mantel–Haenszel method (for ORs). In case of heterogeneity, pooled estimates and 95% confidence intervals were calculated according to DerSimonian and Laird random effects method. Standard errors of HR estimates were indirectly derived from the confidence intervals published in each study. Statistical analyses were done with statistical software STATA (version 8.0).

5.3 Paper V

Identification of randomized trials

We electronically searched the Cochrane Central Trials Registry, PubMed and ISI Web of Science, without year and language restriction, by using the following searching algorithm: (partial OR accelerated partial OR APBI OR PBI OR multicatheter interstitial brachytherapy OR interstitial brachytherapy OR balloon catheter brachytherapy OR mammosite OR intracavitary brachytherapy OR intraoperative OR IORT OR conformal external beam OR 3D-CRT) AND (radiation therapy OR radiotherapy OR irradiation OR brachytherapy) AND (mammary OR breast) AND (cancer OR malign* OR neoplas* OR carcinom*). The last search was updated on June 2008.

In addition, we tried to identify any previous systematic review of randomized trials in this field and we scrutinized the references of all eligible trials in order to find any potentially eligible trial that it was not identified by our searching algorithm. Cross-searches were performed in MEDLINE using the names of investigators who were lead authors in at least one eligible trial.

Eligibility criteria

We considered eligible all randomized controlled studies comparing WBRT versus limited field or partial radiation therapy after BCS in patients with breast cancer.

We excluded trials comparing two different partial irradiation techniques or two different schedules or doses of the same radiation technique. We also excluded meeting abstracts (because they have not undergone yet full peer review), single arm studies, non-randomized and pseudo-randomized trials (e.g. those with alternate allocation of subjects).

Data extraction and outcomes

From each eligible trial we recorded for both arms the following items: authors' names, journal and year of publication, country of origin, years of patient enrollment, and number of centers involved; number of patients randomized and analyzed per arm, age, tumor size, nodal status, histologic type, tumor grade, percentage of estrogen-receptor and progesteron-receptors positive tumors. We also recorded the irradiation technique used, study design, randomization mode, allocation concealment,

withdrawals description, and blinding. Two investigators (Antonis Valachis, Davide Mauri) extracted independently the above items from each eligible trial. In case of discrepancy, consensus was reached by involvement of a third investigator (Nikolaos P Polyzos).

The main outcome was the overall survival rate, whereas secondary outcomes included the number of local recurrences, true and elsewhere breast recurrences (EBR), axillary recurrences, supraclavicular recurrences, and distant recurrences between compared arms.

Local recurrence was defined as any detection of cancer in the treated breast. We defined true recurrences [216] the ipsilateral local recurrences detected within 2 cm from the surgical clips. Recurrences detected at least 2 cm from the surgical clips were defined as elsewhere breast recurrences (EBR).

When data required for analyses were lacking in original reports, we contacted the original authors.

Statistical analysis

For every primary study, two by two tables were constructed and odds ratios (OR) of APBI versus WBRT were calculated for each outcome. Heterogeneity of OR's between trials was analyzed using the chi-squared statistic (with a level of significance of 0.1). Pooled OR's and their 95% confidence intervals (CI) were calculated, in case of homogeneous studies, with a fixed effects model, according to the Mantel-Haenzel method. Statistical analyses were done using STATA statistical software (version 8.0; Stata Corp LP, TX, USA).

5.4 Paper VI

Identification of randomized trials

We searched PubMed, ISI Thompson, and The Cochrane Library to identify all relevant randomized controlled trials (RCTs), comparing chemotherapy with or without bevacizumab in metastatic breast cancer. Keywords employed in the search process included avastin; bevacizumab; breast or mammary; cancer, malign*, neoplasm*, or carcinoma. We set no language or year restriction. The last search update was on June 3, 2009.

The reference lists of all relevant articles of this topic were examined manually for other relevant articles missed by the electronic searches. Because recent trials with bevacizumab may still be unpublished, we also searched electronically the major international congresses proceedings (American Society of Clinical Oncology Annual Meeting, San Antonio Breast Cancer Symposium, European Cancer Conference).

Eligibility criteria

Eligibility and exclusion criteria were prespecified. We included all randomized controlled trials that evaluated the administration of bevacizumab plus chemotherapy versus chemotherapy alone. All cytotoxic chemotherapy regimens were considered eligible for the meta-analysis, provided that the same drugs were given at the same dose in all study arms and that the arms differed systematically only regarding bevacizumab administration.

We excluded dose escalating studies, phase I or I/II trials and non-randomized studies. We also excluded trials testing the neoadjuvant administration of bevacizumab.

Data extraction and outcomes

From each eligible trial, we recorded authors' names, journal and year of publication, country of origin, years of patient enrolment, sample size, regimens used, chemotherapy and bevacizumab dosing and scheduling, line of treatment, additional treatments given to both arms, follow-up period, the number of outcome events and information pertaining to study design, mode of randomization, blinding, allocation concealment, and withdrawals description.

Two authors (Antonis Valachis and Davide Mauri) extracted data independently and reached consensus on all items. Disagreement on specific studies between the two reviewers was resolved through discussion.

The three outcomes of interest were

(1) overall survival (OS), defined as the time from random assignment to death from any cause;

(2) progression-free survival (PFS) defined as the time from randomization to disease progression or death from any cause; and

(3) objective response rate (ORR) defined as the sum of partial and complete response rates.

For the overall survival and progression-free survival we synthesized extracted HRs and respective standard errors, whereas for the outcome of objective response rate we used available data from 2 x 2 tables.

Statistical analysis

Hazard ratios and their 95% confidence intervals (CI) for treated versus control groups were retrieved from each primary study. In case these data were not available in primary reports we derived HR and their 95% CIs using Tierney methodology when applicable. We used the Der-Simonian-Laird random effects model [136] to calculate the overall effect size when more than two studies were available. A random effects model assumes that each study has its own true effect size, whereas a fixed effects model assumes that there is only one true effect size. Since most studies were expected to have differences in clinical settings, methodology, etc., we preferred a random effects model. The presence of statistical heterogeneity was assessed with Cochran's Q test (considered significant for P=0.10) [138] and quantified using I^2 and respective 95% CIs [139]. For I^2 values >/= 50% indicate large heterogeneity and values >/= 75% indicate very large (extreme) heterogeneity [139]. Finally, we synthesized separately studies based on the type of comparative chemotherapy treatment used, e.g. taxanes.

5.5 Paper VII

Identification of randomized trials

We searched MEDLINE and the Cochrane Central Register of Controlled Trials, without year and language restriction, by using the following searching algorithm: (neoadjuvant OR preoperative OR induction OR primary systemic OR primary chemotherapy) AND (trastuzumab OR herceptin). The last search was updated in July 2010.

Because recent trials with trastuzumab in neoadjuvant setting may still be unpublished, we also searched electronically the major international congresses' proceedings (American Society of Clinical Oncology Annual Meeting, San Antonio Breast Cancer Symposium, European Cancer Conference). The reference lists of all studies fulfilling the eligibility criteria

were also examined for other relevant articles missed by the electronic searches.

Eligibility criteria

Eligibility and exclusion criteria were prespecified. Studies were considered eligible for our systematic review if they were randomized phase II or III and evaluated the administration of trastuzumab plus chemotherapy versus chemotherapy alone in the neoadjuvant setting. All cytotoxic chemotherapy regimens were considered eligible for the meta-analysis, provided that the same drugs were given at the same dose in all study arms and that the arms differed systematically only regarding trastuzumab administration.

From the systematic review we excluded all the nonrandomized studies. If multiple publications of the same trial were retrieved or if there was a case mix between publications, only the most recent publication (and the most informative) was included.

Data extraction

Two authors (Antonis Valachis and Davide Mauri) extracted data independently and reached consensus on all items. Disagreement on specific studies between the two reviewers was resolved through discussion.

From each eligible trial we recorded for both arms the following items: authors' name, journal and year of publication, country of origin, years of patient enrollment, and number of centers involved; number of patients randomized and analyzed per arm, age, ER/PR status, node positivity, median follow up, technique used for HER2 identification, type/dose of chemotherapy and dose and duration of trastuzumab therapy. Primary and secondary outcome measures, as described below, were recorded. We also recorded, whenever possible, issues that reveal the quality of included studies: randomization model, allocation concealment, blindness, withdrawals description.

Outcome definition

The primary outcome of our study was the rate of pCR achieved. In case the primary study reported separate pCR rate in breast tissue and in

breast tissue plus axilla, we included the pCR rate from the combination of breast tissue plus axilla.

Secondary objectives were the rate of breast-conserving surgery and the rate of toxicities including grade III-IV neutropenia, febrile neutropenia, overall cardiac adverse events, congestive heart failure (CHF) and treatment-related deaths.

We could not evaluate complete clinical response since only two trials presented such data. In addition, outcomes such as overall survival and disease free survival were not analyzed because only 2 trials presented sufficient data and a meta-analysis of two studies with enormous difference between observed events (deaths and recurrences) has no validity.

Statistical analysis

2X2 tables were constructed, using the intention-to-treat (ITT) assignment when applicable, and risk ratio (RR) was calculated for each primary study to estimate the relative risk of each outcome in patients with HER2-positive breast cancer receiving chemotherapy plus trastuzumab versus chemotherapy alone as neoadjuvant therapy. For each eligible study group, we estimated the relative risk for the outcome measures between the groups in comparison and the 95% confidence interval (CI).

We then synthesized the data across studies using fixed effects (Mantel and Haenszel) or random effects (Der Simonian and Laird) modeling when between-study heterogeneity was present. The significance of the heterogeneity test suggests a preference for the random-effect estimation for a more appropriate evaluation of the results. The RRs are to be interpreted as follows: an RR < 1.0 indicates fewer events in the trastuzumab arm.

To test for heterogeneity between trials, the Q statistic was used. The presence of statistical heterogeneity was assessed with Cochran's Q test (considered significant for $p < 0.10$) and quantified using I^2 and respective 95% confidence intervals. For I^2 values, >50% indicate large heterogeneity and values >75% indicate very large (extreme) heterogeneity. The meta-analysis calculations were accomplished by RevMan v 5.0 (Centre TNC: Review Manager (RevMan); In Version 5 for Windows edn. Copenhagen: The Cochrane Collaboration; 2008). All p-values are two-tailed.

6. Results

6.1 Paper I

Eligible Studies

Literature search identified 21 potentially eligible trials [217-237] evaluating the adjuvant use of bisphosphonates compared with no use. Most of the trials were designed to analyze safety data and the impact of bisphosphonates on bone loss [220-234, 236]; data suitable for our analyses could be retrieved from only 13 studies [217-219,228-237].

A flow chart indicating the identification of randomized controlled trials for inclusion in the meta-analysis is reported in Figure 1.

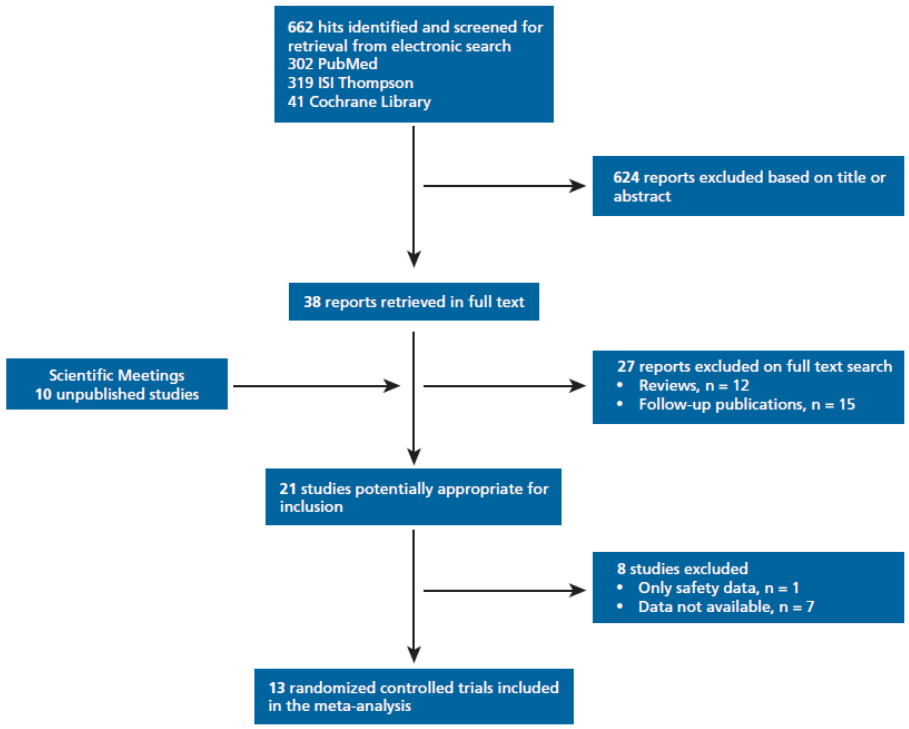

Figure 1. Flowchart diagram of study selection (Paper I)

Characteristics of Trials

Table 1 presents the characteristics of the 13 trials that met the eligibility criteria for this study; 6 studies used the bisphosphonate

zoledronic acid [218, 228,229,232-234], 4 trials used clodronate [219,235-237], 2 trials used pamidronate, [217,230] and 1 used risedronate [231].

Among these trials, 3 were double-blinded [230,231,237], 3 described in detail the mode of randomization [230,232,237], 2 reported allocation concealment [232,237], and 9 trials reported withdrawal description [217-219, 228, 229, 231, 232, 235, 236]. An intent-to-treat analysis was performed in all but 1 trial [236].

Study	Year	Intervention	Dosage of Treatment	Duration (y)	Number of Patients	Follow-Up (mo)	Reporting Modality
Kristensen et al.[10]	2008	Pamidronate No treatment	150 mg orally twice daily	4	460 493	NA	Peer-reviewed manuscript
Gnant et al.[11] (ABCSG-12)	2009	Zoledronic acid No treatment	4 mg IV every 6 mo	3	899 904	47.8	Peer-reviewed manuscript
Diel et al.[12]	2008	Clodronate No treatment	1600 mg daily	2	157 145	103	Peer-reviewed manuscript
Brufsky et al.[21] (Z-FAST)	2007	Upfront zoledronic acid Delayed zoledronic acid	4 mg IV every 6 mo	5	300 300	36 (Survival data retrieved from 12 mo[34])	Conference abstract and peer-reviewed manuscript
Eidtmann et al.[22] (ZO-FAST)	2008	Upfront zoledronic acid Delayed zoledronic acid	4 mg IV every 6 mo	5	532 533	36 (Survival data retrieved from 24 mo[35])	Conference abstracts
Fuleihan et al.[23]	2005	Pamidronate Placebo	60 mg IV every 3 mo	1	21 19	22.8 +/– 9.6 (mean) 24 +/– 9.6	Peer-reviewed manuscript
Delmas et al.[24]	1997	Risedronate Placebo	30 mg daily for 2 wk followed by 10 wk without drug	2	27 26	36	Peer-reviewed manuscript
Hershmann et al.[25]	2008	Zoledronic acid Placebo	4 mg every 6 mo	1	50 53	12	Peer-reviewed manuscript
Tevaarwerk et al.[26]	2007	Zoledronic acid No treatment	4 mg IV every 12 wk	1	26 23	12	Conference abstract
Schenk et al.[27] (EZO-FAST)	2007	Upfront zoledronic acid Delayed zoledronic acid	4 mg IV every 6 mo	5	252 270	12	Conference abstract
Saarto et al.[28]	2004	Clodronate Placebo	1600 mg orally daily	3	139 143	120	Peer-reviewed manuscript
Vehmanen et al.[29]	2004	Clodronate No treatment	1500 mg intermittent IV for 7 consecutive cycles	NA	21 24	12	Peer-reviewed manuscript
Powles et al.[30]	2006	Clodronate Placebo	1600 mg orally daily	2	530 539	66	Peer-reviewed manuscript

Abbreviations: IV, intravenously; NA, not available.

Table 1. Characteristics of eligible trials (Paper I) (number of references refers to citation in original paper)

Outcomes

Meta-analysis included data on 511 deaths from 9 trials (5736 patients) [218,219,228-231,234,235,237], 675 disease recurrences from 11 trials (5631 patients) [218,219,228-235,237], and 545 bone metastases from 8 trials (5571 patients) [217-219,228,230,234,235,237]. Although the primary investigators were contacted, additional data could not be retrieved.

Pooled results showed no statistical significant differences with the use of bisphosphonates in early breast cancer versus non-use for the overall number of deaths (summary OR, 0.708; 95% CI, 0.482–1.041; P =0.079), disease recurrences (summary OR, 0.843; 95% CI, 0.602–1.181; P =0.321), and bone metastases (summary OR, 0.925; 95% CI, 0.768–1.114; P =0.413; Figure 2). A statistically significant heterogeneity was seen among trials in estimates for deaths and disease recurrences (P =0.034 and P =0.016 for heterogeneity, for deaths and disease recurrences, respectively). No statistically significant difference between study heterogeneity was observed for bone metastases.

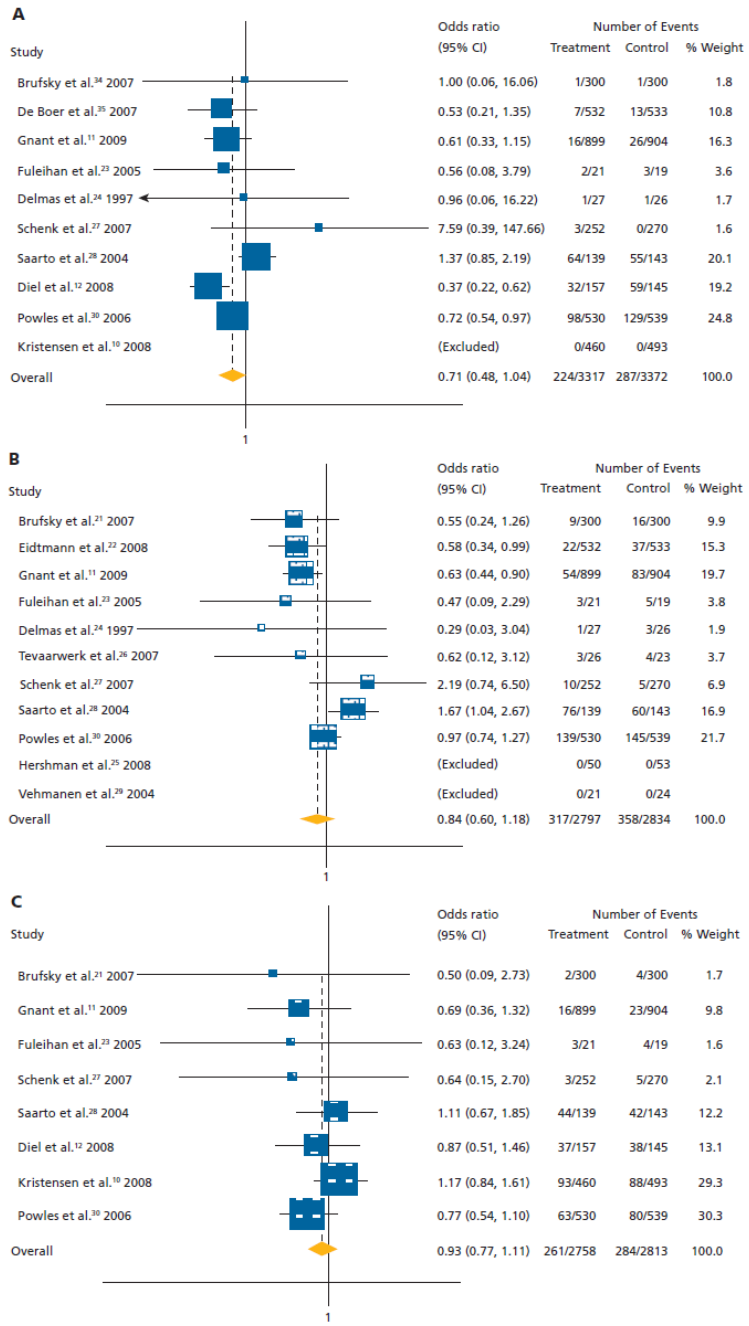

Figure 2: Forest plot for overall survival (A), disease recurrence (B) and bone metastasis (C) between bisphosphonates and control group (number of references refers to citation in original paper)

Adjuvant treatment with bisphosphonates compared with no use was not associated with any statistically significant differences between arms for type of recurrences, including distant metastases (7 trials, 4618 patients; OR, 0.896; 95% CI, 0.674–1.192; P =0..453) [218,219,228,230,234,235,237], visceral recurrences (4 trials, 1693 patients; OR, 1.051; 95% CI, 0.686–1.609; P =0.820) [219, 230, 235,237], and local relapses (5 trials, 4276 patients; OR, 1.056; 95% CI, 0.750–1.487; P =0.756) [218,228,234,235,237]. No significant between-study heterogeneity was observed.

Subgroup analyses for disease recurrences according to the type of bisphosphonate used showed a statistically significant lower risk for disease recurrences with zoledronic acid (6 trials, OR, 0.675; 95% CI, 0.479–0.952; P =0.025) [218,228,229,232-234]. Use of zoledronic acid was not associated with any significant difference in death rate (OR, 0.642; 95% CI, 0.388–1.063) or bone metastasis rate (OR, 0.661; 95% CI, 0.379–1.151; Table 2).

Outcome	Bisphosphonate	Number of Studies	Number of Patients	OR (95% CI)	*P* Value
Death	Zoledronic acid	4	3990	0.642 (0.388–1.063)	.085
	Clodronate	3	1653	0.721 (0.384–1.351)	.307
	Pamidronate	2	993	0.561 (0.083–3.787)	.553
	Risedronate	1	53	0.926 (0.057–16.223)	.978
Disease recurrence	Zoledronic acid	6	4142	0.675 (0.479–0.952)	.025
	Clodronate	3	1396	1.226 (0.720–2.085)	.453
	Pamidronate	1	40	0.476 (0.095–2.295)	.348
	Risedronate	1	53	0.295 (0.029–3.036)	.305
Bone metastasis	Zoledronic acid	3	2925	0.661 (0.379–1.151)	.144
	Clodronate	3	1653	0.871 (0.676–1.122)	.286
	Pamidronate	2	993	1.139 (0.829–1.565)	.422

Abbreviations: CI, confidence interval; OR, odds ratio.

Table 2: Subgroup meta-analyses by type of bisphosphonates: Estimates of effect in 23 comparisons (Paper I)

6.2 Paper II

Eligible trials characteristics

A flow chart indicating the identification of RCTs for inclusion in the meta-analysis is reported in Fig. 3.

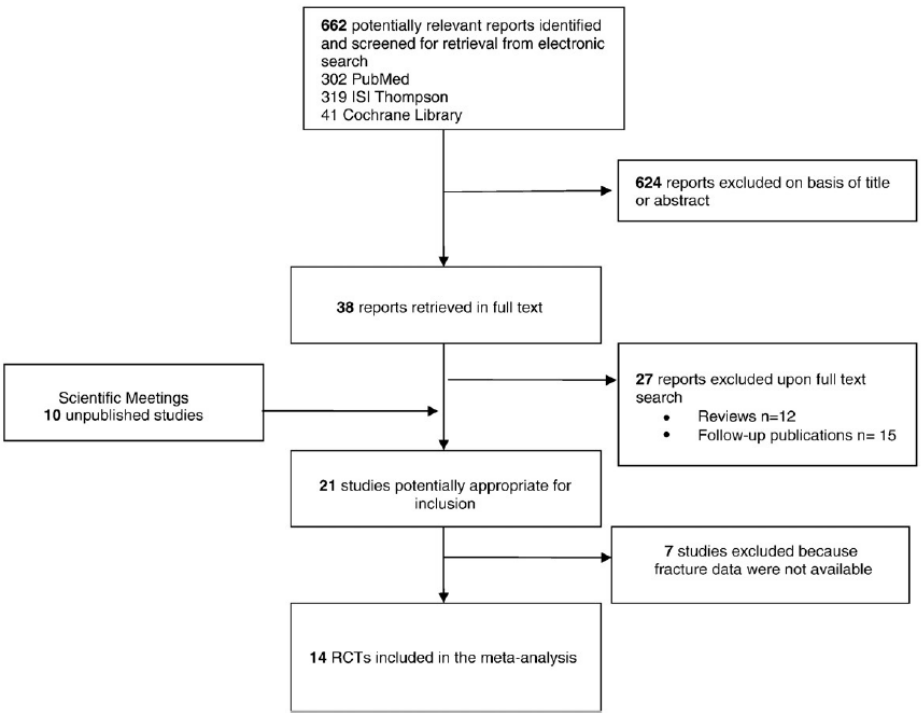

Figure 3: Flowchart diagram of study selection (Paper II)

We identified 21 different trials that were potentially eligible for our study [217,218,221-226,228-235,237-240]. Of these trials, 7 were excluded since data regarding fractures could not be retrieved. Finally, 14 trials were included in the meta-analysis [217,218,221-226,228,229,232,234,237,240]. A total of 7461 randomized women were included, of whom 3691 received bisphosphonates and 3770 received either placebo or no treatment.

Table 3 presents the characteristics of the 14 trials that met our eligibility criteria. Seven studies used the bisphosphonate zoledronic acid [218,223,225,228,229,232,234], three trials used risedronate [221,224,226], two used clodronate [237,240], one pamidronate [217] and one ibandronate [222].

Author (trail) [ref.]	Year	Intervention	Bisphosphonate administration	Duration (yr)	F-up (mo)	No	% Menopause	% AIs	Outcome measures
Brufsky A (Z-FAST) [13]	2007	Upfront zoledronic acid	4 mg IV every 6 mo	5	24	300	99.6	100	LS and TH BMD
		Delayed zoledronic acid				300	99.6	100	
Eidtmann H (ZO-FAST) [14]	2008	Upfront zoledronic acid	4 mg IV every 6 mo	5	36	532	83.6	100	LS and TH BMD
		Delayed zoledronic acid				533	83.1	100	
Gnant M (ABCSG-12) [32]	2009	Zoledronic acid	4 mg IV every 6 mo	3	47.8	899	0	50	DFS, OS, RFS, LS BMD
		No treatment				904	0	50	
Greenspan SL (ReBBeCa) [15]	2008	Risedronate	35 mg orally weekly	1[a]	24	43	100	18.6	PA spine, lateral spine,
		Placebo				44	100	13.6	hip, forearm, total body BMD
Lester JE (ARIBON) [16]	2008	Ibandronate	150 mg orally every	2	24	25	100	100	LS and TH BMD,
		Placebo	28 days			25	100	100	adverse events, fractures
Mincey BA (N03CC) [17]	2008	Upfront zoledronic acid	4 mg IV every 6 mo	5	12	197	100	100	LS BMD
		Delayed zoledronic acid				198	100	100	
Van Poznak (SABRE) [18]	2008	Risedronate	35 mg orally weekly	1	24	77	100	100	LS and TH BMD
		Placebo				77	100	100	
Schenk N (EZO-FAST) [19]	2007	Upfront zoledronic acid	4 mg IV every 6 mo	5	12	252	100	100	LS BMD, fractures,
		Delayed zoledronic acid				270	100	100	time to recurrence, OS, safety
Saarto T [29]	2001	Clodronate	1600 mg orally daily	3	60	139	52	0	Bone metastasis
		Placebo				143	43	0	
Kristensen B [30]	2008	Pamidronate	150 mg orally twice daily	4	Na	460	33	0	Skeletal events,
		No treatment				493	34	0	fractures, OS
Powles T [31]	2006	Clodronate	1600 mg orally daily	2	66	530	50	0	Bone metastases,
		Placebo				539	51	0	non-skeletal metastases, OS
Hines SL (N02C1) [22]	2008	Risedronate	35 mg orally weekly	1	12	106	0	0	LS BMD
		Placebo				106	0	0	
Shapiro CL (CALGB 79809) [20]	2008	Zoledronic acid	4 mg IV every 3 mo	1	12	81	0	0	LS BMD
		No treatment				85	0	0	
Hershman DL [21]	2008	Zoledronic acid	4 mg IV every 6 mo	1	12	50	0	25	LS BMD, tolerability
		Placebo				53	0	27	

Abbreviations: ref, reference; mg, milligrams; IV, intravenously; mo, months; yr, year; No, number; AIs, aromatase inhibitors; LS, lumbar spine; TH, total hip; OS, overall survival; PA, posteroanterior.

[a] With a 12-month extension for 40 women in placebo and 38 women in risedronate group.

Table 3: Characteristics of eligible trials (Paper II) (number of references refers to citation in original paper)

Five of the trials were double blinded [221,223,226,232,237]. Randomization model, allocation concealment, withdrawal description and intention-to-treat analysis were evaluated in only nine eligible trials published in full text. Four [218,221,232,237] out of nine trials described in detail the mode of randomization and three trials [221,232,237] described in detail the mode of allocation concealment. Withdrawals were described in detail in all nine trials [217, 218, 221, 222, 228, 229, 232, 237, 240]. All but one trial [222] specifically reported performing an intention-to-treat analysis.

Primary outcome

Fig. 4 shows the pooled data from the 14 randomized controlled trials evaluating the number of fractures in breast cancer patients receiving bisphosphonates versus no bisphosphonate treatment.

According to our analysis, treatment with bisphosphonates did not reduce the fracture rate compared to no treatment (OR=0.84, 95% CI=0.65–1.09). No between-study heterogeneity was observed (Q=11.97, P=0.287).

Two eligible trials [237, 240] faced a serious bias when considering the number of fractures in each arm; according to the authors, fractures were evaluated and reported only in patients who developed bone metastasis during the study period. Consequently, we performed a meta-analysis for the remaining 12 studies including all the patients in an intention-to-treat analysis (Fig. 4). When pooling the data for these 12 trials, bisphosphonates did not reduce fracture rate (OR=0.99, 95% CI=0.73–1.34). No between-study heterogeneity was observed (Q=5.75, P=0.675).

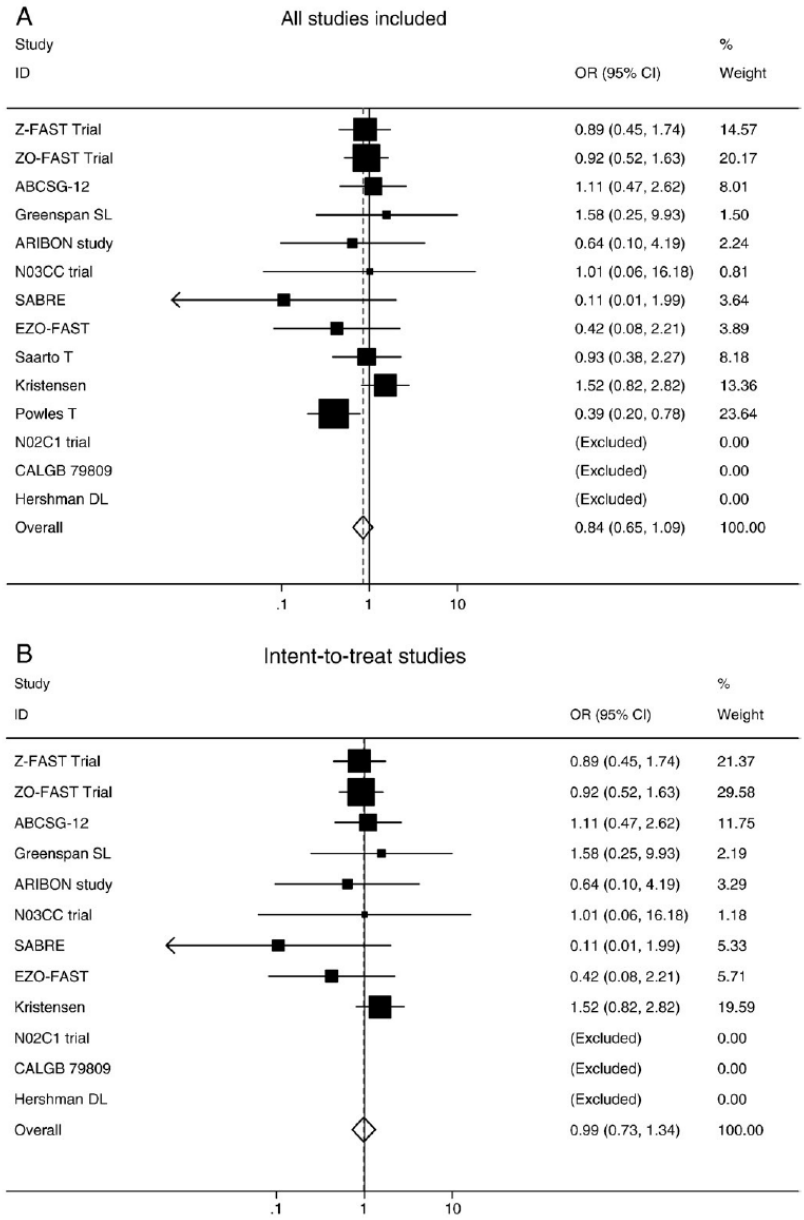

Figure 4: Meta-analysis plots comparing the risk of fractures between bisphosphonates and control group: all studies (A) and ITT studies (B)

According to our protocol, we performed subgroup analysis in groups of patients with accelerated bone loss and increased fracture risk. Treatment with bisphosphonates did not reduce the fracture rate neither in postmenopausal women (OR=0.82, 95% CI=0.55–1.20) (Fig. 5) nor in women with breast cancer receiving AIs (OR=0.79, 95% CI=0.53–1.17)

(Fig. 5). For both outcomes, no between-study heterogeneity was observed (Q=3.28, P=0.772, and Q=2.83, P=0.727, respectively).

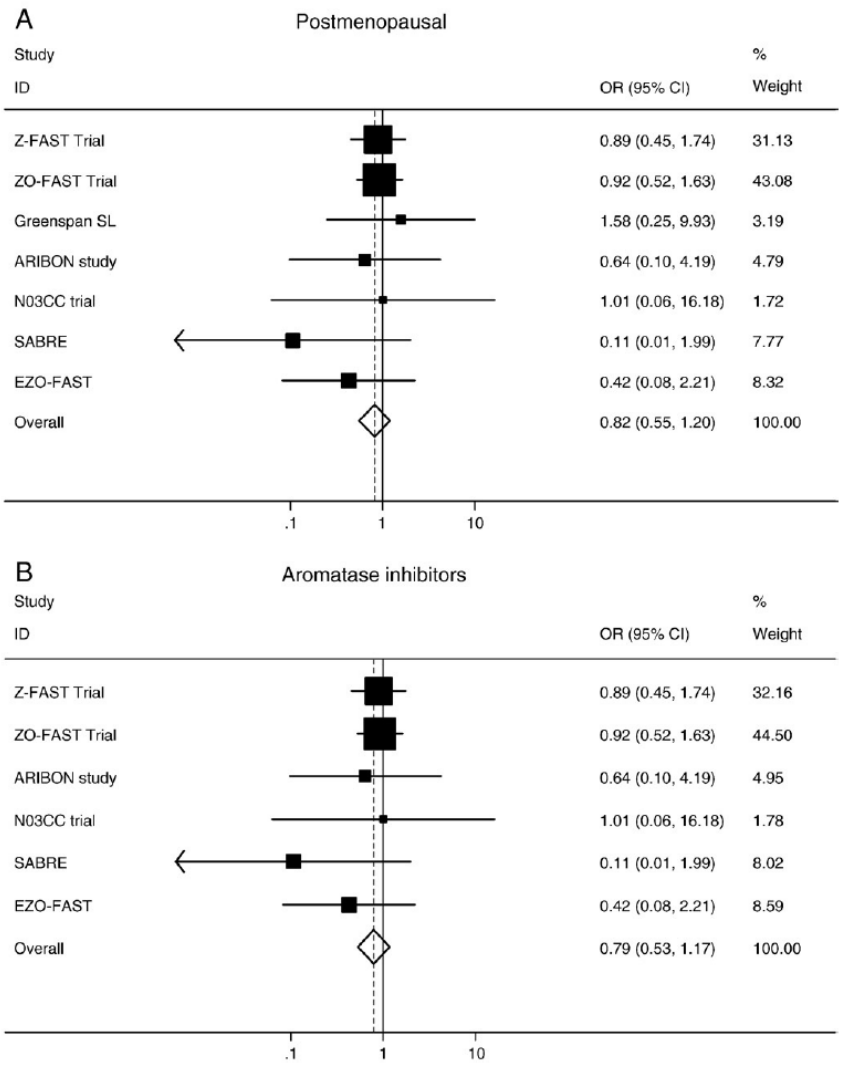

Figure 5: Meta-analysis plots for fractures in postmenopausal women (A) and women who received aromatase inhibitors (B)

6.3 Paper III

A flow chart indicating the identification of RCTs for inclusion in the meta-analysis is reported in Fig. 6. We identified 21 different trials that were potentially eligible for our study [217-237]. Of these trials, six were excluded since data regarding the osteonecrosis of the jaw could not be retrieved. Finally, 15 trials were included in the meta-analysis [217, 218, 220, 222-226, 228, 229, 232-235, 237].

A total of 10,694 randomized women were included, of whom 5,312 received bisphosphonates and 5,382 received either placebo or no treatment.

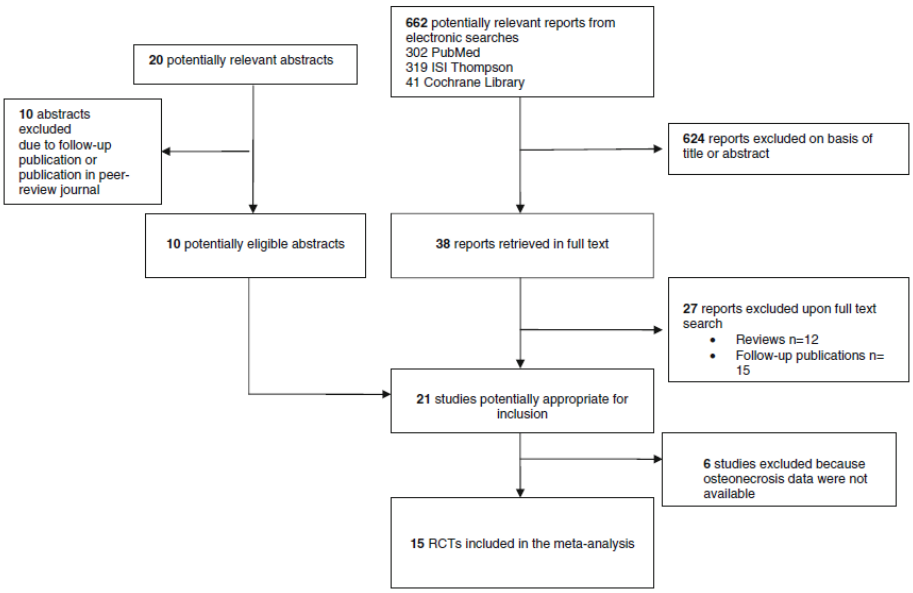

Figure 6. Flow chart diagram of study selection (Paper III)

Trials characteristics

Table 4 presents the characteristics of the 15 trials that met our eligibility criteria. Nine studies (7,990 patients) used the bisphosphonate zoledronic acid [218, 220, 223, 225, 228, 229, 232-234], two (1,351 patients) clodronate [235, 237], two (350 patients) risedronate [224, 226], one pamidronate (953 patients) [217] and one ibandronate (50 patients) [222].

Four of the trials were double-blinded [222, 224, 226, 237]. Randomization model, allocation concealment, and withdrawal description were evaluated in only eight eligible trials published in full text. Two trials [232, 237] described in detail the mode of randomization and the mode of allocation concealment. Withdrawals were described in detail in all eight trials published in full text [217, 218, 222, 228, 229, 232, 235, 237].

Author (Study) [ref]	Year	Intervention	Dosage of treatment	Duration (yr)	Number of patients	Follow-up (mo)
Brufsky A (Z-FAST) [7]	2007	Upfront zoledronic acid	4 mg IV every 6 months	5	300	36
		Delayed zoledronic acid			300	
De Boer R (ZO-FAST) [8]	2007	Upfront zoledronic acid	4 mg IV every 6 months	5	532	24
		Delayed zoledronic acid			533	
Lester JE (ARIBON) [10]	2008	Ibandronate	150 mg orally monthly	2	25	24
		Placebo			25	
Gnant M (ABCSG-12) [17]	2009	Zoledronic acid	4 mg IV every 6 months	3	873	47.8
		No treatment			871	
Mincey BA (N03CC) [22]	2008	Zoledronic acid	4 mg IV every 6 months	5	197	12
		No treatment			198	
Van Poznak C (SABRE) [23]	2008	Risedronate	35 mg/week orally	2	65	24
		Placebo			73	
Schenk N (EZO-FAST) [24]	2007	Upfront zoledronic acid	4 mg IV every 6 months	5	252	12
		Delayed zoledronic acid			270	
Shapiro CL (CALGB-79809) [25]	2008	Zoledronic acid	4 mg IV every 3 months	NA	81	12
		No treatment			85	
Hershman DL et al. [26]	2008	Zoledronic acid	4 mg every 6 months	1	50	12
		Placebo			53	
Hines SL (N02C1) [27]	2008	Risedronate	35 mg weekly	1	106	12
		Placebo			106	
Tevaarwerk A et al. [28]	2007	Zoledronic acid	4 mg IV every 12 weeks	1	26	12
		No treatment			23	
Saarto T [29]	2004	Clodronate	1,600 mg orally daily	3	139	120
		Placebo			143	
Kristensen B [30]	2008	Pamidronate	150 mg orally twice daily	4	460	NA
		No treatment			493	
Powles T et al. [31]	2006	Clodronate	1,600 mg orally daily	2	530	66
		Placebo			539	
Coleman R (AZURE) [32]	2006	Zoledronic acid	4 mg IV monthly for 6 months, then every 3 months for 8 doses and then every 6 months for 5 doses	5	1,681	Within 6 months from randomization
			No treatment		1,678	

ref Reference, *yr* year, *No* number, *mo* months, *IV* intravenous, *NA* not assessable

Table 4. Characteristics of eligible trials (Paper III) (number of references refers to citation in original paper)

Descriptive results

Overall, osteonecrosis of the jaw was a rare event, occurring in 13 (0.24%) of the 5,312 patients receiving bisphosphonates, and in one of the 5,382 patients in the control group.

All the 13 events of osteonecrosis of the jaw reported among bisphosphonates arms occur in patients undergoing treatment with zoledronic acid (13/3,987, 0.33%). No events of osteonecrosis of the jaw were reported among patients randomized to receive clodronate (n = 669), pamidronate (n = 460), risedronate (n = 171), and ibandronate (n = 25).

Due to the absence of events among patients randomized to receive treatment other than zoledronic acid, these studies were excluded from analysis since they provide no information concerning the odds ratio of developing osteonecrosis of the jaw. All they can tell us is that it is a rare or relatively rare event for both arms.

Meta-analysis

Fig. 7 shows the pooled data from the nine randomized controlled trials evaluating the number of osteonecrosis of the jaw in breast cancer patients receiving adjuvant treatment with zoledronic acid versus no use.

If the cells of the studies are all summed together as if they were a single trial, the difference in risk is statistically significant with a CI of (0.1–0.5%), and the risk estimated for the control arm is 0.02.

When meta-analysis was performed, using M-H pooled odds ratio with a continuity correction of 0.5, we found that treatment with zoledronic acid was significantly associated to the occurrence of osteonecrosis of the jaw (OR = 3.23, 95% CI = 1.7–8) compared with no use. This means that, in the worst case, the probability of ONJ occurrence in treated patients may have eight times the odds of the event to happen.

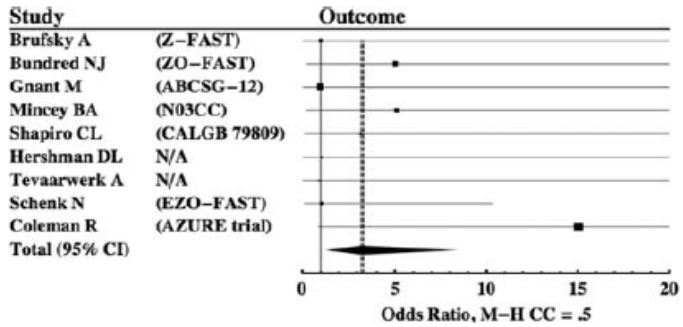

Figure 7: Meta-analysis plots comparing the risk of osteonecrosis of the jaw between zoledronic acid and control group. Mantel-Haenszel (M-H) statistics.

Multi-tests sensitivity analyses are reported in Table 5. No significant between-study heterogeneity was observed.

Method	Estimate	CI	*Q*	Chi^2 for *Q*
M-H, CC = 0	13.6	1.74–105	NA	NA
M-H, CC = .5	3.23	1.23–8.47	2.99	.96
M-H, IOA CC = 1	12.5	1.74–89.9	3.21	.96
M-H, NN, CC = .5	4.50	1.42–14.6	1.90	.86
M-H, NN, CC = .01	12.96	1.75–96.2	3.20	.67
M-H, NN, EC, CC = .1	9.37	1.66–52.8	2.90	.71
Peto, CC = 0	5.64	1.97–16.1	NA	NA

See Sweeting et al. [19] for details about the methods

Q statistic, *CC* continuity correction, *M-H* Mantel-Haenszel, *IOA* inverse of opposite arm weight, *NN* no null studies (with no events), *EC* empirical correction

Table 5: Sensitivity analysis (Paper III)

6.4 Paper IV

Eligible trials characteristics

The electronic search yielded 1020 reports; 607 from MEDLINE, 374 from ISI Thompson and 39 from Cochrane Central. Of those, 15 reports were scrutinized in full text. Seven reports were considered ineligible. Overall, eight reports [241-248] pertaining four randomized trials [245-248] were recorded. Two trials [245, 246] were double reported and in four reports [241-244] combined analysis of overall survival, time to tumor progression, clinical benefit and objective response were analyzed. In all, the four eligible trials included in the meta-analysis pertained a total of 2125 patients; 1089 had been randomized to fulvestrant, and 1036 to other hormonal agents. All four studies included in the meta-analysis pertained to postmenopausal breast cancer patients. A flow diagram outlining the results from the search strategy is provided in Fig. 8.

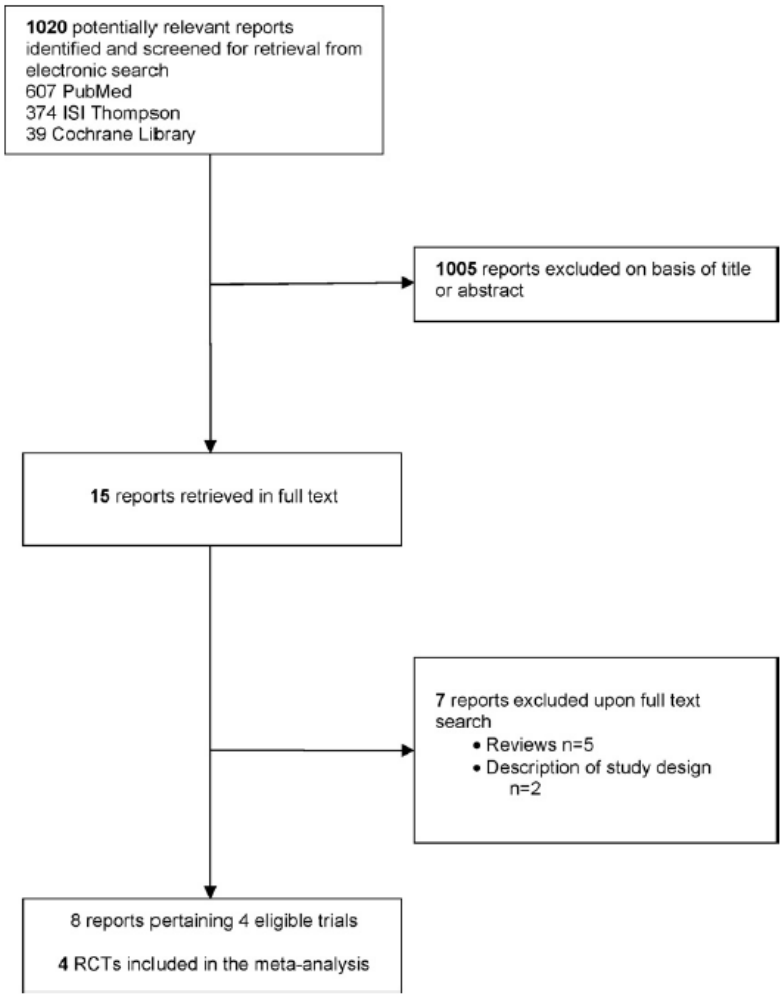

Figure 8: Flow chart diagram of study selection (Paper IV)

Design and quality characteristics

Table 6 shows the key characteristics of the four included studies. Three out of the trials were double blind [246-248]. Only one described in detail the mode of randomization [248] and some methods for ensuring allocation concealment.

Withdrawals were described in detail in three trials [245, 246, 248]. None of the trials were stopped early because of statistically significant outcome differences in an interim analysis. The definition of both objective response and clinical benefit recurrence was similar across studies.

Author [Ref.]	Year	Regimen	Line	No. of pts.	Median age, years	Receptor status (%) (unknown)	Blinding	Randomization model/allocation concealment
Howell et al. [15]	2002	Fulvestrant 250 mg (IM) once monthly	Second	222	63	73.4/3.6/23	Non-blinded	NR
		Anastrozole 1 mg/day orally		229	64	79.9/3.9/16.2		
Osborne et al. [16]	2002	Fulvestrant 250 mg (IM) once monthly	Second	206	72 (mean)	86.9/6.8/6.3	Double-blind	NR
		Anastrozole 1 mg/day orally		194	73 (mean)	87.1/5.2/7.7		
Chia et al. [17]	2008	Fulvestrant 250 mg (IM) once monthly	Second or greater	351	63	98.3/NA/NA	Double-blind	NR
		Exemestane 25 mg/day orally		342	63	98.2/NA/NA		
Howell et al. [18]	2004	Fulvestrant 250 mg (IM) once monthly	First	313	67	78.9/2.2/17.9	Double-blind	Software-generated random numbers
		Tamoxifen 20 mg/day orally		274	66	77.4/1.8/19.7		

Abbreviations: IM, intra muscular; NR, not reported; NA, not applicable.

Table 6: Characteristics of eligible trials (Paper IV) (number of references refers to citation in original paper)

Primary outcomes

All of the eligible trials reported data regarding overall survival, time to progression, objective response and clinical benefit.

In the meta-analysis, we found no difference between fulvestrant versus other hormonal agents regarding overall survival (HR: 1.047, 95% CI: 0.688–1.592; P = 0.830) and time to tumor progression (HR: 0.994, 95% CI: 0.691–1.431; P = 0.975) (Fig. 9). Additionally, there was no evidence of any difference in objective response (pooled OR: 1.044, 95% CI: 0.828–1.315; P = 0.716) or clinical benefit (pooled OR: 0.949, 95% CI: 0.736–1.224; P = 0.687) between the two arms (Fig. 10).

No statistically significant between study heterogeneity was observed for any of the four primary outcomes.

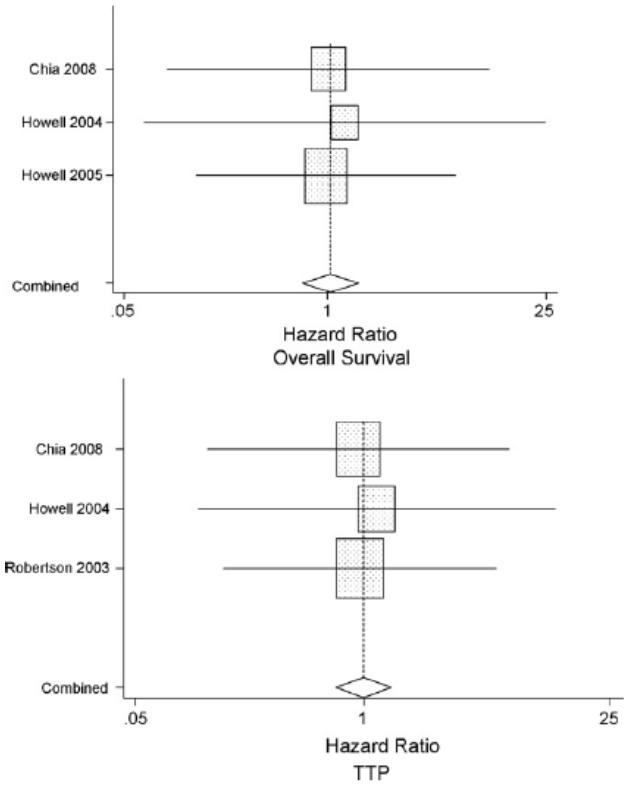

Figure 9. Meta-analysis plots for overall survival and time to progression

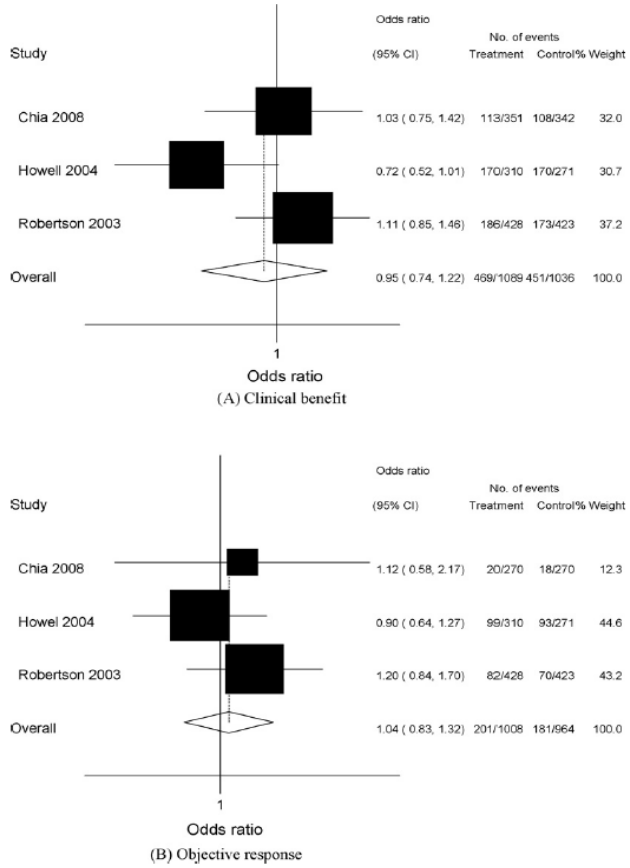

Figure 10: Meta-analysis plots for clinical benefit (A) and objective response (B)

Secondary outcomes

Secondary outcomes are shown in Table 7. There was no greater reporting of either adverse events or serious adverse events in breast cancer patients taking fulvestrant compared with those taking other hormonal agents. Furthermore, deaths due to adverse events were not significantly different between the two arms (pooled OR: 0.754, 95% CI: 0.267–2.127; P =0.594).

	No. of studies	Total no. of patients	Patients in fulvestrant arm	Patients in other hormonal agents arm	Pooled OR	95% CI	*P*-value
Adverse events	3	1432	650	616	0.940	0.679–1.300	0.707
Serious AEs	3	1544	11	7	1.549	0.595–4.031	0.370
Death due to AEs	4	2125	15	19	0.754	0.267–2.127	0.594
Thromboembolic disease	4	2125	22	17	1.139	0.645–2.011	0.653
Hot flushes	4	2125	178	200	0.807	0.637–1.021	0.074
Vaginitis	3	1432	23	25	0.869	0.390–1.935	0.731
Joint disorders	3	1544	48	73	0.621	0.424–0.909	0.014
GI disturbance	3	1432	321	309	0.944	0.662–1.346	0.749
Nausea	4	2125	198	202	0.954	0.762–1.193	0.678
Asthenia	4	2125	168	176	0.879	0.691–1.117	0.291
Vasodilatation	3	1432	122	131	0.827	0.516–1.323	0.428
Headache	4	2125	84	87	0.930	0.627–1.287	0.662
Pain	3	1432	124	138	0.816	0.614–1.085	0.161
Weight gain	3	1432	13	13	0.938	0.409–2.151	0.880

Abbreviations: AE, sadverse events; GI, gastrointestinal; OR, odds ratio; CI, confidence interval.

Table 7: Secondary outcomes: Tolerability of fulvestrand compared with other hormonal agents

Regarding specific treatment-related adverse events, it was observed that fulvestrant had significantly fewer joint disorders than those using either exemestane [246] or anastrozole [241] (pooled OR: 0.621, 95% CI: 0.424–0.909; P =0.014).

6.5 Paper V

Eligible trials characteristics

The electronic search yielded 3,093 hits; 1,776 from MEDLINE, 1,160 from ISI Thompson and 157 from Cochrane Central. Of those 29 trials were scrutinized in full text. Twenty five reports were considered ineligible (Fig 11). Of the remaining four trials [249-252], three [250-252] presented updated results and cosmetic outcomes from the same Hungarian study. Two studies [253, 254] which presented updated results from the Manchester study were retrieved from cross-checking of references. For the meta-analysis, we used the last peer-reviewed report for each study. Thus, three eligible trials [249, 250, 253] were included in the meta-analysis with a total of 1,140 patients: 575 had been randomized to whole breast irradiation and 565 to limited field or APBI (Fig 11).

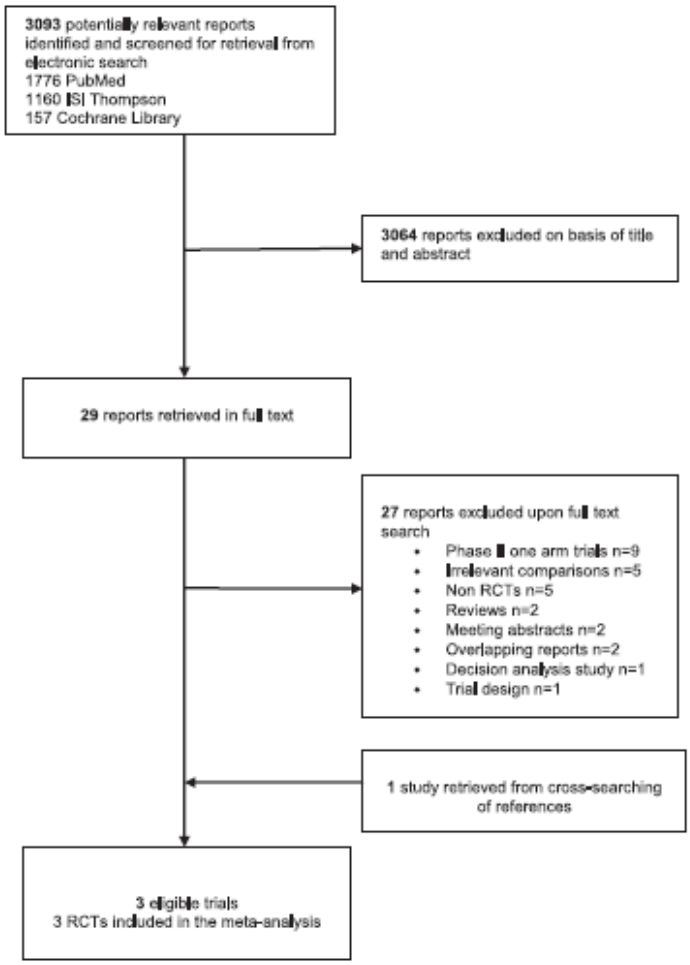

Figure 11: Flow chart diagram of study selection (Paper V)

Table 8 shows the key characteristics of the included trials. Two out of three trials were conducted according to ‘‘intention-to-treat’’ analysis and described reasons for withdrawal from the study. In one trial, five patients were excluded for the analysis with description of the withdrawal reasons. Two trials described model of randomization and the model of allocation concealment.

Study [ref]	Year	Enrollment interval (year)	Arms	No. patients analyzed	Median age (mean), year	Median follow-up, months	Quality of studies
Ribeiro et al. (20)	1993	1982–1987	Wide-field radiation Limited-field radiation	355 353	52 53	96 96	Randomization: balanced block design Allocation concealment: central randomization by telephone call
Dodwell et al. (21)	2005	1986–1990	Whole-breast radiation Tumor-bed radiation	90 84	51.5 52	65 65	Randomization: no declare Allocation concealment: none
Polgar et al. (22)	2007	1998–2004	Whole-breast radiation Partial breast irradiation	130 128	(58) (59)	66 66	Randomization: sealed envelopes in block of 10 Allocation concealment: sealed-envelope

Table 8: *Baseline characteristics of eligible trials (Paper V)* (number of references refers to citation in original paper)

Meta-analysis of primary and secondary outcomes

Survival data were available for all the eligible studies. Overall 214 deaths were included in the meta-analysis. No significant differences for the risk of death were observed between compared arms [OR 0.912, 95% CI, 0.674–1.234, P =0.550] (Fig. 12). Odds Ratios [OR] were homogeneous between studies (heterogeneity x^2 = 1.11, P =0.575).

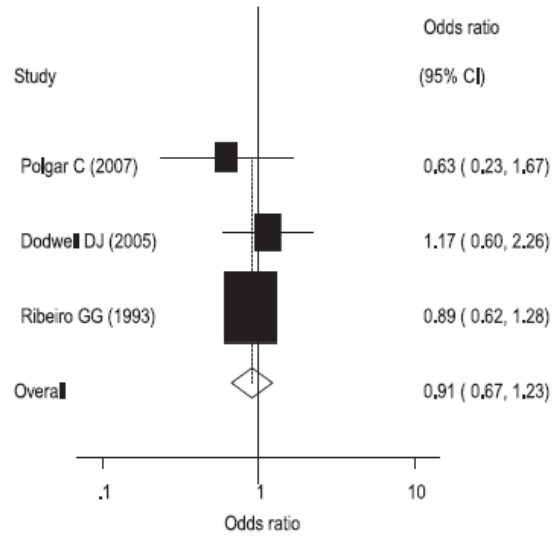

Figure 12: Meta-analysis of survival for the comparison between partial breast irradiation or whole breast radiation therapy

Forest plots for secondary outcomes are presented in Fig 13. Partial breast radiation therapy resulted in a statistically significant higher risk for developing local recurrences [pooled OR 2.150, 95% CI, 1.396–3.312; P

=0.001] and axillary recurrences [pooled OR 3.430, 95% CI, 2.058–5.715; P <0.0001]. No difference was observed for supraclavicular recurrences [pooled OR 1.415, 95% CI 0.278–7.202, P =0.560] and distant recurrences [pooled OR 0.740, 95% CI, 0.506–1.082, P =0.120] among investigational arms.

No statistically significant between-study heterogeneity was observed for any of the four secondary outcomes.

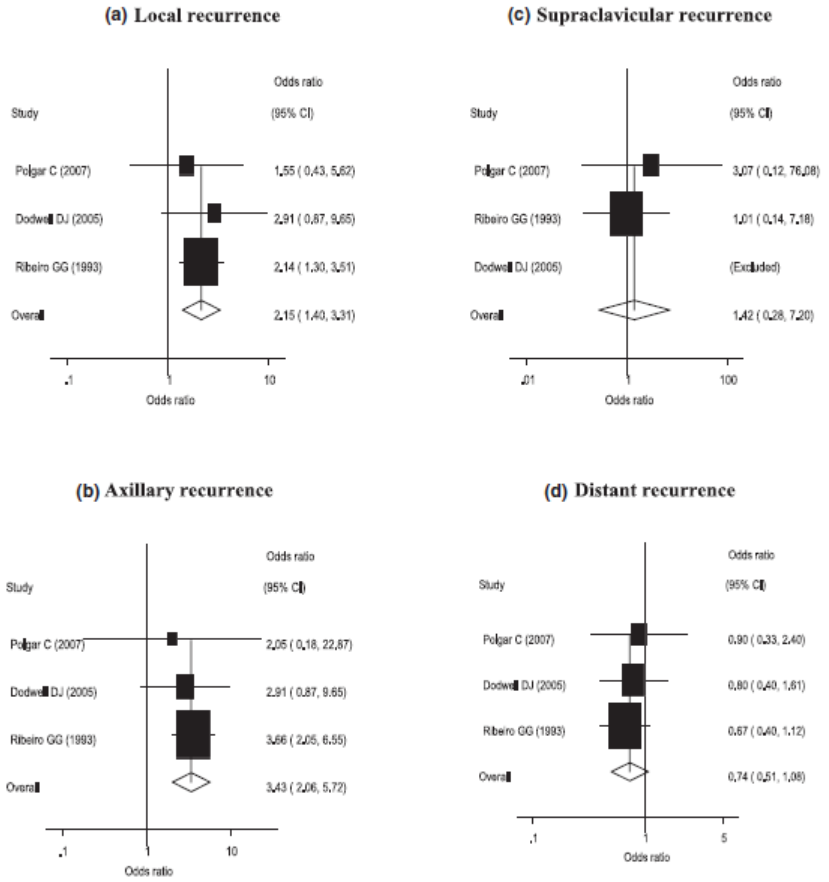

Figure 13: Meta-analysis for secondary outcomes with partial breast irradiation compared with whole breast radiation. (a) Local recurrence, (b) Axillary recurrence, (c) Distant recurrence, (d) Supraclavicular recurrence

Since only one study [250] clearly set the true recurrence and EBR rates, these issues were not included in final analyses.

6.6 Paper VI

Description of eligible trials

The trial flow is summarized in Fig. 14. The search yielded 723 studies and after scrutinizing title and abstract 15 papers were selected for full text evaluation (Fig. 14). We identified two eligible trials published in full text [255, 256] and three eligible trials presented only in major conferences [257-259]. Even considering that survival data for two trials (AVADO and RIBBON-1) [257, 258] was premature at the time of abstract presentation (ASCO 2009), since only 2 months intercourse between abstract presentation and our data-analyses, no further data were requested from primary investigators. Thus, we included five studies in the meta-analysis, including a total of 3,163 patients, with 1,932 patients in the chemotherapy-plus bevacizumab arm and 1,231 patients in the chemotherapy alone arm.

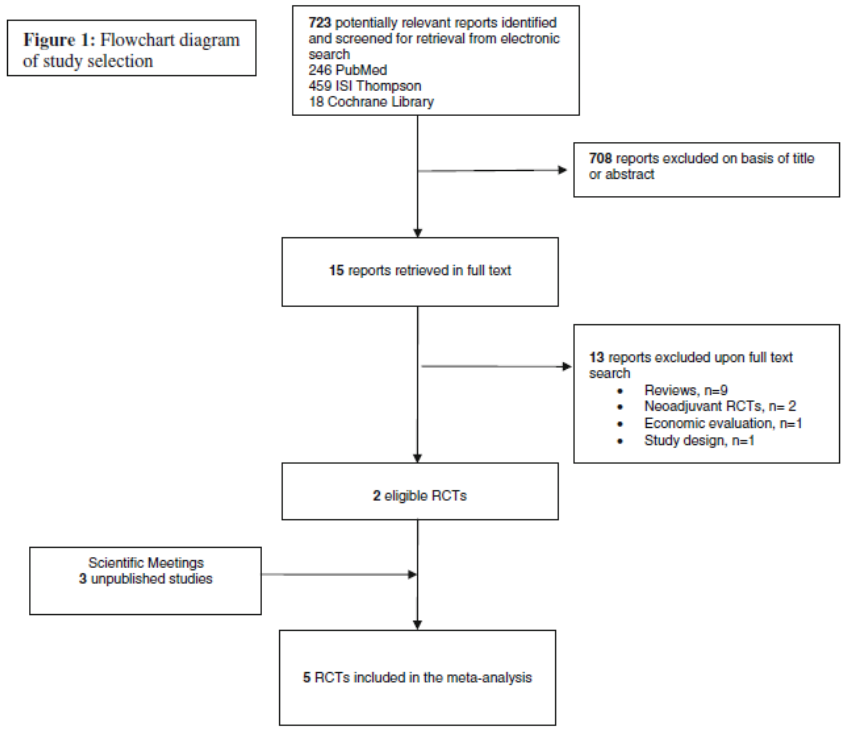

Figure 14: Flow chart diagram of study selection (Paper VI)

Two of the studies were described as double-blinded [257, 258]. We were able to evaluate other quality characteristics of eligible trials in only two studies published in full text [255, 256]. Both studies provided in detail withdrawals description, only one [255] described in detail the mode of randomization while none of them reported allocation concealment.

One of the studies was a three-arm trial [256]. In this trial, patients were randomized to receive placebo or bevacizumab in two different doses, 15 or 7.5 mg/kg. Since hazard ratios for OS and PFS were given separately for the two dosage bevacizumab arms versus the placebo comparator arm, to avoid double counting of placebo arm, only data from 15 mg/kg dosage versus placebo arms were included in the meta-analyses for OS and PFS. The choice was driven by the fact that the same dose density of 15 mg/kg bevacizumab was used in all the other trials included in present meta-analysis. The arm of 7.5 mg/kg was used instead of the 15 mg/kg in sensitivity analyses.

In addition, one study [257] reported separate analyses for patients receiving capecitabine plus bevacizumab versus placebo and those who received anthracycline-based chemotherapy or taxanes plus bevacizumab versus placebo. We also considered this trial as two different two-arm studies. The key trial characteristics are listed in Table 9.

Author, study name [ref]	Year	Study arms	Bevacizumab schedule	No. of patients	Line of treatment	Primary endpoint
Miller, E2100 [7]	2007	Paclitaxel		326	1st	PFS
		Paclitaxel + bevacizumab	10 mg/kg iv every 2 weeks	347		
Pivot, AVADO [8]	2009	Docetaxel		241	1st	PFS
		Docetaxel + bevacizumab	7.5 mg/kg iv every 3 weeks	248		
		Docetaxel + bevacizumab	15 mg/kg iv every 3 weeks	247		
Robert, RIBBON-1 [9]	2009	Capecitabine + placebo		206	1st	PFS
		Capecitabine + bevacizumab	15 mg/kg iv every 3 weeks	409		
Robert, RIBBON-1 [11]	2009	Taxanes or anthracyclines + placebo		207	1st	PFS
		Taxanes or anthracyclines + bevacizumab	15 mg/kg iv every 3 weeks	415		
Miller, AVF2119g [12]	2005	Capecitabine		230	>1st	PFS
		Capecitabine + bevacizumab	15 mg/kg iv every 3 weeks	232		
Burstein [13]	2005	Methotrexate + cyclophosfamide		21	>1st	RR
		Methotrexate + cyclophosphamide + bevacizumab	10 mg/kg iv every 2 weeks	34		

ref reference, *mg* milligrams, *kg* kilograms, *PFS* progression-free survival, *RR* response rate

Table 9: Key trial characteristics (Paper VI) (number of references refers to citation in original paper)

Progression-Free Survival

All but one trial [258] reported this outcome representing a total of 3,108 patients. The combination of bevacizumab and chemotherapy resulted in a statistically significant improvement in PFS compared with chemotherapy alone (HR = 0.70, 95% CI = 0.60–0.82, P =$9.3x10^{-6}$) (Fig. 15). There was significant statistical heterogeneity between individual trials (Q test P =0.023, I^2 = 65%; 95% CI 8–87%). Using the 7.5 mg/kg instead yielded similar results.

The subgroup analysis based on type of chemotherapy showed that the combination of bevacizumab with taxanes led to statistical significant improvement of PFS (HR = 0.63, 95% CI 0.56–0.71, P =$2.4x10^{-15}$) while combination of bevacizumab with capecitabine does not improve PFS statistically significant (HR = 0.82, 95% CI 0.58–1.15, P =0.249). However, this difference did not reach statistical significance (P-value of difference between groups: 0.158).

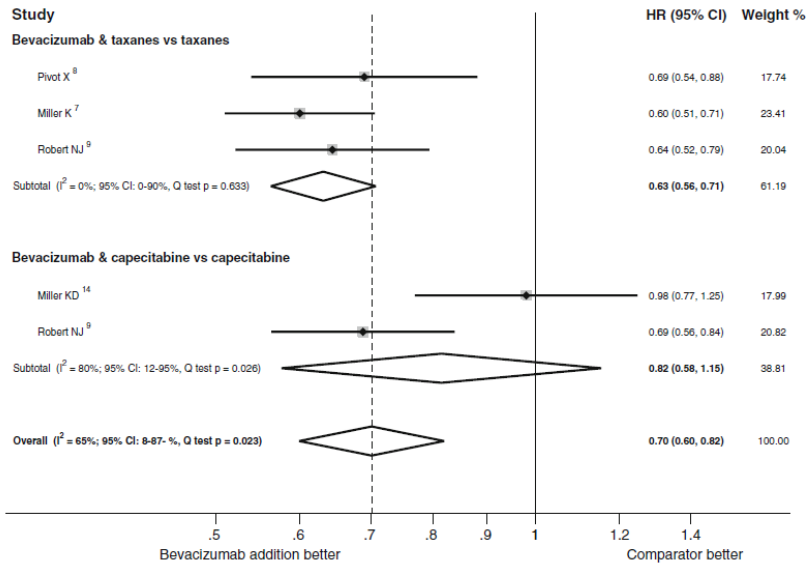

Figure 15: Forest plots for progression-free survival (PFS) for the use of bevacizumab versus non-use

Overall Survival

Four studies [255-258] reported or had data available to calculate hazard ratio including totally 2,646 patients.

The pooled HR for overall survival did not show significant advantage for the use of bevacizumab compared to placebo arm (pooled HR = 0.90, 95% CI 0.80–1.03, P =0.119; Q test P =0.777; I^2 = 0%, 95% CI 0–85%) (Fig.16). Using the 7.5 mg/kg arm yielded borderline statistically significant results (HR =0.88, 95% CI 0.76–1.00, P =0.046; Q test P =0.433; I^2 = 0%, 95% CI 0–85%). Only the subgroup of taxanes had enough studies to synthesize. The pooled HR was very similar to the overall one (HR: 0.92, 95% CI 0.80–1.06).

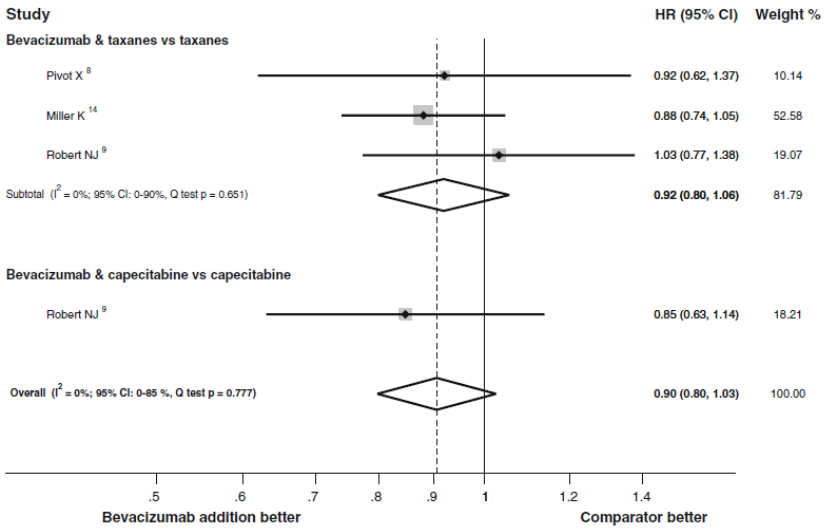

Figure 16: Forest plots for overall survival (OS) for the use of bevacizumab versus non-use

Objective Response Rate

All the five studies [255-259] included in the meta-analysis reported data on objective response rate. The RR was 1.26 with an associated 95% CI of 1.17–1.37 (P =9.96×10^{-9}; Q test P =0.091; I^2 = 47%; 95% CI 0– 79%), corresponding to a statistically significant increase in response for the addition of bevacizumab therapy (Fig 17). Differences in objective response rates were substantial independently by the type of chemotherapy use in combination with bevacizumab (taxanes agents, capecitabine or metronomic treatment with Methotrexate + Cyclophosfamide) (Fig 17).

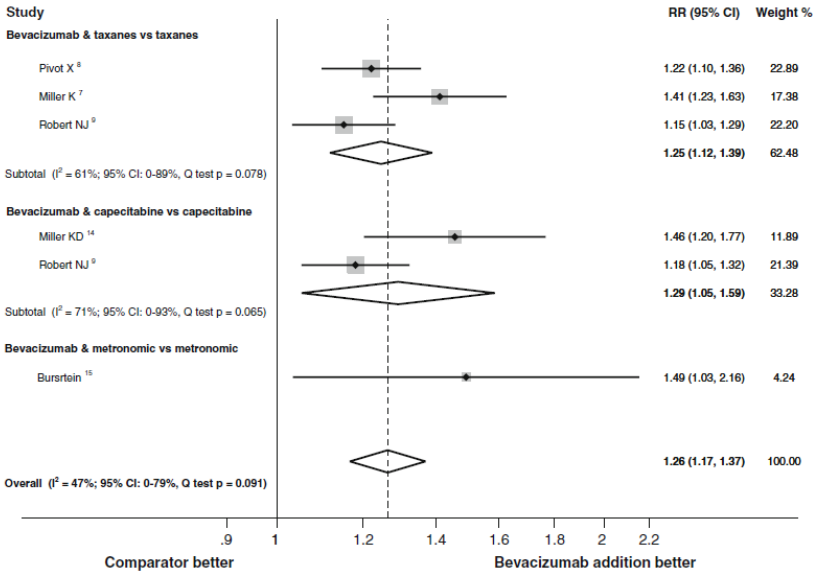

Figure 17: Forest plot for objective response rate (ORR) for the use of bevacizumab versus non-use

First and second line setting

Only few data were available to evaluate the use of bevacizumab in first and second line of treatment. When response rate data were analyzed we found that three studies included in the meta-analysis reported first line data (n=2,646) [255, 257, 258] and two studies reported second-line data (n=517) [256, 259].

The objective response rate RR for the use of bevacizumab in the 1st line of treatment was 1.23 with an associated 95% CI of 1.13–1.33 (P =3.87x10^{-7}; Q test P = 0.136; I^2 = 46%; 95% CI 0–82%) corresponding to a statistical significant increase in response for the addition of bevacizumab therapy. Analogous objective response rate advantages were observed for the use of bevacizumab in the second line of treatment: RR 1.466 with an associated 95% CI of 1.24–1.34 (P= 0.000011; Q test P =0.91; I^2 = 0%).

Unfortunately, no study reported OS data for second line of treatment, thereafter the retrieved data (HR=0.90, 95% CI 0.80–1.03, P =0.119; Q test P =0.777; I_2 = 0%, 95% CI 0–85%) strictly pertain the first-line setting. Similarly, when PFS was analyzed, only one study [256] reported data on second-line setting; consequently, no subgroup analysis was performed.

6.7 Paper VII

Study selection

Electronic search yielded 650 hits from PubMed and 32 from Cochrane. Five eligible randomized trials were retrieved, four from peer-reviewed reports [260-263] and one from congress abstracts [264]. One eligible trial [261] was also presented in the American Society of Clinical Oncology Annual Meeting on two occasions, 2006 [265] and 2008 [266], but the data included in our meta-analysis was retrieved from a review article [261] from the same author, in which updated and unpublished data of the study were included. Furthermore, one eligible trial [260] has published updated results [267] and data regarding toxicity were extracted from the most recent publication.

A flow chart indicating the identification of randomized controlled trials for inclusion in the meta-analysis is reported in Fig. 18. In total, 515 patients were included in the meta-analysis; of those 259 patients had been randomized to trastuzumab plus chemotherapy arm, and 256 to chemotherapy alone arm.

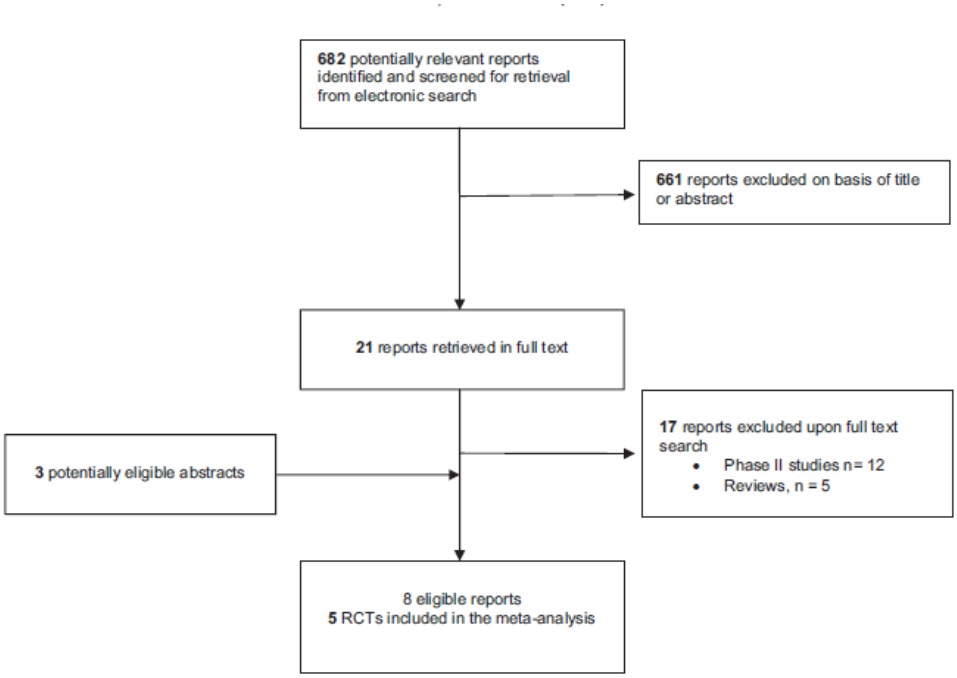

Figure 18: Flow chart diagram of study selection (Paper VII)

Study characteristics

Table 10 presents the characteristics of the 5 trials that met the eligibility criteria for this study. Regarding study design, two studies [261, 263] were phase II and three phase III [260, 262, 264]. Two studies [260, 261] were single-institutional while 3 [262-264] were multicentre. We were able to evaluate quality of included studies only in those published in full text (4 studies). Two studies [260, 262] describe adequately the randomization model and only one [262] the allocation concealment. One study [260] was terminated early because the primary objective of the study had been reached in interim analysis.

Study [ref]	No of pts analyzed	Clinical stage	HER2 status	Follow up, months	Arms	Neoadjuvant chemotherapy	Neoadjuvant Trastuzumab schedule	Adjuvant chemotherapy
Buzdar AU, 2005[11,18]	42	II-IIIA, non-inflammatory	IHC +3 or FISH	36.1	23	P (4 c) → FEC (4 c)	4 mg/kg → 2 mg/kg weekly for 24 wks	None
					19	P (4 c) → FEC (4 c)	No	None
H2269s, 2010[12]	29	T2-4, any N, M0	FISH	NR	15	Doc + Carbo 4 c	4 mg/kg weekly during chemotherapy	Doc + Carbo 4 c + trastuzumab
					14	Doc + Carbo 4 c	No	Doc + Carbo 4 c + trastuzumab for 52 weeks
ABCSG-24, 2009[13]	89	T1-4, any N, M0	NR	NR	42	ED ± Cap (6 c)	8 mg/kg → 6 mg/kg every 3 wks during chemotherapy	NR
					47	ED ± Cap (6 c)	No	
NOAH, 2010[14]	235	T3N1 or T4 or any T N2-3	IHC +3 or FISH	38.4	117	Doxo + P (3 c) → P (4 c) → CMF (3 c)	8 mg/kg → 6 mg/kg every 3 wks for 10 c	Trastuzumab (overall 1 year)
					118	Doxo + P (3 c) → P (4 c) → CMF (3 c)	No	Trastuzumab 1 year (only 17% of pts)
Pierga JY, 2010[15]	120	II-III	IHC +3 or FISH	NR	62	EC (4 c) → Doc (4c)	8 mg/kg → 6 mg/kg every 3 wks during chemotherapy	Trastuzumab (overall 18 c) ± chemotherapy
					58	EC (4 c) → Doc (4c)	No	Trastuzumab (18 c) ± chemotherapy

Abbreviations: ref, reference; No, number; pCR, pathologic Complete Remission; IHC, immunohistochemistry; FISH, Fluorescent In Situ Hybridization, P, paclitaxel; FEC, Fluoruracil–Epirubicin–Cyclophosphamide; c, cycles; mg, milligram; kg, kilogram; wks, weeks; Doc, Docetaxel; Carbo, Carboplatin; ED, Epirubicin, Docetaxel; Cap, Capecitabine; NR, Not-Reported; Doxo, Doxorubicin; CMF, Cyclophosphamide–Methotrexate–Fluoruracil; pts, patients; EC, Epirubicin–Cyclophosphamide.

Table 10: Characteristics of the incuded studies (Paper VII) (number of references refers to citation in original paper)

In all trials, except one [261], the treatment was based on a combination of anthracycline and taxanes concomitantly or sequentially. In two trials [261, 263] all HER2-positive patients received trastuzumab in the adjuvant setting, while in one study [262] adjuvant trastuzumab was administered only in patients randomized to receive trastuzumab preoperatively and only 16% of the HER2-positive patients in the chemotherapy alone arm received adjuvant trastuzumab.

Table 11 presents the characteristics of the analyzed patients in each eligible table and an overview of the outcomes in each study.

Study [ref]	Arms	Age, median (range)	ER/PR positive (%)	Inflammatory breast cancer (%)	pCR (%)	cCR (%)	OS	DFS
Buzdar AU, 2005[11,18]	Trastuzumab	52 (29–71)	13 (57)	0	15 (65)	20 (87)	NC[a]	100%
	No trastuzumab	48 (25–75)	11 (58)	0	5 (26)	9 (47)		85.3%[b]
H2269s, 2010[12]	Trastuzumab	NR	NR	11[c]	6 (40)	NR	NR	NR
	No trastuzumab				1 (7)			
ABCSG-24, 2009[13]	Trastuzumab	51 (26–70)	17 (40)	0	17 (40)	NR	NR	NR
	No trastuzumab	48 (29–68)	18 (38)	0	13 (28)			
NOAH, 2010[14]	Trastuzumab	NR	42 (36)	32 (27)	45 (38)	102 (87)	87%	71%
	No trastuzumab		42 (36)	31 (26)	23 (19)	87 (74)[d]	79%[e]	56%[f]
Pierga JY, 2010[15]	Trastuzumab	47	34 (55)	NR	16 (26)	21 (34)	NR	NR
	No trastuzumab	46.5	34 (59)		11 (19)	13 (22)		

Abbreviations: ref, reference; ER, estrogen receptor; PR, progesterone receptor; pCR, pathologic complete response; cCR, clinical complete response; OS, overall survival; DFS, disease-free survival; NC, not calculated; NR, not reported.
[a] One death in no trastuzumab arm and 0 in trastuzumab arm.
[b] Disease free survival at 3-years (*P*-value = 0.041).
[c] In both arms.
[d] Overall response: clinical complete and partial response.
[e] At 3 years. No significant difference.
[f] At 3 years (*P*-value = 0.013).

Table 11: Characteristics of analyzed patients and outcomes in eligible studies (Paper VII) (number of references refers to citation in original paper)

Overall effect of trastuzumab on pathologic complete response

The pCR rates for each eligible trial were available. The pCR was defined as no evidence of residual invasive cancer, both in breast and axilla in two studies [260,263], while in two studies [261, 264] the definition of pCR was unclear. In one study [262] a separate pCR rate in breast tissue and in breast tissue plus axilla was reported and, based on our study protocol, we used in meta-analysis the pCR rate from the combination of breast tissue plus axilla (Figs. 19 and 20).

In the overall population (515 patients; 5 RCTs), the absolute pCR rate was 38% (99 out of 259 patients) in trastuzumab arm in comparison with 21% (53 out of 256 patients). As a result, the probability to achieve pCR was higher for the trastuzumab plus chemotherapy arm (RR 1.85, 95% CI: 1.39-2.46; P =< 0.001).

No recurrence and survival analysis was performed due to the short term follow up and lack of such data.

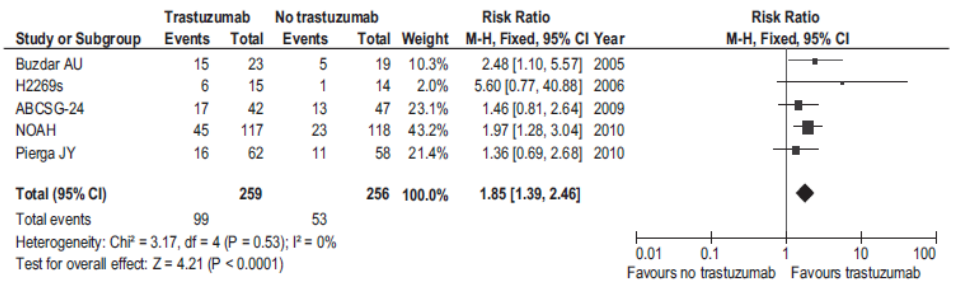

Figure 19: Meta-analysis evaluating the pathologic complete response (pCR) rate

Overall effect of trastuzumab on breast-conserving surgery and toxicity profile

The number of patients who underwent BCS were available in four trials (280 patients).We found no difference in terms of breast-conserving surgery between the two treatment arms (OR: 0.98, 95% CI: 0.80-1.19, P =0.82).

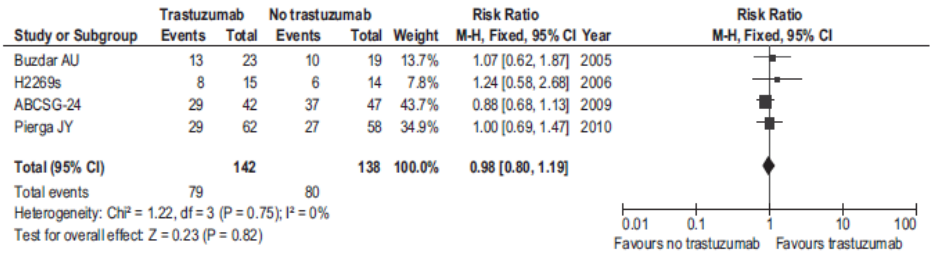

Figure 20: Meta-analysis evaluating the breast-conserving surgery rate

Regarding toxicity, pooled relative ratios with 95% CI for the use of trastuzumab versus no trastuzumab in neoadjuvant setting are reported in Table 12.

	No of studies	Total no of pts	Events/Total no of pts in trastuzumab arm	Events/Total no of pts in no trastuzumab arm	Pooled RR	95% CI	*P*-value	I-square (%)
Neutropenia gr III-IV	3	397	40/202	37/195	0.95	0.45–2.03	0.12	0
Febrile neutropenia	3	397	15/202	13/195	1.02	0.53–1.94	0.96	0
Cardiac adverse events	4	486	22/240	18/246	1.16	0.66–2.06	0.61	0
CHF	4	426	2/217	0/209	NC	NC	NC	NC
Treatment-related deaths	3	191	0/100	0/91	NC	NC	NC	NC

Abbreviations. No, number; pts, patients; RR, Relative Risk; CI, Confidence Interval; gr, grade; CHF, Congestive Heart Failure; NC, Not-Calculated.

Table 12: Pooled analysis of adverse events (Paper VII)

Overall, the incidence of neutropenia, neutropenic fever, and cardiac adverse events were similar between the two arms. Two out of 217 (0.9%) patients in the trastuzumab arms presented CHF compared with none in the chemotherapy alone arms. None of the patients either in trastuzumab or the chemotherapy only arm died due to treatment-related toxicities.

7. General discussion

7.1 Modification of natural course of breast cancer due to bisphosphonate use as adjuvant therapy (Paper I)

This meta-analysis showed no significant differences in terms of overall survival, overall disease recurrence, distant and visceral relapses, local relapses, and bone metastases rates for the use or non-use of bisphosphonates in the adjuvant setting of early breast cancer. Therefore, the currently available randomized evidence does not support the hypothesis that use of adjuvant bisphosphonates may alter the natural course of breast cancer. Nonetheless, because a positive but nonsignificant trend was seen favoring bisphosphonates use for reduction in death rate (OR, 0.708; 95% CI, 0.482– 1.041), further randomized studies are needed before the use of bisphosphonates in the adjuvant setting of breast cancer can be definitively supported or discouraged.

Of interest, when different bisphosphonates agents were considered, the investigators noted that the use of zoledronic acid may be of benefit in decreasing the number of disease recurrences. However, these results should be interpreted cautiously because the statistical significance for this association was not adequately strong (OR=0.675; 95% CI, 0.479–0.952; *P* =0.025) and may be largely attributed to chance because of multitest analyses (1/21 analyses = 4.7%). Moreover, the observed decrease in the number of disease recurrences was not associated with a decrease in the occurrence of bone metastases. Conversely, the trend for overall survival improvement (OR=0.642; 95% CI, 0.388–1.063; *P*=0.085) noted in the zoledronic acid arm was far from reaching statistical significance.

Currently, evidence on the role of zoledronic acid in the adjuvant setting is more reliable since larger randomized studies and studies with longer follow-up have been presented [268, 269]. Data from the AZURE trial, in which 3360 patients randomized to receive standard adjuvant systemic therapy either with or without zoledronic acid, did not reveal any statistically significant differences in terms of disease-free or overall survival in the overall population; however, a trend towards a better survival was observed in postmenopausal women who received zoledronic acid [268]. On the other hand, updated data from the ABCSG-12 randomised trial revealed that the addition of zoledronic acid improved disease-free survival in patients treated with anastrozole or tamoxifen [269]. A recent meta-

analysis of all randomized trials regarding zoledronic acid in the adjuvant setting revealed a survival benefit (18% reduced risk for death) with the use of zoledrnonic acid compared with placebo or no use [270].

Overall, this meta-analysis is consistent with the results of a previous study reported by Ha and Li [271] that examined the potential benefit of adjuvant oral clodronate in patients with early breast cancer. However, data from this early meta-analysis were feeble and unlikely to detect small differences between the arms because the study was relatively under-powered (only 3 trials were included involving 1653 patients and reflecting low number of events). Another limitation of the meta-analysis by Ha and Li [271] was that the results could not be extrapolated to bisphosphonates other than clodronate.

Therefore, this more recent meta-analysis is the first to present solid data on the use of bisphosphonates in the adjuvant setting of early breast cancer.

Several limitations must be considered when interpreting these results. First, this meta-analysis is based on published data and a meta-analysis of individual level data might define more clearly treatment benefits [272]. Second, many trials identified were reported in abstract form only, making complete data difficult to extract for analyses. Third, small time-dependent differences may have been lost in these analyses because most of the included studies did not report hazard ratios for overall and disease-free survival; therefore, this meta-analysis was mainly based on OR statistics [273]. Finally, 7 studies were excluded from survival analysis because no survival data could be retrieved. Furthermore, all but one studies were either small or had short follow-up, and were therefore unlikely to invalidate the conclusions. Likewise, the large study by Kristensen et al. [217] was unlikely to change the results because no overall survival differences were reported between the arms.

7.2 Role of adjuvant bisphosphonates in fracture risk (Paper II)

Recent data in adjuvant treatment suggest that fractures are increasingly recognized as important clinical issues for breast cancer patients since they might not only affect quality of life but also survival [160,274].

Our meta-analysis provides substantial evidence that bisphosphonates in the adjuvant setting among women with breast cancer do

not decrease the number of fractures compared with placebo or no treatment. Moreover, current evidence does not support bisphosphonate use neither in postmenopausal patients nor in patients receiving adjuvant AIs who are at increased risk for fractures. Nevertheless, since randomization process included all breast cancer patients and it was not limited to patients at higher bone fracture risk (e.g. patient with osteoporosis, osteomalacia, prior fracture history, low body weight or inactive lifestyle), we cannot exclude that patients at a higher risk for osteoporotic fractures could derive some benefit from the prophylactic use of bisphosphonate.

Our results are in contrast with the solid hypotheses suggesting possible beneficial effects for bisphosphonate use in early breast cancer patients. First of all, large population-based studies have shown that BMD is a marker for fracture risk [275, 276]. Secondly, among breast cancer patients, rapid and profound bone loss may arise in both premenopausal individuals due to chemotherapy-induced ovarian failure or adjuvant ovarian suppression/ablation [277, 278] and in postmenopausal setting due to the increasingly use of adjuvant AIs [158]. Finally, there is a growing evidence from randomized studies that both intravenous and oral bisphosphonates can be effective in maintaining bone mineral density (BMD) in both premenopausal and postmenopausal patients with early breast cancer either if treatment with AIs [228,229] or chemotherapy [225, 226, 232] are considered. All this evidence leads to the belief that the use of bisphosphonates in early breast cancer may decrease the number of fractures. However, most of the abovementioned studies were small phase III studies evaluating bone mineral density changes, but none of them was designed to evaluate the fracture rate as primary outcome.

Bone mineral density is only a surrogate outcome for the estimation of bone fracture and might not be a valuable predictive marker for fractures among patients receiving bisphosphonates. New indicators are therefore needed to indicate which breast cancer patients may benefit from the adjuvant use of bisphosphonates.

Several limitations do exist in our meta-analysis. None of the eligible trials was prospectively designed or was powered enough to measure the risk of fractures as primary outcome. Moreover, no sufficient data were available to either determine the role of each bisphosphonate in reducing fracture rate or evaluate the activity of bisphosphonate among patients with different stage and at different risk for metastatic disease. Another limitation

is the short follow-up length, since most of the studies had 3 years or less follow-up period, narrowing the possibility to reliably detect a difference in fracture rates between treatment groups. In addition, there was a wide variation (ranged from 1 to 5 years) among the included studies regarding the duration of prophylactic bisphosphonates and this could have an impact on both BMD and osteoporotic fracture rates. Finally, we excluded seven eligible trials evaluating the role of bisphosphonates in the adjuvant setting due to lack of fracture data. Nonetheless, these studies are unlikely to change significantly our results, since they were either small or with short follow-up.

Considering the short follow-up and the fact that most of the randomized trials were under-powered to detect differences in fracture rate, studies with longer follow up or with larger number of randomized patients may influence the results of our meta-analysis. Indeed, data from the large AZURE trial, including 3360 patients, showed a 1,9% reduced risk for fracture in patients who received zoledronic acid [279] compared with no treatment. Consequently, further randomized trials with larger number of patients are essential in order to clarify if the lack of protective effect of bisphosphonates against fractures in adjuvant breast cancer setting observed in our meta-analysis is true or due to under-powered data.

7.3 Risk for osteonecrosis of the jaw by using bisphosphonates in adjuvant setting (Paper III)

Our study indicates that the occurrence of osteonecrosis of the jaw during the adjuvant treatment of breast cancer is a rare event. Indeed data from pooled-randomized evidences indicated that it occurred in only 13 of the 5,312 patients receiving bisphosphonates (0.24%). The observed incidence of this phenomenon was up to 20 times lower than those observed in patients treated for metastatic disease or multiple myeloma [170]. This could be explained by the dose intensity of bisphosphonates regimens used in the treatment of primary breast cancer, that are remarkably lower than those used in metastatic disease.

Updated results from AZURE trial, the largest trial that has been published about the role of bisphosphonates, specifically zoledronic acid, in adjuvant breast cancer setting, reported an incidence of 0,7 % (95% confidence interval (CI) 0.3–1.2%) which is somewhat higher than ours but still low [280]. In addition, a meta-analysis of all randomized trials

examining the use of zoledronic acid as adjuvant therapy in breast cancer revealed an incidence of ONJ 0,54% [270].

The occurrence of osteonecrosis of the jaw appeared to be lower among patient receiving bisphosphonates different than zoledronic acid since no event was reported. This might be largely attributed to the low number of patients included in investigational arms, especially if pamidronate (n = 460), risedronate (n = 171), and ibandronate (n = 25) are considered (the number of expected events, considering the .estimated incidence of 0.24%, is about one or smaller for these trials and still smaller than two evens for clodronate). Another explanation could be found in the greater potency of zoledronic acid as demonstrated by its larger reductions in collagen type-I degradation products (N-telopeptide) [281]. However, the data currently available do not allow us to address this question.

Our meta-analysis provides substantial evidence that the use of zoledronic acid in the adjuvant setting among women with breast cancer statistically increase the number of events of osteonecrosis of the jaw. Even with this increment, the phenomenon remains rare with a maximum estimated risk (upper bound of risk CI of 0.5%).

Several limitations do exist in our meta-analysis. None of the eligible trials was prospectively designed or was powered enough to measure the risk of osteonecrosis of the jaw as primary outcome. Analyses were based on rare events, thereafter sensitivity analysis was needed to confirm these results. Finally, we excluded six trials due to lack of data on jaw osteonecrosis events. Nonetheless, these studies were unlikely to change significantly our results, since they were either small or with short follow-up.

7.4 Role of fulvestrant in advance breast cancer (Paper IV)

This meta-analysis showed that there is no difference in terms of efficacy parameters including overall survival, time to tumor progression, objective response and clinical benefit between fulvestrant, a new estrogen receptor antagonist, and other hormonal agents (tamoxifen, anastrozole and exemestane) in postmenopausal patients with advanced breast cancer. In terms of tolerability, the two treatment arms seem to have a similar tolerability profile with the exception of a higher incidence of joint disorders

in patients treated with AIs (anastrozole or exemestane) compared with those treated with fulvestrant.

After the publication of our meta-analysis, an additional randomized study [282] performed in a Chinese population and compared fulvestrant versus anastrazole in patients with advanced breast cancer whose disease had progressed following prior endocrine treatment, revealed similar efficacy and tolerability among the 2 treatment arms.

Fulvestrant is a new ER antagonist with a unique mode of action which offers several advantages compared with tamoxifen and AIs. First, fulvestrant lacks estrogen agonist effects in the endometrium [283] and is therefore unlikely to lead to an increased risk of endometrial cancer such as that produced by tamoxifen [284]. Second, due to the novel mode of action through binding, blockage and acceleration of degradation of ER protein [176], fulvestrant lacks of cross-resistance with other antioestrogens. Considering the fact that breast tumors often remain sensitive to subsequent endocrine therapy, despite disease progression to prior endocrine therapies, a different endocrine treatment with lack of cross-resistance would be of value.

Another approach that makes fulvestrant an attractive endocrine therapy is the potential ability for combination endocrine therapy, a case which is supported by preclinical data [181]. It has been proposed that the circulating estrogen might be a potential competitive inhibitor of fulvestrant. Therefore, the use of an AI so as to minimize the estrogen could have a synergistic effect with fulvestrant. Currently, there are several ongoing trials evaluating fulvestrant plus anastrazole combination regimens. The first results from the FACT study could not reveal a superiority of combination fulvestrant and anastrazole versus anastrazole alone but further randomized data is coming to clarify this possibility [285]. Furthermore, preliminary data demonstrate that fulvestrant may be active in patients with HER-2-positive disease [286].

Thereafter, fulvestrant showed to be at least not inferior to actual standard hormonal treatment in metastatic breast cancer and considering its pharmacological advantages, it constitutes a major drug for the management of hormone-sensitive metastatic breast cancer. Based on these randomized trials, fulvestrant gained FDA approval in 2002 and was licensed in Europe

in 2004 as an option in patients with hormone-sensitive advanced breast cancer.

In all the studies included in our meta-analysis fulvestrant either at dose of 250 mg given by intramuscular injection every 28 days or loading-dose regimen, consisting of 500 mg on day 1 and 250 mg on days 14 and 28 and monthly thereafter. This dose was initially chosen because it was associated with substantial reductions in tumor expression of ER and proliferation (Ki-67) in a preoperative trial [287] and because of clinical trial evidence that a 125-mg dose was suboptimal [245,246]. However, a growing body of evidence supports that a higher dose of fulvestrant might be associated with increased efficacy. Indeed, two randomized trials have been published and indicate efficacy benefit in favor of fulvestrant 500 mg [288,289]. The first study by Di Leo et al (CONFIRM study) compared fulvestrant 500 mg versus 250 mg in women previously treated with adjuvant endocrine therapy. Fulvestrant 500 mg was associated with significantly longer PFS and improvement in clinical benefit [288]. In addition, Robertson et al in FIRST study, compared fulvestrant 500 mg versus anastrazole as first-line endocrine therapy for advanced hormone receptor–positive breast cancer [289]. Fulvestrant was at least as effective as anastrazole and was associated with significantly longer time to progression. Interestingly, the tolerability profile in both trials was comparable between treatment arms and even the injection site reactions were comparable despite there being twice as many injections per month with the 500 mg regimen [288,289].

Based on these results, fulvestrant 500 mg became the approved monthly dose in 29 European countries and in the US [290].

Some limitations exist in our meta-analysis and should be discussed. Firstly, only four studies were available for the meta-analysis, at the time of last searching update, and there was considerable heterogeneity in the design and modes of treatment used in each study. Only one study evaluated fulvestrant as first-line treatment in advanced breast cancer compared with tamoxifen [248], while the other three trials [245-247] investigated the role of fulvestrant versus AIs as second or greater line of treatment. Furthermore only two trials were similar (comparing with anastrozole as second line therapy) and prospectively planned for combined analysis. The reported heterogeneity decreased the quality of our meta-analysis; anyhow, it should be taken into account that heterogeneity is not necessarily a disadvantage in

meta-analysis [291]. Secondly, in one study [247], close to one-third of randomized patients had hormone-resistant disease, so as to undermine the power of the study. Furthermore, some quality parameters of the studies included in our meta-analyses were not sufficiently reported in primary reports. Allocation concealment and randomization model were adequate in only one trial [248]. Moreover, our meta-analysis is based on data from trials that have published results in the literature and not on individual data. The use of such data might have further enhanced the accuracy and reduced the uncertainty of the estimates [272]. Finally, our meta-analysis was based on randomized data of fulvestrant 250 mg. The role of the currently approved higher dose of fulvestrant 500 mg in the sequencing of endocrine therapy in patients advanced breast cancer is still under investigation.

7.5 Efficacy and safety of partial breast irradiation vs. whole breast radiotherapy (Paper V)

Our meta-analysis supports that PBI is a safe treatment modality since it does not seem to jeopardize survival when compared to standard WBRT. Indeed, risk for death is comparable among both treatment modalities. Anyhow, considering the relative short follow-up of the studies included in meta-analysis, studies with longer follow-up might be of value in order to confirm our result after a long term follow up. Supraclavicular and distant recurrences did not differ among PBI arms and WBRT arms. However, APBI was associated with statistically significant increased risk for local and axillary recurrences.

Partial breast irradiation is a new radiation technique which offers some advantages compared with WBRT. First, it offers increased convenience, due to shorter duration of radiation therapy (5–7 days versus 6 weeks). Considering that limited access to radiation facilities is a major barrier for women to be treated with RT, the reduction of the period needed for radiation delivering is extremely important. One study in the United States found that when the travel distance was less than 10 miles, 82% of patients received RT after BCS; when it was 50 to 75 miles, 69% received it; and when it was more than 100 miles, only 42% received it [292]. By using accelerated PBI, more breast cancer patients will have the opportunity to undergo RT and conserve their breast. Furthermore, in countries with limited RT centers, patients treated with BCS may wait a prolonged time before beginning RT. As a consequence, there is a delay in the initiation of postoperative treatment when indicated. Two large population-based cohort

studies suggest that delaying the initiation of conventional RT more than 20 weeks after surgery was associated with decreased survival [188, 293]. In addition, Punglia et al [186], in a retrospective cohort study that includes 18,050 breast cancer patients, revealed a continuous relation between the interval from breast conserving surgery to radiotherapy and local recurrence in women with breast cancer, suggesting that starting radiotherapy as soon as possible could minimize the risk of local recurrence. Moreover, in APBI less radiation is delivered throughout normal breast tissue and other organs since treatment targets are more narrowly focused, and this could result in less adverse events and better cosmetic outcomes. Indeed, a preliminary analysis of an Italian study including 259 patients who were randomized to receive either WBRT or APBI demonstrated that APBI has very low acute skin toxicity [294].

Although APBI seems to offer several advantages over WBRT, there are also data from studies that raise concerns in terms of the rationale for this new technology. Data from clinical [295] and histopathological [296] studies show that a considerable proportion of local recurrences after BCS occurred away from the primary tumor. Several studies have attempted to identify the pattern of ipsilateral breast tumour relapse after conservative surgery; however, the results are contradictory and not easily comparable, since the definition of same-site relapse has no generally accepted criteria and the extension of surgery varies. This means that a local recurrence close to the surgical cavity after a quadrantectomy corresponds to breast recurrence elsewhere if a lumpectomy had been performed [297]. Another concern is raised from detailed histopathological analysis of the entire specimen after mastectomy [296, 298] and magnetic resonance imaging [299] studies suggesting that multifocal-same-quadrant or multicentric-other-quadrant foci are relatively common in patients with early-stage breast cancer. The extent of the disease in these patients cannot be encompassed using partial breast irradiation techniques. The clinical significance of multifocal and multicentric foci are uncertain; however, the reason these foci did not reach clinical significance may be that patients who suffer from the first local recurrence, which in the vast majority of cases occurs close to primary tumor, undergo mastectomy before a tumour focus becomes clinically apparent. Finally, evidence suggests that rates of ipsilateral recurrence away from the primary site after BCS and WBRT are lower than new breast cancer in the controlateral cancer [300]. Even if radiotherapy seems to increase the development of new primary cancer in the

controlateral breast, these data suggest that WBRT could have a protective effect on other areas of the breast.

In respect of the concerns that accompany APBI, the key research question is whether the potential benefit gained with this technique is significant enough to outweigh a potential risk of recurrence within the untreated tissue in the breast receiving PBI, which could affect survival in breast cancer patients.

In our study, we found that a statistically significantly higher risk of both local and axillary recurrences were associated with partial than with whole breast radiation. The recurrence risk needs to be considered with caution in terms of biases of the eligible studies. In two studies [249, 253] there were difficulties with target-volume definition. Furthermore, the study of Ribeiro et al. [253] has been heavily criticized for its older radiation technique and poor quality control, inadequate axillary and systemic management, and incomplete pathologic examination [301]. In the same study, a single field-size was used for all patients in limited-field arm, irrespective of the tumor dimensions or other characteristics, which could have resulted in several instances of ‘‘geographical miss’’. When the results were analyzed according to the type of the primary tumor, it was found that limited-field RT was inadequate only for patients with infiltrating lobular cancers or cancers with an extensive intra-ductal component. Polgar et al.’s [250] trial, which is the only study included in the meta-analysis with sufficient target-volume definition and modern radiation technique, showed no statistical significant differences in either local or axillary breast recurrences between the two arms.

Recently, after the performance of this meta-analysis, the results of the Targeted Intraoperative Radiotherapy (TARGIT-A) trial, a large international randomized controlled trial of targeted IORT versus WBRT for breast cancer, were published [302]. In this study, 2,232 patients were randomised to receive either intraoperative targeted radiotherapy or whole breast external beam radiotherapy. With targeted radiation technique the surface of the tumour bed typically received 20 Gy that attenuated to 5–7 Gy at 1 cm depth. The study presents data in terms of local recurrences and toxicity with a median follow-up of four years. There were six local recurrences in the IORT group and five in the external beam radiotherapy group, with no difference between the two arms. In terms of frequency of complications and major toxicities, no differences were observed between

the two arms, while radiotherapy-related toxicities were significantly lower in targeted radiotherapy arm. This study represents the largest randomized trial on this topic and provides additional evidence about the safety of PBI and the potential benefits from the use of this technique in women with early breast cancer. In addition, this study supports our hypothesis that the higher rate of locoregional recurrences in APBI arm observed in the meta-analysis might be a result of biases (difficulties of target-volume definition, poor quality, and older radiation techniques) in two of the included studies [249, 253] rather than an actual difference in outcome.

The key question that needs to be clarified is whether APBI negatively affects survival of breast cancer patients. Our results indicate that APBI is a safe radiation delivering technique since the overall death rate compared with WBRT is comparable. Regarding supraclavicular and distant recurrences, there were no differences between the two radiation techniques.

Some limitations do exist in our meta-analysis. First, the available randomized data for APBI is still limited, since only three randomized trials are available. Second, in the eligible studies a variety of APBI techniques were used and there may be differences in dosimetry, delivery, adverse effects, and outcomes among different modalities. Furthermore, despite the fact that the median follow-up time in eligible studies were 5 years in two studies [249, 255] and 8 years in one [253], median time needed to compare ipsilateral breast true recurrence frequencies after BCS plus APBI with those after BCS plus WBRT is thought to be at least 7 to 8 years and even longer in order to demonstrate the impact on mortality [302]. Our meta-analysis is based on data from trials whose results have been published, and we note that publication bias is a potential threat to the validity of the results. Moreover, we did not obtain up-dated individual patient data, the use of such data might have further enhanced the accuracy and reduced the uncertainty of the estimates [272]. However, we found no evidence of between-study heterogeneity. Finally, considerable shortcomings emerge regarding the methodological quality of the trials included in our systematic review. Several quality parameters of the studies were not sufficiently reported. Allocation concealment and randomization model were adequate in two trials [253, 255]. Additionally while two of the trials described scientific hypothesis and statistical power calculations [249, 255], none of the trials was powered enough due to low accrual rates.

7.6 Role of bevacizumab in metastatic breast cancer (Paper VI)

This meta-analysis summarizes all the current randomized evidence of the potential benefit with the addition of bevacizumab to chemotherapy for metastatic breast cancer. Data from our meta-analysis confirm that the addition of bevacizumab to chemotherapy regimens provides substantial benefit for women with metastatic breast cancer in terms of progression-free survival and objective response but not in overall survival. We also found that the combination of bevacizumab with taxanes has beneficial effect in PFS while the combination of bevacizumab with capecitabine does not reach statistical differences. It is possible that taxanes may have synergistic effect with bevacizumab as proposed by preclinical data [304]. In any case, it should be considered that pooled HR results for capecitabine were influenced by Miller report [256], which used bevacizumab in heavily pretreated patients. Indeed, individually, all but Miller [256] trials contained in this study reported that bevacizumab improves PFS when added to chemotherapy for MBC, but all positive PFS trials used bevacizumab in the first line settimg. A possible explanation is that VEGF alone decreases in importance when more numerous and redundant pathways of angiogenesis become effective as breast cancer progresses [305, 306]. A direct consequence of this theory is that anti-VEGF agents might provide their greatest benefit in patients with early disease, which might be the rationale for recommending bevacizumab in first line setting.

On the other hand, overall survival is the gold standard endpoint for clinical benefit in cancer patients, while PFS is a surrogate endpoint. In this setting, none of the trials included in our analyses revealed improvement in overall survival, and pooled HR analyses could not reach statistical significance.

Surprisingly, based on E2100 study [255] both EMEA and FDA, with “accelerated approval”, approved the combination of bevacizumab plus paclitaxel as first-line therapy in patients with HER-2 negative metastatic breast cancer. This decision has a great impact in the decision making for the regulatory approval of oncological drugs since it recognizes progression-free survival as a surrogate end point for survival. The major concern with this approval is that if progression-free survival was accepted as a primary end point for approval of first-line therapies, data on overall survival might never be obtained, since no studies of sufficient duration would be conducted [307].

On the contrary, when data from 2 additional studies (RIBBON-1 and AVADO) [258,259] were presented and demonstrated that the adsministration of bevacizumab was associated with a better response rate and a statistically significant but very small (<1 month) and clinically meaningless benefit in terms of progression-free survival, with no difference in overall survival, the Oncologic Drugs Advisory Committee (ODAC) of FDA recommended almost unanimously to remove this licensed indication from the product's label [308]. FDA commission has accepted the Committee's recommendation and the approval of Bevacizumab in breast cancer has been withdrawn.

Our meta-analysis confirms the positive effect of bevacizumab in PFS as long as the lack of effect in overall survival and the authors, based on the results of the meta-analysis, share the skepticism about the approval of bevacizumab in metastatic breast cancer, based on the current randomized data. The final decision about bevacizumab is a matter of a complex risk-benefit calculation and the decision-makers should take into account not only the effectiveness of bevacizumab but also the toxicity and the cost implications. Regarding toxicity, several safety concerns have arisen from the recent clinical trials and it has been suggested that the risk of serious side effects of bevacizumab overshadows the benefit of the drug [309]. In terms of cost implications, a recent economic evaluation concluded that the combination bevacizumab-paclitaxel was not cost-effective [310]. Under these assumptions, solid recommendation of bevacizumab in first-line MBC could not be given until significant advantage in overall survival will be revealed.

Several limitations need to be considered when interpreting our results. First, our approach was based on data abstracted from publications and not on individual patient data (IPD) which might define more clearly treatment benefits [272]. Second, most trials that we identified were reported in an abstract form only, which made it difficult both to extract complete data for analyses and to examine study quality characteristics. Furthermore, two studies were excluded from survival analysis since we could not retrieve any survival data. In any case, these studies are unlikely to change our results since one study [259] had extremely small sample size and in the second one [256] no overall survival differences between the two arms were reported. Finally, we did not test formally for publication bias because we had few studies [311], but we cannot exclude the possibility of delayed

publication of negative studies or no publication at all. Furthermore, we have to consider that if different benefits are expected as first versus refractory therapy (or with different chemotherapy partners), the meta-analysis may decrease important differences, thus limiting the benefits of more effective settings (e.g. combinations or line of treatment). Similarly if the survival benefits of a drug are maintained in second or more line of treatment, the eventual crossover of the drug after disease progression may obscure and render null the survival benefits that were amenable with the former line of treatment. Thereafter, meta-analysis technique may sometimes be inappropriate to quantify the eventual benefits of a certain drugs.

7.7 Role of trastuzumab as neoadjuvant therapy (Paper VII)

This study, with the inclusion of all available randomized data regarding trastuzumab as neoadjuvant therapy, provides evidence that the addition of trastuzumab in the neoadjuvant setting in HER2-positive breast cancer patients offers significant benefit in terms of pCR with no additional toxicity. Nevertheless, this benefit could not change the rate of breast-conserving surgery in favor of trastuzumab. Moreover, we were unable to analyze recurrences and survival for the neoadjuvant use of trastuzumab vs non use, due to the lack of data and short follow up of original studies.

Several non-randomized phase II trials have examined the potential benefit of neoadjuvant trastuzumab combined with chemotherapeutic agents in patients with HER2-positive tumors and have reported pCR rates ranging from 7% to 78% [261, 312] with the largest Geparquattro trial, revealing a pCR rate of 31.7% [313]. Recently, an exploratory pooled analysis of eight German, both randomized and non-randomized, neoadjuvant studies was presented and showed a 3.2-fold improvement in pCR in HER2- positive patients receiving trastuzumab when compared with HER2-positive patients who did not received trastuzumab [314]. In addition, a Chinese meta-analysis of three studies showed that neoadjuvant regimens combined with trastuzumab can significantly improve the pCR without increasing the toxicity [315]. However, this meta-analysis had several drawbacks, since one of the analyzed study was a cohort study and only two small randomized trials were included in the analyses. Therefore, no firm conclusions could be driven from the Liao's analysis.

Our meta-analysis, by including only randomized data, confirms and underscores the possible benefits for the use of trastuzumab in the

neoadjuvant setting when compared with no use. Therefore the actual available cumulative randomized evidence supports the current guidelines of the National Comprehensive Cancer Network (NCCN) for the inclusion of trastuzumab as a standard drug in neoadjuvant regimens for the treatment of HER2-positive breast cancer [316].

Interestingly, despite the trastuzumab-related benefit in terms of pCR rate in patients with HER2-positive breast cancer does not result in a higher rate of breast-conserving surgery, which is one of the theoretical advantages of neoadjuvant treatment. This could partially be explained by the fact that data from only 280 patients was available for this analysis. It has been suggested that the use of trastuzumab prior to surgery might be of great benefit by limiting the proliferation and by improving the control of eventually sub-clinical not-detectable residual tumor. Residual HER2-positive breast carcinomas show a significant increase in proliferation within 48 days after surgery [317]. Indeed, wound drainage fluid and postsurgical serum samples from patients after breast cancer surgery stimulate the in-vitro growth of HER2-overexpressing breast carcinoma cells. It seems, therefore, that surgery promotes the production of factors which can potentially stimulate the growth of HER2-positive breast cancer cells. The in-vitro proliferative activity is inhibited by the treatment of HER2 positive tumor cells with trastuzumab before adding drainage fluid [317]. Unfortunately, early available clinical data from our review are not enough to confirm whether the observed preclinical benefits for the use of trastuzumab in neoadjuvant setting might be translated into better long-term tumor control by reducing the proportion of local and distant recurrences and prolonging overall survival. Larger trials and longer follow up are needed to confirm the promising data that have emerged from the lab with breast cancer cell lines.

Concerning toxicity, the addition of trastuzumab to neoadjuvant therapy does not appear to compromise the safety profile of neoadjuvant therapy. The use of trastuzumab, especially in conjunction with anthracyclines has raised concerns about increased cardiotoxicity. In this meta-analysis, less than 1% of the patients with neoadjuvant trastuzumab presented with CHF despite the fact that in most of the eligible studies a combination of trastuzumab and anthracyclines was used. This finding is in accordance with the results of 2 recently published studies which evaluated the cardiotoxicity of trastuzumab in adjuvant setting with a longer follow up.

Both studies concluded that the incidence of symptomatic cardiac failure is less than 2% and in the majority of cases reversible [318,319].

Our meta-analysis has certain limitations which should be discussed. First, the number of studies and the number of patients included are relatively small and, thus, could affect the power of the meta-analysis to reveal statistically significant results. Nonetheless, we have systematically identified all the available randomized studies, either published in peer-reviewed journals or presented in major international cancer congresses, so as to include in our analysis all the available randomized evidence on this topic. Second, the definitions of pCR used by each study are not the same and the pathological methods and criteria of assessment are not standardized. In order to minimize the risk for heterogeneity regarding pCR definition, in studies which reported separate pCR in breast tissue and in breast tissue plus axilla, we included the pCR rate from the combination of breast tissue plus axilla because this definition is more appropriate and is proposed by current recommendations [320]. Furthermore, due to the limited number of the patients included in the analyses and the fact that primary studies did not present adequate data regarding patient (age, menopausal status) or tumor (ER status, node involvement) characteristics, we did not proceed to subgroup analysis in order to investigate the role of trastuzumab in different subgroups. Finally, the follow up of the included studies is relatively short with a median follow up no more than 3.5 years. The limited follow up does not allow a reliable evaluation of the potential benefit of neoadjuvant trastuzumab on overall survival or disease-free survival. The only studies with survival data available were NOAH trial [262] and Buzdar et al [260]. The NOAH trial revealed significantly better event-free survival in favor of trastuzumab and non-significant differences on overall survival [262]. However, according to study protocol, the non-trastuzumab arm received no trastuzumab as adjuvant therapy despite the fact that patients were HER2-positive. As a result, since adjuvant trastuzumab is of recognized beneficial effects in HER positive breast cancer, the NOAH study is unable to evaluate the additional effects of neoadjuvant trastuzumab use on survival outcomes. Buzdar et al. presented survival data in an update of their previous publication but the number of events was limited (1 death and 3 recurrences) and the follow up short so the interpretation of the results is questionable [267]. Despite survival outcomes are the most important endpoint when assessing the benefit of any therapy, the tumor response assessment in

neoadjuvant therapy is considered also crucial. Indeed, patients achieving a pCR may have a highly favorable long-term outcome

8. Conclusions

8.1 Paper I

This meta-analysis showed that currently available randomized evidence does not support the hypothesis that using bisphosphonates in adjuvant treatment of early breast cancer alters the natural course of the disease, because it does not affect overall survival or disease relapse and does not prevent bone metastases. Nonetheless, patients undergoing bisphosphonate treatment seem to show a nonsignificant trend toward better outcomes. Until further evidence from new clinical trials becomes available, adjuvant bisphosphonates should not be routinely recommended as agents that may potentially alter the course of breast cancer when administered in the adjuvant setting.

8.2 Paper II

Our meta-analysis provides substantial evidence suggesting that the use of bisphosphonates in early breast cancer patients does not prevent bone fracture. Probably, only breast cancer patients at a higher risk for osteoporotic fractures may derive benefit from prophylactic bisphosphonate use; however, this should be demonstrated in future trials.

8.3 Paper III

Our study provides substantial evidence suggesting that osteonecrosis of the jaw is a rare event in breast cancer patients treated with adjuvant use of bisphosphonates. Despite the fact that the use of zoledronic acid is associated with a higher number of events compared with the non-use, the overall incidence of this phenomenon continues to be low. Thereafter, at current dosage, adjuvant use of bisphosphonates is relatively safe in adjuvant treatment of breast cancer, even if the risks are not entirely negligible.

8.4 Paper IV

Our study suggests that fulvestrant 250 mg is similar to other hormonal agents with respect to efficacy measures with equal or even better tolerability profile compared with other hormonal agents. As a result, fulvestrant provides a useful additional option for the treatment of hormone-sensitive advanced breast cancer. Considering the current promising

evidence about the efficacy of fulvestrant at 500 mg, further randomized trials would be important to define the correct positioning of fulvestrant 500 mg in relation to aromatase inhibitors and tamoxifen in the sequencing of drugs for endocrine-responsive disease in the metastatic setting.

8.5 Paper V

APBI is a new technology that offers potential advantages compared with WBRT. The most valid concern regarding APBI, as a new treatment modality in radiation oncology, is its oncological safety. Our meta-analysis, despite the fact that it is based on limited randomized evidence, suggests that partial breast RT is a safe treatment modality as it does not seem to jeopardize survival compared with standard WBRT. Nevertheless, the issue of locoregional recurrence needs to be further addressed. This radiation-delivering technique is unlikely to replace WBRT as the 'gold standard' treatment for all early breast cancer patients. Ongoing large phase III randomized trials [321,322] will further clarify whether APBI offers high efficacy with better cosmetic outcomes and will identify the subgroups of patients who will benefit from APBI. Until then, APBI methods remain investigational and should be performed only in patients enrolled in controlled clinical trials.

8.6 Paper VI

The results of this meta-analysis show that the addition of bevacizumab to chemotherapy offers a statistically significant improvement in PFS and ORR in patients with metastatic breast cancer but does not benefit overall survival. In addition clinical significance of this improvement is questionable. Bevacizumab treatment cannot be suggested for treatment of 1st line metastatic breast cancer, but more data are needed until statistical overall survival differences will be documented and firm guideline recommendation could be given.

8.7 Paper VII

This meta-analysis summarizes all available randomized evidence for the use of trastuzumab in the neoadjuvant setting versus no use. Our results underscore the beneficial effects of trastuzumab treatment in neoadjuvant regimens among HER2-positive breast cancer patients in terms of pCR. Of interest, no additional cardiotoxicity was documented in the

trastuzumab arms. However, although the data are still limited, based on the present evidence the combination of trastuzumab to neoadjuvant chemotherapy improves pCR in HER2-positive breast cancer patients without adding clear survival benefit which remains unknown.

References

[1] Rosenberg W, Donald A. Evidence based medicine: an approach to clinical problem-solving. BMJ 1995;310:1122–1126

[2] Timmermans S, Mauck A. The promises and pitfalls of evidence-based medicine. Health Aff (Millwood) 2005;24:18-28

[3] U.S. Preventive Services Task Force. Guide to clinical preventive services: report of the U.S. Preventive Services Task Force. DIANE Publishing. 1989, p 21–25

[4] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61:69-90

[5] Bray F, McCarron P, Parkin DM. The changing global patterns of female breast cancer incidence and mortality. Breast Cancer Res. 2004;6:229-39

[6] Althuis MD, Dozier JD, Anderson WF, Devesa SS, Brinton LA. Global trends in breast cancer incidence and mortality 1973-1997. Int J Epidemiol. 2005;34:405-412

[7] Glass AG, Lacey Jr JV, Carreon JD, Hoover RN. Breast cancer incidence, 1980–2006: combined roles of menopausal hormone therapy, screening mammography, and estrogen receptor status. J Natl Cancer Inst 2007;99:1152–61

[8] Karim-Kos HE, de Vries E, Soerjomataram I, Lemmens V, Siesling S, Coebergh JW. Recent trends of cancer in Europe: a combined approach of incidence, survival and mortality for 17 cancer sites since the 1990s. Eur J Cancer. 2008;44:1345-89

[9] Parkin DM. Is the recent fall in incidence of post-menopausal breast cancer in UK related to changes in use of hormone replacement therapy? Eur J Cancer. 2009; 45:1649-1653

[10] Canfell K, Banks E, Moa AM, Beral V. Decrease in breast cancer incidence following a rapid fall in use of hormone replacement therapy in Australia. Med J Aust. 2008;188:641-644

[11] Tryggvadóttir L, Gislum M, Bray F, Klint A, Hakulinen T, Storm HH, Engholm G. Trends in the survival of patients diagnosed with breast cancer in the Nordic countries 1964-2003 followed up to the end of 2006. Acta Oncol. 2010;49:624-31

[12] Malvezzi M, Arfé A, Bertuccio P, Levi F, La Vecchia C, Negri E. European cancer mortality predictions for the year 2011. Ann Oncol. 2011 Apr;22(4):947-56

[13] Dawood S, Merajver SD, Viens P, et al. International expert panel on inflammatory breast cancer: consensus statement for standardized diagnosis and treatment. Ann Oncol 2011; 22:515

[14] Nelson HD, Tyne K, Naik A, Bougatsos C, Chan B, Nygren P, Humphrey L. Screening for Breast Cancer: Systematic Evidence Review Update for the U.S. Preventive Services Task Force. Evidence Review Update No. 74. AHRQ Publication No. 10-05142-EF-1. Rockville, MD: Agency for Healthcare Research and Quality; 2009

[15] Baxter N, Canadian Task Force on Preventive Health Care. Preventive health care 2001 update: should women be routinely taught breast self-examination to screen for breast cancer? CMAJ. 2001;164:1837-1846

[16] Roth MY, Elmore JG, Yi-Frazier JP, Reisch LM, Oster NV, Miglioretti DL. Self-detection remains a key method of breast cancer detection for U.S. women. J Womens Health (Larchmt). 2011;20:1135-9

[17] Rosenberg A, Burke L, Vos P, Liles D. Self-exam is the most common method of breast cancer identification. J Clin Oncol 2007;25:1543

[18] Smith RA, Saslow D, Sawyer KA, et al. American Cancer Society guidelines for breast cancer screening: update 2003. CA Cancer J Clin 2003;53:141–169

[19] Miller AB, To T, Baines CJ, Wall C. The Canadian National Breast Screening Study-1: breast cancer mortality after 11 to 16 years of follow-up: a randomized screening trial of mammography in women age 40 to 49 years. Ann Intern Med 2002;137:305–312

[20] Sardanelli F, Boetes C, Borisch B et al. Magnetic resonance imaging of the breast: recommendations from the EUSOMA working group. Eur J Cancer 2010;46: 1296–1316

[21] Saslow D, Boetes C, Burke W, Harms S, Leach MO, Lehman CD, Morris E, Pisano E, Schnall M, Sener S, Smith RA, Warner E, Yaffe M, Andrews KS, Russell CA; American Cancer Society Breast Cancer Advisory Group. American Cancer Society guidelines for breast screening with MRI as an adjunct to mammography. CA Cancer J Clin. 2007;57:75-89

[22] Morris KT, Pommier RF, Morris A, Schmidt WA, Beagle G, Alexander PW, et al. Usefulness of the triple test score for palpable breast masses. Arch Surg. 2001;136:1008-13

[23] Liberman L. Percutaneous imaging-guided core breast biopsy: state of the art at the millennium. AJR Am J Roentgenol 2000; 174:1191–1199

[24] Wells C. Quality assurance in breast cancer screening cytology A review of the literature and a report on the U.K. national cytology scheme. Eur J Cancer 1995;31A:273-280

[25] Fornage BD, Coan JD, David CL. Ultrasound-guided needle biopsy of the breast and other interventional procedures. Radiol Clin North Am 1992; 30:167–185

[26] Verkooijen HM. Diagnostic accuracy of stereotactic largecore needle biopsy for nonpalpable breast disease. Results of a multicenter prospective study with 95% surgical confirmation. Int J Cancer 2002;99:853- 859

[27] Kettritz U, Rotter K, Schreer I, Murauer M, Schulz-Wendtland R, Peter D, Heywang-Köbrunner SH. Stereotactic vacuum-assisted breast biopsy in 2874 patients A multicenter study. Cancer 2004;100:245-251

[28] Parker SH, Burbank F, Jackman RJ, et al. Percutaneous large-core breast biopsy: a multi-institutional study. Radiology 1994;193:359–364

[29] Perlet C, Heywang-Kobrunner SH, Heinig A, Sittek H, Casselman J, Anderson I, Taourel P. Magnetic resonanceguided, vacuum-assisted breast biopsy Results from a European multicenter study of 538 lesions. Cancer 2006;106:982-990

[30] Edge SB, Byrd DR, Cpmton CC, et al. eds. AJCC Cancer Staging Manual, 7th edition New York: Springer; 2010

[31] Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B, Senn HJ; Panel members. Strategies for subtypes--dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Ann Oncol. 2011;22:1736-47

[32] Goldhirsch A, Ingle JN, Gelber RD, Coates AS, Thürlimann B, Senn HJ; Panel members. Thresholds for therapies: highlights of the St Gallen International Expert Consensus on the primary therapy of early breast cancer 2009. Ann Oncol. 2009;20:1319-29

[33] Eifel P, Axelson JA, Costa J, et al. National Institutes of Health Consensus Development Conference Statement: adjuvant therapy for breast cancer, November 1–3, 2000. J Natl Cancer Inst 2001;93:979–989

[34] Galea MH, Blamey RW, Elston CE, et al. The Nottingham Prognostic Index in primary breast cancer. Breast Cancer Res Treat 1992;22:207–219

[35] Fisher B, Bauer M, Wickerham DL et al. Relation of the number of positive axillary nodes to the prognosis of patients with primary breast cancer. An NSABP update. Cancer 1983;52:1551-1557

[36] Carter CL, Allen C, Henson DE. Relation of tumor size, lymph node status and survival in 24,740 breast cancer cases. Cancer 1989;63:181-187.

[37] Rosen PP, Groshen S, Kinne DW et al. Factors influencing prognosis in node-negative breast carcinoma: analysis of 767 T1N0M0/T2N0M0 patients with long-term follow-up. J Clin Oncol 1993;11:2090-2100

[38] Carstens PH, Greenberg RA, Francis D et al. Tubular carcinoma of the breast. A long term follow-up. Histopathol 1985;99:271-280

[39] Clayton F. Pure mucinous carcinomas of the breast: morphologic features and prognostic correlates. Hum Pathol 1986;17:34-38

[40] Ridolfi RL, Rosen PP, Port A et al. Medullary carcinoma of the breast: a clinicopathologic study with a ten year follow-up. Cancer 1977;40:1365-1385

[41] Elston CW, Ellis IO: Pathological prognostic factors in breast cancer I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 1991;19:403-410

[42] Rakha EA, El-Sayed ME, Lee AH, Elston CW, Grainge MJ, Hodi Z, Blamey RW, Ellis IO. Prognostic significance of Nottingham histologic grade in invasive breast carcinoma. J Clin Oncol. 2008;26:3153-8

[43] de Azambuja E, Cardoso F, de Castro G Jr, et al. Ki-67 as prognostic marker in early breast cancer: a meta-analysis of published studies involving 12,155 patients. Br J Cancer. 2007;96:1504-13

[44] Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet. 2011;378:771-84

[45] Bardou VJ, Arpino G, Elledge RM, et al. Progesterone receptor status significantly improves outcome prediction over estrogen receptor status alone for adjuvant endocrine therapy in two large breast cancer databases. J Clin Oncol 2003;21:1973–1979

[46] Schechter AL, Stern DF, Vaidyanathan L et al. The neu oncogene: an erb-B-related gene encoding a 185,000-Mr tumour antigen. Nature 1984;312:513-516

[47] Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science. 1987;235:177-82

[48] Ross JS, Slodkowska EA, Symmans WF, Pusztai L, Ravdin PM, Hortobagyi GN. The HER-2 receptor and breast cancer: ten years of targeted anti-HER-2 therapy and personalized medicine. Oncologist. 2009;14:320-68

[49] Pritchard KI, Shepherd LE, O'Malley FP, Andrulis IL, Tu D, Bramwell VH, Levine MN; National Cancer Institute of Canada Clinical Trials Group. HER2 and responsiveness of breast cancer to adjuvant chemotherapy. N Engl J Med. 2006;354:2103-11

[50] Hayes DF, Thor AD, Dressler LG, et al; Cancer and Leukemia Group B (CALGB) Investigators. HER2 and response to paclitaxel in node-positive breast cancer. N Engl J Med. 2007;357:1496-506

[51] Dawood S, Broglio K, Buzdar AU, Hortobagyi GN, Giordano SH. Prognosis of women with metastatic breast cancer by HER2 status and trastuzumab treatment: an institutional-based review. J Clin Oncol. 2010;28:92-8

[52] Ryan Q, Ibrahim A, Cohen MH, et al. FDA drug approval summary: lapatinib in combination with capecitabine for previously treated metastatic breast cancer that overexpresses HER-2. Oncologist. 2008;13:1114-9

[53] Perou CM, Sørlie T, Eisen MB, et al. Molecular portraits of human breast tumours. Nature 2000;406:747–752

[54] Sørlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A. 2001;98:10869-74

[55] Rouzier R, Perou CM, Symmans WF, et al. Breast cancer molecular subtypes respond differently to preoperative chemotherapy. Clin Cancer Res. 2005;11:5678-85

[56] Albain KS, Paik S, van't Veer L: Prediction of adjuvant chemotherapy benefi t in endocrine responsive, early breast cancer using multigene assays. Breast 2009;Suppl 3:S141-145

[57] Ross JS, Hatzis C, Symmans WF, Pusztai L, Hortobagyi GN. Commercialized multigene predictors of clinical outcome for breast cancer. Oncologist 2008;13:477-493

[58] Tang G, Shak S, Paik S, et al. Comparison of the prognostic and predictive utilities of the 21-gene Recurrence Score assay and Adjuvant! for women with node-negative, ER-positive breast cancer: results from NSABP B-14 and NSABP B-20. Breast Cancer Res Treat. 2011;127:133-42

[59] Parisi F, González AM, Nadler Y, Camp RL, Rimm DL, Kluger HM, Kluger Y. Benefits of biomarker selection and clinico-pathological covariate inclusion in breast cancer prognostic models. Breast Cancer Res. 2010;12:R66

[60] Aebi S, Davidson T, Gruber G, Cardoso F; ESMO Guidelines Working Group. Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2011;22 Suppl 6:vi12-24

[61] Veronesi U, Cascinelli N, Mariani L, et al. Twenty-year follow-up of a randomized study comparing breastconserving surgery with radical mastectomy for early breast cancer. N Engl J Med 2002;347:1227-32

[62] Fisher B, Anderson S, Bryant J, et al. Twenty-year follow-up of a randomized trial comparing total mastectomy, lumpectomy, and lumpectomy plus irradiation for the treatment of invasive breast cancer. N Engl J Med. 2002;347:1233- 41

[63] Association of Breast Surgery at Baso 2009. Surgical guidelines for the management of breast cancer. Eur J Surg Oncol 2009; 35 Suppl 1: 1–22

[64] EUSOMA. The requirements of a specialist breast unit. Eur J Cancer 2000;36:2288–2293

[65] Boneti C, Yuen J, Santiago C, et al. Oncologic safety of nipple skin-sparing or total skin-sparing mastectomies with immediate reconstruction. J Am Coll Surg. 2011;212:686-93

[66] Gerber B, Krause A, Dieterich M, Kundt G, Reimer T. The oncological safety of skin sparing mastectomy with conservation of the nipple-areola complex and autologous reconstruction: an extended follow-up study. Ann Surg. 2009;249:461-8

[67] Salhab M, Al Sarakbi W, Joseph A, Sheards S, Travers J, Mokbel K. Skin-sparing mastectomy and immediate breast reconstruction: patient satisfaction and clinical outcome. Int J Clin Oncol. 2006;11:51-4

[68] Heneghan HM, Prichard RS, Lyons R, et al. Quality of life after immediate breast reconstruction and skin-sparing mastectomy - A comparison with patients undergoing breast conserving surgery. Eur J Surg Oncol. 2011;37:937-43.

[69] Gill G; SNAC Trial Group of the Royal Australasian College of Surgeons (RACS) and NHMRC Clinical Trials Centre. Sentinel-lymph-node-based management or routine axillary clearance? One-year outcomes of sentinel node biopsy versus axillary clearance (SNAC): a randomized controlled surgical trial. Ann Surg Oncol. 2009;16:266-75

[70] Mansel RE, Fallowfield L, Kissin M, et al. Randomized multicenter trial of sentinel node biopsy versus standard axillary treatment in operable breast cancer: the ALMANAC Trial. J Natl Cancer Inst. 2006;98:599-609

[71] Canavese G, Catturich A, Vecchio C, et al. Sentinel node biopsy compared with complete axillary dissection for staging early breast cancer with clinically negative lymph nodes: results of randomized trial. Ann Oncol. 2009;20:1001-7

[72] Veronesi U, Viale G, Paganelli G, et al. Sentinel lymph node biopsy in breast cancer: ten-year results of a randomized controlled study. Ann Surg 2010;251:595–600

[73] Krag DN, Anderson SJ, Julian TB, et al. Sentinel-lymph-node resection compared with conventional axillary-lymph-node dissection in clinically node-negative patients with breast cancer: overall survival findings from the NSABP B-32 randomised phase 3 trial. Lancet Oncol. 2010;11:927-33

[74] Giuliano AE, Hunt KK, Ballman KV et al. Axillary dissection vs no axillary dissection in women with invasive breast cancer and sentinel node metastasis. JAMA 2011;305: 569–575

[75] Lyman GH, Giuliano AE, Somerfield MR, et al. American Society of Clinical Oncology Guideline Recommendations for sentinel lymph node biopsy in early-stage breast cancer. J Clin Oncol 2005;23:7703–20

[76] Weaver DL, Ashikaga T, Krag DN, et al. Effect of occult metastases on survival in node-negative breast cancer. N Engl J Med. 2011;364:412-21

[77] Giuliano AE, Hawes D, Ballman KV, et al. Association of occult metastases in sentinel lymph nodes and bone marrow with survival among women with early-stage invasive breast cancer. JAMA. 2011;306:385-93

[78] NCCN Clinical Practice Guidelines for breast cancer. Available at: http://www.nccn.org/professionals/physician_gls/f_guidelines.asp

[79] Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effect of radiotherapy after breast-conserving surgery on 10-year recurrence and 15-year breast cancer death: meta-analysis of individual patient data for 10 801 women in 17 randomised trials. Lancet. 2011 Oct 19

[80] Fyles AW, McCready DR, Manchul LA et al. Tamoxifen with or without breast irradiation in women 50 years of age or older with early breast cancer. N Engl J Med 2004; 351: 963–970

[81] Hughes KS, Schnaper LA, Berry D et al. Lumpectomy plus tamoxifen with or without irradiation in women 70 years of age or older with early breast cancer. N Engl J Med 2004; 351: 971–977

[82] Danish Breast Cancer Cooperative Group, Nielsen HM, Overgaard M, Grabu C, Jensen AR, Overgaard J. Study of failure pattern among high-risk breast cancer patients with or without postmastectomy radiotherapy in addition to adjuvant systemic therapy: long-term results from the Danish Breast Cancer Cooperative Group DBCG 82 b and c randomized studies. J Clin Oncol. 2006;24:2268-75

[83] Overgaard M, Nielsen HM, Overgaard J. Is the benefit of postmastectomy irradiation limited to patients with four or more positive nodes, as recommended in international consensus reports? A subgroup analysis of the DBCG 82 b&c randomized trials. Radiother Oncol. 2007;82:247-53

[84] Whelan TJ, Olivotto I, Ackerman, JW, et al. NCIC-CTG MA.20: An intergroup trial of regional nodal irradiation in early breast cancer. J Clin Oncol 2011;29 (suppl; abstr LBA1003)

[85] Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet. 2005;365:1687-717

[86] Albain KS, Barlow WE, Ravdin PM, et al; Breast Cancer Intergroup of North America. Adjuvant chemotherapy and timing of tamoxifen in postmenopausal patients with endocrine-responsive, node-positive breast cancer: a phase 3, open-label, randomised controlled trial. Lancet. 2009;374:2055-63

[87] LHRH-agonists in Early Breast Cancer Overview group, Cuzick J, Ambroisine L, Davidson N, Jakesz R, Kaufmann M, Regan M, Sainsbury R. Use of luteinising-hormone-releasing hormone agonists as adjuvant treatment in premenopausal patients with hormone-receptor-positive breast cancer: a meta-analysis of individual patient data from randomised adjuvant trials. Lancet. 2007;369:1711-23

[88] Dowsett M, Cuzick J, Ingle J, et al. Meta-analysis of breast cancer outcomes in adjuvant trials of aromatase inhibitors versus tamoxifen. J Clin Oncol. 2010;28:509-18

[89] Burstein HJ, Prestrud AA, Seidenfeld J, et al; American Society of Clinical Oncology. American Society of Clinical Oncology clinical practice guideline: update on adjuvant endocrine therapy for women with hormone receptor-positive breast cancer. J Clin Oncol. 2010;28:3784-96

[90] Muss HB, Tu D, Ingle JN, et al. Efficacy, toxicity, and quality of life in older women with early-stage breast cancer treated with letrozole or placebo after 5 years of tamoxifen: NCIC CTG intergroup trial MA.17. J Clin Oncol. 2008;26:1956-64

[91] Jakesz R, Greil R, Gnant M, et al; Austrian Breast and Colorectal Cancer Study Group. Extended adjuvant therapy with anastrozole among postmenopausal breast cancer patients: results from the randomized Austrian Breast and Colorectal Cancer Study Group Trial 6a. J Natl Cancer Inst. 2007;99:1845-53

[92] Mamounas EP, Jeong JH, Wickerham DL, et al. Benefit from exemestane as extended adjuvant therapy after 5 years of adjuvant tamoxifen: intention-to-treat analysis of the National Surgical Adjuvant Breast And Bowel Project B-33 trial. J Clin Oncol. 2008;26:1965-71

[93] Francis P, Crown J, Di Leo A et al. Adjuvant chemotherapy with sequential or concurrent anthracycline and docetaxel: Breast International Group 02-98 randomized trial. J Natl Cancer Inst 2008;100:121–133

[94] Joensuu H, Kellokumpu-Lehtinen PL, Huovinen R, et al; FinXX Study Investigators. Adjuvant capecitabine in combination with docetaxel and cyclophosphamide plus epirubicin for breast cancer: an open-label, randomised controlled trial. Lancet Oncol. 2009;10:1145-51

[95] O'Shaughnessy J,Paul D, Stokoe C, et al. First efficacy results of a randomized, open-label, phase III study of adjuvant doxorubicin plus cyclophosphamide, followed by docetaxel with or without capecitabine, in high-risk early breast cancer. Cancer Research 2011;70:S4-2

[96] Lindman H, Kellokumpu-Lehtinen PL, Huovinen R. Integration of Capecitabine into Anthracycline- and Taxane-Based Adjuvant Therapy for Triple-Negative Early Breast Cancer: Final Subgroup Analysis of the FinXX Study. Cancer Research 2011;70:PD01-02

[97] Pippen JE, Paul D, Stokoe T, et al. Randomized, phase III study of adjuvant doxorubicin plus cyclophosphamide (AC) followed by docetaxel (T) with or without

capecitabine (X) in high-risk early breast cancer: Exploratory Ki-67 analyses. J Clin Oncol 2011;29:suppl; abstr 500

[98] Yin W, Jiang Y, Shen Z, Shao Z, Lu J. Trastuzumab in the adjuvant treatment of HER2-positive early breast cancer patients: a meta-analysis of published randomized controlled trials. PLoS One. 2011;6:e21030

[99] Petrelli F, Barni S. Meta-analysis of concomitant compared to sequential adjuvant trastuzumab in breast cancer: the sooner the better. Med Oncol. 2011 Mar 13

[100] Slamon D, Eiermann W, Robert N, et al; Breast Cancer International Research Group. Adjuvant trastuzumab in HER2-positive breast cancer. N Engl J Med. 2011;365:1273-83

[101] Banerjee S, Smith IE. Management of small HER2-positive breast cancers. Lancet Oncol. 2010;11:1193-9

[102] Mauri D, Pavlidis N, Ioannidis JP. Neoadjuvant versus adjuvant systemic treatment in breast cancer: a meta-analysis. J Natl Cancer Inst. 2005;97:188-94

[103] Ellis MJ, Coop A, Singh B, et al. Letrozole is more effective neoadjuvant endocrine therapy than tamoxifen for ErbB-1- and/or ErbB-2-positive, estrogen receptor-positive primary breast cancer: evidence from a phase III randomized trial. J Clin Oncol. 2001;19:3808-16

[104] Smith IE, Dowsett M, Ebbs SR, et al; IMPACT Trialists Group. Neoadjuvant treatment of postmenopausal breast cancer with anastrozole, tamoxifen, or both in combination: the Immediate Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen (IMPACT) multicenter double-blind randomized trial. J Clin Oncol. 2005;23:5108-16

[105] Curigliano G, Bagnardi V, Viale G, et al. Should liver metastases of breast cancer be biopsied to improve treatment choice? Ann Oncol. 2011;22:2227-33

[106] Guarneri V, Giovannelli S, Ficarra G, et al. Comparison of HER-2 and hormone receptor expression in primary breast cancers and asynchronous paired metastases: impact on patient management. Oncologist. 2008;13:838-44

[107] Liedtke C, Broglio K, Moulder S, et al. Prognostic impact of discordance between triple-receptor measurements in primary and recurrent breast cancer. Ann Oncol. 2009;20:1953-8

[108] Simmons C, Miller N, Geddie W, Gianfelice D, Oldfield M, Dranitsaris G, Clemons MJ. Does confirmatory tumor biopsy alter the management of breast cancer patients with distant metastases? Ann Oncol. 2009;20:1499-50

[109] Schwartzberg LS, Franco SX, Florance A, O'Rourke L, Maltzman J, Johnston S. Lapatinib plus letrozole as first-line therapy for HER-2+ hormone receptor-positive metastatic breast cancer. Oncologist. 2010;15:122-9

[110] Kaufman B, Mackey JR, Clemens MR, et al. Trastuzumab plus anastrozole versus anastrozole alone for the treatment of postmenopausal women with human epidermal growth factor receptor 2-positive, hormone receptor-positive metastatic breast cancer: results from the randomized phase III TAnDEM study. J Clin Oncol. 2009;27:5529-37

[111] Cameron D, Casey M, Oliva C, Newstat B, Imwalle B, Geyer CE. Lapatinib plus capecitabine in women with HER-2-positive advanced breast cancer: final survival analysis of a phase III randomized trial. Oncologist. 2010;15:924-34

[112] Kohno N, Aogi K, Minami H, et al. Zoledronic acid significantly reduces skeletal complications compared with placebo in Japanese women with bone metastases from breast cancer: a randomized, placebo-controlled trial. J Clin Oncol. 2005;23:3314-21

[113] Rosen LS, Gordon D, Kaminski M, et al. Long-term efficacy and safety of zoledronic acid compared with pamidronate disodium in the treatment of skeletal complications in patients with advanced multiple myeloma or breast carcinoma: a randomized, double-blind, multicenter, comparative trial. Cancer. 2003;98:1735-44

[114] Stopeck AT, Lipton A, Body JJ, et al. Denosumab compared with zoledronic acid for the treatment of bone metastases in patients with advanced breast cancer: a randomized, double-blind study. J Clin Oncol. 2010;28:5132-9

[115] Rojas MPMP, Telaro E, Moschetti I, Coe L, Fossati R, Liberati A, Rosselli MDT. Follow-up strategies for women treated for early breast cancer. Cochrane Database Syst Rev. 2005;1:CD001768

[116] Glass GV. Primary, secondary and meta-analysis of research. Educ Res 1976;5:3-8

[117] L'Abbe KA, Detsky AS, O'Rourke K. Meta-analysis in clinical research. Ann Intern Med 1987;107:224-233

[118] Phillips B, Ball C, Badenoch D, Straus S, Haynes B, Dawes M: Oxford Centre for Evidence-based Medicine Levels of Evidence (May 2001). BJU International 2008;102:920

[119] Oxman AD, Cook DJ, Guyatt GH. Users' guides to the medical literature. VI. How to use an overview. Evidence-Based Medicine Working Group. JAMA. 1994;272:1367-71

[120] Swingler GH, Volmink J, Ioannidis JP. Number of published systematic reviews and global burden of disease: database analysis. BMJ. 2003;327:1083-4

[121] Sacks HS, Berrier J, Reitman D, Ancona-Berk VA, Chalmers TC. Meta-analyses of randomized controlled trials. N Engl J Med. 1987;316:450-5

[122] Moher D, Tetzlaff J, Tricco AC, Sampson M, Altman DG. Epidemiology and reporting characteristics of systematic reviews. PLoS Med. 2007;4:e78

[123] Kelly KD, Travers A, Dorgan M, Slater L, Rowe BH. Evaluating the quality of systematic reviews in the emergency medicine literature. Ann Emerg Med. 2001;38:518-26

[124] Delaney A, Bagshaw SM, Ferland A, Manns B, Laupland KB, Doig CJ. A systematic evaluation of the quality of meta-analyses in the critical care literature. Crit Care. 2005;9:R575-82

[125] Moher D, Hopewell S, Schulz KF, et al; Consolidated Standards of Reporting Trials Group. CONSORT 2010 Explanation and Elaboration: Updated guidelines for reporting parallel group randomised trials. J Clin Epidemiol. 2010;63:e1-37

[126] Moher D, Cook DJ, Eastwood S, Olkin I, Rennie D, Stroup DF. Improving the quality of reports of meta-analyses of randomised controlled trials: the QUOROM statement. Quality of Reporting of Meta-analyses. Lancet. 1999;354:1896-900

[127] Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. Ann Intern Med. 2009;151:W65-94

[128] Berman NG, Parker RA. Meta-analysis: neither quick nor easy. BMC Med Res Methodol. 2002;2:10

[129] Pignon JP, Hill C. Meta-analyses of randomised clinical trials in oncology. Lancet Oncol. 2001;2:475-82

[130] Dickersin K, Scherer R, Lefebvre C. Identifying relevant studies for systematic reviews. Br Med J 1994;309:1286–91

[131] Hopewell S, McDonald S, Clarke M, Egger M. Grey literature in meta-analyses of randomized trials of health care interventions. Cochrane Database Syst Rev. 2007;2:MR000010

[132] Dickersin K, Min YI, Meinert CL. Factors influencing publication of research results. Follow-up of applications submitted to two institutional review boards. JAMA 1992;267:374–78

[133] McAuley L, Pham B, Tugwell P, Moher D. Does the inclusion of grey literature influence estimates of intervention effectiveness reported in meta-analyses? Lancet 2000;356:1228–31

[134] Higgins JP, Altman DG, Gøtzsche PC, et al; Cochrane Bias Methods Group; Cochrane Statistical Methods Group. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011;343:d5928

[135] Egger M, Smith GD, Phillips AN. Meta-analysis: principles and procedures. BMJ. 1997;315:1533-1537

[136] Der Simonian R, Laird N. Meta-analysis in clinical trials. Controlled Clin Trials 1986; 7:177–88

[137] Engels EA, Schmid CH, Terrin N, Olkin I, Lau J. Heterogeneity and statistical significance in meta-analysis: an empirical study of 125 meta-analyses. Stat med 2000;19:1707–28

[138] Cochran WG. The combination of estimates from different experiments. Biometrics 1954;10:101-29

[139] Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003;327:557–560

[140] Crowther M, Lim W, Crowther MA. Systematic review and meta-analysis methodology. Blood. 2010;116:3140-6

[141] Thompson SG, Higgins JP. How should metaregression analyses be undertaken and interpreted? Stat Med. 2002;21:1559-1573

[142] Lewis S, Clarke M: Forest plots: trying to see the wood and the trees. BMJ 2001;322:1479-1480

[143] Lawrentschuk N, McCall J, Güller U. Critical appraisal of meta-analyses: an introductory guide for the practicing surgeon. Patient Saf Surg. 2009;3:16

[144] Bax L, Yu LM, Ikeda N, Moons KG. A systematic comparison of software dedicated to meta-analysis of causal studies. BMC Med Res Methodol. 2007;7:40

[145] Zwahlen M, Renehan A, Egger M. Meta-analysis in medical research: potentials and limitations. Urol Oncol. 2008;26:320-9

[146] Montori VM, Smieja M, Guyatt GH: Publication bias: A brief review for clinicians. Mayo Clin Proc 2000;75:1284–1288

[147] Stern JM, Simes RJ Publication bias: evidence of delayed publication in a cohort study of clinical research projects. BMJ 1997;315:640–5

[148] Egger M, Smith GD. Bias in location and selection of studies. BMJ. 1998;316:61-6

[149] Grégoire G, Derderian F, LeLorier J Selecting the language of the publications included in a meta-analysis: is there a Tower of Babel bias? J Clin Epidemiol 1995;48:159–63

[150] Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315:629-34

[151] Deangelis CD, Drazen JM, Frizelle FA, et al: Is this clinical trial fully registered? A statement from the International Committee of Medical Journal Editors. JAMA 2005;293:2927-2929

[152] Lyman GH, Kuderer NM. The strengths and limitations of meta-analyses based on aggregate data. BMC Med Res Methodol. 2005;5:14

[153] Ioannidis JP. Integration of evidence from multiple meta-analyses: a primer on umbrella reviews, treatment networks and multiple treatments meta-analyses. CMAJ. 2009;181:488-93

[154] Valsecchi MG, Masera G. A new challenge in clinical research in childhood ALL: The prospective meta-analysis strategy for intergroup collaboration. Ann Oncol 1996;7:1005–08

[155] Pfeilschifter J, Diel IJ. Osteoporosis due to cancer treatment: pathogenesis and management. J Clin Oncol 2000;18:1570–93.

[156] Shapiro CL, Manola J, Leboff M. Ovarian failure after adjuvant chemotherapy is associated with rapid bone loss in women with early-stage breast cancer. J Clin Oncol 2001;19:3306–11

[157] Delmas PD, Fontana A. Bone loss induced by cancer treatment and its management. Eur J Cancer 1998;34:260–2

[158] Perez EA, Josse RG, Pritchard KI, et al. Effect of letrozole versus placebo on bone mineral density in women with primary breast cancer completing 5 or more years of

adjuvant tamoxifen: a companion study to NCIC CTG MA. 17. J Clin Oncol 2006;24:3629–35.

[159] Coleman RE, Banks LM, Girgis SI, et al. Skeletal effects of exemestane on bone-mineral density, bone biomarkers, and fracture incidence in post-menopausal women with early breast cancer participating in the Intergroup Exemestane Study (IES): a randomized controlled study. Lancet Oncol 2007;8:119–27

[160] Hoff AO, Gagel RF. Osteoporosis in breast and prostate cancer survivors. Oncology (Williston Park) 2005;19:651–8

[161] Kimmel, D. Mechanism of action, pharmakinetic and pharmacodynamic profile, and clinical applications of nitrogen-containing bisphosphonates. Journal of Dental Research 2007;86: 1022-1033

[162] Pavlakis N, Schmidt R, Stockler M. Bisphosphonates for breast cancer. Cochrane Database Syst Rev. 2005;3:CD003474

[163] Brufsky A. Cancer treatment-induced bone loss: pathophysiology and clinical perspectives. Oncologist 2008;13:187–195

[164] Aapro M, Abrahamsson PA, Body JJ, et al. Guidance on the use of bisphosphonates in solid tumours: recommendations of an international expert panel. Ann Oncol 2008;19:420–32

[165] Daubine F, Le Gall C, Gasser J, et al. Antitumor effects of clinical dosing regimens of bisphosphonates in experimental breast cancer bone metastasis. J Natl Cancer Inst 2007;99:322–330

[166] van der Pluijm G, Vloedgraven H, van Beek E, et al. Bisphosphonates inhibit the adhesion of breast cancer cells to bone matrices in vitro. J Clin Invest 1996;98:698–705

[167] Senaratne SG, Pirianov G, Mansi JL, et al. Bisphosphonates induce apoptosis in human breast cancer cell lines. Br J Cancer 2000;82:1459–1468

[168] Fournier P, Boissier S, Filleur S, et al. Bisphosphonates inhibit angiogenesis in vitro and testosterone-stimulated vascular regrowth in the ventral prostate in castrated rats. Cancer Res 2002;62:6538–6544

[169] Gallo M, De Luca A, Lamura L, Normanno N. Zoledronic acid blocks the interaction between mesenchymal stem cells and breast cancer cells: implications for adjuvant therapy of breast cancer. Ann Oncol. 2011 May 12

[170] Bamias A, Kastritis E, Bamia C, et al. Osteonecrosis of the jaw in cancer after treatment with bisphosphonates: incidence and risk factors. J Clin Oncol 2005;23:8580–8587

[171] van de Velde CJ, Rea D, Seynaeve C, et al. Adjuvant tamoxifen and exemestane in early breast cancer (TEAM): a randomised phase 3 trial. Lancet. 2011;377:321-31

[172] BIG 1-98 Collaborative Group, Mouridsen H, Giobbie-Hurder A, Goldhirsch A, et al. Letrozole therapy alone or in sequence with tamoxifen in women with breast cancer. N Engl J Med. 2009;361:766-76

[173] Arimidex, Tamoxifen, Alone or in Combination (ATAC) Trialists' Group, Forbes JF, Cuzick J, Buzdar A, Howell A, Tobias JS, Baum M. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 100-month analysis of the ATAC trial. Lancet Oncol. 2008;9:45-53

[174] Mauri D, Pavlidis N, Polyzos NP, Ioannidis JP. Survival with aromatase inhibitors and inactivators versus standard hormonal therapy in advanced breast cancer: meta-analysis. J Natl Cancer Inst. 2006;98:1285-91

[175] Wakeling AE, Bowler J. Steroidal pure antioestrogens. J Endocrinol 1987;112:R7-10

[176] Wakeling AE. The future of new pure antioestrogens in clinical breast cancer. 285 Breast Cancer Res Treat 1993;25:1–9

[177] Lupien M, Meyer CA, Bailey ST, et al. Growth factor stimulation induces a distinct ER(alpha) cistrome underlying breast cancer endocrine resistance. Genes Dev. 2010;24:2219-27

[178] Massarweh S, Schiff R. Resistance to endocrine therapy in breast cancer: exploiting estrogen receptor/growth factor signaling crosstalk. Endocr Relat Cancer 2006;13(Suppl 1):S15-24

[179] Osborne CK, Coronado-Heinsohn EB, Hilsenbeck SG, et al. Comparison of the effects of a pure steroidal antiestrogen with those of tamoxifen in a model of human breast cancer. J Natl Cancer Inst. 1995;87:746-50

[180] Hu XF, Veroni M, De Luise M, et al. Circumvention of tamoxifen resistance by the pure anti-estrogen ICI 182,780. Int J Cancer. 1993;55:873-6

[181] Jelovac D, Macedo L, Goloubeva OG, Handratta V, Brodie AM. Additive antitumor effect of aromatase inhibitor letrozole and antiestrogen fulvestrant in a postmenopausal breast cancer model. Cancer Res. 2005;65:5439-44

[182] Nattinger AB, Gottlieb MS, Veum J, Yahnke D, Goodwin JS. Geographic variation in the use of breast-conserving treatment for breast cancer. N Engl J Med 1992;326:1102-1107

[183] Lazovich DA, White E, Thomas DB, Moe RE. Underutilization of breastconserving surgery and radiation therapy among women with stage I or II breast cancer. JAMA 1991;266:3433-3438

[184] Morrow M, White J, Moughan J, et al. Factors predicting the use of breast-conserving therapy in stage I and II breast carcinoma. J Clin Oncol 2001;19:2254–62

[185] Nattinger AB, Hoffmann RG, Kneusel RT, Schapira MM. Relation between appropriateness of primary therapy for early-stage breast carcinoma and increased use of breast-conserving surgery. Lancet 2000;356:1148–53

[186] Punglia RS, Saito AM, Neville BA, Earle CC, Weeks JC. Impact of interval from breast conserving surgery to radiotherapy on local recurrence in older women with breast cancer: retrospective cohort analysis. BMJ. 2010;340:c845

[187] Jobsen JJ, van der Palen J, Ong F, Meerwaldt JH. Timing of radiotherapy and survival benefit in breast cancer. Breast Cancer Res Treat. 2006;99:289-94

[188] Olivotto IA, Lesperance ML, Truong PT, et al. Intervals longer than 20 weeks from breast-conserving surgery to radiation therapy are associated with inferior outcome for women with early-stage breast cancer who are not receiving chemotherapy. J Clin Oncol. 2009;27:16-23

[189] Malmström P, Holmberg L, Anderson H, et al; Swedish Breast Cancer Group. Breast conservation surgery, with and without radiotherapy, in women with lymph node-negative breast cancer: a randomized clinical trial in a population with access to public mammography screening. Eur J Cancer. 2003;39:1690-7

[190] Vicini FA, Kestin LL, Goldstein NS. Defining the clinical target volume for patients with early-stage breast cancer treated with lumpectomy and accelerated partial breast irradiation: a pathologic analysis. Int J Radiat Oncol Biol Phys. 2004;60:722-30

[191] Fowble B, Solin LJ, Schultz DJ, Rubenstein J, Goodman RL. Breast recurrence following conservative surgery and radiation: patterns of failure, prognosis, and pathologic findings from mastectomy specimens with implications for treatment. Int J Radiat Oncol Biol Phys. 1990;19:833-42

[192] Weed DW, Edmundson GK, Vicini FA, Chen PY, Martinez AA. Accelerated partial breast irradiation: a dosimetric comparison of three different techniques. Brachytherapy. 2005;4:121-9

[193] Sauer R, Sautter-Bihl ML, Budach W, et al. Accelerated partial breast irradiation: consensus statement of 3 German Oncology societies. Cancer. 2007;110:1187-94

[194] Veronesi U, Gatti G, Luini A, Intra M, Ciocca M, Sanchez D, Zurrida S, Navarro S, Orecchia R. Full-dose intraoperative radiotherapy with electrons during breast-conserving surgery. Arch Surg. 2003;138:1253-6

[195] Vaidya JS, Tobias JS, Baum M, et al. Intraoperative radiotherapy for breast cancer. Lancet Oncol. 2004;5:165-73

[196] Formenti SC: External-beam partial-breast irradiation. Semin Radiat Oncol 2005;15:92-99

[197] Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. Endocr Rev 1997;18:4−25

[198] Gasparini G, Toi M, Gion M, et al. Prognostic significance of vascular endothelial growth factor protein in node-negative breast carcinoma. J Natl Cancer Inst 1997;89:139−47

[199] Linderholm B, Grankvist K,Wilking N, Johansson M, Tavelin B, Henriksson R. Correlation of vascular endothelial growth factor content with recurrences, survival, and first relapse site in primary node-positive breast carcinoma after adjuvant treatment. J Clin Oncol 2000;18:1423−31

[200] Jain RK. Normalizing tumor vasculature with antiangiogenic therapy: a new paradigm for combination therapy. Nat Med 2001;7:987−9

[201] Netti PA, Hamberg LM, Babich JW, et al. Enhancement of fluid filtration across tumor vessels: implication for delivery of macromolecules. Proc Natl Acad Sci USA 1999;96:3137–42

[202] Benjamin LE, Golijanin D, Itin A, et al. Selective ablation of immature blood vessels in established human tumors follows vascular endothelial growth factor withdrawal. J Clin Invest 1999;103:159–65

[203] Guo P, Fang Q, Tao HQ, et al. Overexpression of vascular endothelial growth factor by MCF-7 breast cancer cells promotes estrogen-independent tumor growth in vivo. Cancer Res 2003;63:4684–91

[204] Ellis LM, Hicklin DJ: VEGF-targeted therapy: Mechanisms of anti-tumour activity. Nat Rev Cancer 2008;8:579-591

[205] Sweeney CJ, Miller KD, Sissons SE, et al. The antiangiogenic property of docetaxel is synergistic with a recombinant humanized monoclonal antibody against vascular endothelial growth factor or 2-methoxyestradiol but antagonized by endothelial growth factors. Cancer Res 2001;61:3369–3372

[206] Borgstrom P, Hillan KJ, Sriramarao P, Ferrara N. Complete inhibition of angiogenesis and growth of microtumors by anti-vascular endothelial growth factor neutralizing antibody: novel concepts of angiostatic therapy from intravital videomicroscopy. Cancer Res 1996;56:4032–9

[207] Eskens FALM, Sleijfer S. The use of bevacizumab in colorectal, lung, breast, renal and ovarian cancer: Where does it fit? Eur J Cancer 2008;44:2350-2356

[208] Chia S, Swain SM, Byrd DR, Mankoff DA. Locally advanced and inflammatory breast cancer. J Clin Oncol 2008;26:786e90

[209] Fisher B, Bryant J, Wolmark N, Mamounas E, Brown A, Fisher ER, et al. Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. J Clin Oncol 1998;16:2672e85

[210] Ménard S, Pupa SM, Campiglio M, Tagliabue E. Biologic and therapeutic role of HER2 in cancer. Oncogene. 2003;22:6570-8

[211] Rubin I, Yarden Y. The basic biology of HER2. Ann Oncol. 2001;12 Suppl 1:S3-8

[212] Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer. 2005;5:341-54

[213] Viani GA, Afonso SL, Stefano EJ, De Fendi LI, Soares FV. Adjuvant trastuzumab in the treatment of her-2-positive early breast cancer: a meta-analysis of published randomized trials. BMC Cancer 2007;7:153

[214] Harris CA,Ward RL,Dobbins TA, Drew AK, Pearson S.The efficacy of HER2-targeted agents in metastatic breast cancer: a meta-analysis. Ann Oncol; 2010 Nov 30

[215] Sweeting MJ, Sutton AJ, Lambert PC. What to add to nothing? Use and avoidance of continuity corrections in metaanalysis of sparse data. Stat Med 2004;23:1351–1375

[216] Recht A, Silver B, Schnitt SJ, Connolly J, Hellman S, Harris JR. Breast relapse following primary radiation therapy for early breast cancer: I. Classification, frequency and salvage. Int J Radiat Oncol Biol Phys 1985;31:1341–6

[217] Kristensen B, Ejlertsen B, Mouridsen HT, et al. Bisphosphonate treatment in primary breast cancer: results from a randomized comparison of oral pamidronate versus no pamidronate in patients with primary breast cancer. Acta Oncol 2008;47:740–746.

[218] Gnant M, Mlineritsch B, Schippinger W, et al. Endocrine therapy plus zoledronic acid in premenopausal breast cancer. N Engl J Med 2009;360:679–691.

[219] Diel IJ, Jaschke A, Solomayer EF, et al. Adjuvant oral clodronate improves the overall survival of primary breast cancer patients with micrometastases to the bone marrow: a long-term follow-up. Ann Oncol 2008;19:2007–2011.

[220] Coleman R, Thorpe H, Cameron D, et al. Zoledronic acid is well tolerated and can be safely administered with adjuvant chemotherapy—first safety data from the AZURE trial (BIG01/04) [poster]. Presented at the 29th Annual San Antonio Breast Cancer Symposium; December 14–17, 2006; San Antonio, Texas. Abstract 2080

[221] Greenspan SL, Brufsky A, Lembersky BC, et al. Risedronate prevents bone loss in breast cancer survivors: a 2-year, randomized, double-blind, placebo-controlled clinical trial. J Clin Oncol 2008;26:2644–2652

[222] Lester JE, Dodwell D, Purohit OP, et al. Prevention of anastrazoleinduced bone loss with monthly oral ibandronate during adjuvant aromatase inhibitor therapy for breast cancer. Clin Cancer Res 2008;14:6336–6342

[223] Mincey BA, Dentchev T, Sloan JA, et al. N03CC—a randomized, controlled, open-label trial of upfront vs. delayed zoledronic acid for prevention of bone loss in postmenopausal (PM) women with primary breast cancer (PBC) starting letrozole after tamoxifen [abstract]. J Clin Oncol 2008;26(Suppl):Abstract 564

[224] Van Poznak C, Hannon R, Clack G, et al. Managing cancer treatment-induced bone loss: 24-month results from the Study of Anastrozole with the Bisphosphonate RisedronatE (SABRE). Presented at the San Antonio Breast Cancer Symposium; December 10–14, 2008; San Antonio, Texas. Abstract 1137

[225] Shapiro CL, Halabi S, Gibson G, et al. Effect of zoledronic acid (ZA) on bone mineral density (BMD) in premenopausal women who developed ovarian failure (OF) due to adjuvant chemotherapy (AdC): first results of GALGB trial 79809 [abstract]. J Clin Oncol 2008;26(Suppl):Abstract 512

[226] Hines SL, Mincey BA, Sloan JA, et al. N02C1: a phase III randomized, placebo-controlled, double-blind trial of risedronate for prevention of bone loss in premenopausal women undergoing adjuvant chemotherapy for breast cancer (BC) [abstract]. J Clin Oncol 2008;26(Suppl):Abstract 525

[227] Bryce CJ, Prior JC, Sill K, et al. Does short-term, intravenous, low dose clodronate, administered with adjuvant chemotherapy for premenopausal breast cancer reduce bone loss in the first year in patients [abstract]? Proc Am Soc Clin Oncol 2002;21:Abstract 270

[228] Brufsky A, Bosserman L, Caradonna R, et al. The effect of zoledronic acid on aromatase inhibitor associated bone loss in postmenopausal women with early breast cancer receiving adjuvant letrozole: the Z-FAST study 36-month follow-up [abstract]. Presented at the San Antonio Breast Cancer Symposium; December 13–16, 2007; San Antonio, Texas. Abstract 27

[229] Eidtmann H, Bundred NJ, DeBoer R, et al. The effect of zoledronic acid on aromatase inhibitor associated bone loss in postmenopausal women with early breast cancer receiving adjuvant letrozole: 36 months follow-up of ZO-FAST [abstract]. Presented at the San Antonio Breast Cancer Symposium; December 10–14, 2008; San Antonio, Texas. Abstract 44

[230] Fuleihan Gel-H, Salamoun M, Mourad YA, et al. Pamidronate in the prevention of chemotherapy-induced bone loss in premenopausal women with breast cancer: a randomized controlled trial. J Clin Endocrinol Metab 2005;90:3209–3214

[231] Delmas PD, Balena R, Confravreux E, et al. Bisphosphonate risedronate prevents bone loss in women with artificial menopause due to chemotherapy of breast cancer: a double-blind, placebocontrolled study. J Clin Oncol 1997;15:955–962

[232] Hershman DL, McMahon DJ, Crew KD, et al. Zoledronic acid prevents bone loss in premenopausal women undergoing adjuvant chemotherapy for early-stage breast cancer. J Clin Oncol 2008;26:4739–4745

[233] Tevaarwerk A, Stewart JA, Love R, et al. Randomized trial to assess bone mineral density (BMD) effects of zoledronic acid (ZA) in postmenopausal women with breast cancer [abstract]. J Clin Oncol 2007;25(Suppl 1):Abstract 19558

[234] Schenk N, Lombart A, Frassoladti A, et al. The E-ZO-FAST trial: zoledronic acid (ZA) effectively inhibits aromatase inhibitor associated bone loss (AIBL) in postmenopausal women (PMW) with early breast cancer (EBC) receiving adjuvant letrozole (Let). Eur J Cancer 2007;5(Suppl 5):186–187. Abstract 2008

[235] Saarto T, Vehmanen L, Virkkunen P, Blomqvist C. Ten-year follow-up of a randomized controlled trial of adjuvant clodronate treatment in node-positive breast cancer patients. Acta Oncol 2004;43:650–656

[236] Vehmanen L, Saarto T, Risteli J, et al. Short-term intermittent intravenous clodronate in the prevention of bone loss related to chemotherapy-induced ovarian failure. Breast Cancer Res Treat 2004;87:181–188

[237] Powles T, Paterson S, McCloskey E, et al. Reduction in bone relapse and improved survival with oral clodronate for adjuvant treatment of operable breast cancer. Breast Cancer Res 2006;82:R13

[238] Gnant M, Mlineritsch B, Luschin-Ebengreuth G, Kainberger F, Kassmann H, Piswanger-Solkner JD, et al. Adjuvant endocrine therapy plus zoledronic acid in premenopausal women with early-stage breast cancer: 5-year follow-up of the ABCSG-12 bone-mineral density substudy. Lancet Oncol 2008;9:840–9

[239] Vehmanen L, Saarto T, Elomaa I, Makela P, Valimaki M, Blomqvist C. Long-term impact of chemotherapy-induced ovarian failure on bone mineral density (BMD) in premenopausal breast cancer patients. The effect of adjuvant clodronate treatment. Eur J Cancer 2001;37:2373–8

[240] Saarto T, Blomqvist T, Virkkunen P, Elomaa I. Adjuvant clodronate treatment does not reduce the frequency of skeletal metastases in node-positive breast cancer patients: 5-year results of a randomized controlled trial. J Clin Oncol 2001;19:10–7

[241] Robertson JF, Osborne CK, Howell A, et al. Fulvestrant versus anastrozole for the treatment of advanced breast carcinoma in postmenopausal women: a prospective combined analysis of two multicenter trials. Cancer 2003;98:229–38

[242] Howell A, Pippen J, Elledge RM, et al. Fulvestrant versus anastrozole for the treatment of advanced breast carcinoma: a prospectively planned combined survival analysis of two multicenter trials. Cancer 2005;104:236–9

[243] Mauriac L, Pippen JE, Quaresma Albano J, Gertler SZ, Osborne CK. Fulvestrant (Faslodex) versus anastrozole for the second-line treatment of advanced breast cancer in subgroups of postmenopausal women with visceral and non-visceral metastases: combined results from two multicentre trials. Eur J Cancer 2003;39:1228–33

[244] Dodwell D, Pippen J. Time to response: comparison of fulvestrant and oral endocrine agents. Clin Breast Cancer 2006;7:244–7

[245] Howell A, Robertson JF, Quaresma Albano J, et al. Fulvestrant, formerly ICI 182, 780, is as effective as anastrozole in postmenopausal women with advanced breast cancer progressing after prior endocrine treatment. J Clin Oncol 2002;20(16):3396–403

[246] Osborne CK, Pippen J, Jones SE, et al.Adouble-blind, randomized trial comparing the efficacy and tolerability of fulvestrant versus anastrozole in postmenopausal women with advanced breast cancer progressing on prior endocrine therapy: results of a North American trial. J Clin Oncol 2002;20:3386–95

[247] Chia S, Gradishar W, Mauriac L, et al. Double-blind, randomized placebo controlled trial of fulvestrant compared with exemestane after prior nonsteroidal aromatase inhibitor therapy in postmenopausal women with hormone receptor-positive, advanced breast cancer: results from EFECT. J Clin Oncol 2008;26:1664–70

[248] Howell A, Robertson JF, Abram P, et al. Comparison of fulvestrant versus tamoxifen for the treatment of advanced breast cancer in postmenopausal women previously untreated with endocrine therapy: a multinational, double-blind, randomized trial. J Clin Oncol 2004;22:1605–13

[249] Dodwell DJ, Dyker K, Brown J, et al. A randomized study of whole-breast vs. tumour-bed irradiation after local excision and axillary dissection for early breast cancer. Clin Oncol 2005;17:618–22

[250] Polgar C, Fodor J, Major T, et al. Breast-conserving treatment with partial or whole breast irradiation for low-risk invasive breast carcinoma- 5-year results of a randomized trial. Int J Radiat Oncol Biol Phys 2007;69:694–702

[251] Polgar C, Sulyok Z, Fodor J, et al. Sole brachytherapy of the tumor bed after conservative surgery for T1 breast cancer: five year results of a phase I-II study and initial findings of a randomized phase III trial. J Surg Oncol 2002;80:121–8.

[252] Lovey K, Fodor J, Major T, et al. Fat necrosis after partialbreast irradiation with brachytherapy or electron irradiation versus standard whole-breast radiotherapy- 4-year results of a randomized trial. Int J Radiat Oncol Biol Phys 2007;69:724–31

[253] Ribeiro GG, Magee B, Swindell R, Harris M, Banerjee SS. The Christie Hospital Breast Conservation trial: an update at 8 years from inception. Clin Oncol 1993;5:278–83

[254] Ribeiro GG, Dunn G, Swindell R, Harris M, Banerjee SS. Conservation of the breast using two different radiotherapy techniques: interim report of a clinical trial. Clin Oncol 1990;2:27–34

[255] Miller K, Wang D, Gralow J, et al. Paclitaxel plus bevacizumab versus paclitacel alone for metastatic breast cancer. N Engl J Med 2007;357:2666–2676

[256] Miller KD, Chap LI, Holmes FA, et al. Randomized phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer. J Clin Oncol 2005;23:792–799

[257] Pivot X, Verma S, Thomssen C, et al. Clinical benefit of bevacizumab plus first-line docetaxel in elderly patients with locally recurrent or Metastatic breast cancer. J Clin Oncol 2009;27:15s (abstr 1094)

[258] Robert NJ, Dieras V, Glaspy J, et al. RIBBON-1: randomized, double-blind, placebo-controlled, phase III trial of chemotherapy with or without bevacizumab for first-line treatment of HER2-negative locally recurrent or metastatic breast cancer. J Clin Oncol 2009;27:15s (abstr 1005)

[259] Burstein HJ, Spigel D, Kindsvogel K, Parker LM, Bunnell CA, Partridge AH, Come SE, Ryan PD, Gelman R, Winer EP. Metronomic chemotherapy with and without bevacizumab for advanced breast cancer: a randomized phase II study. Breast Cancer Res Treat 2005;94(Suppl 1):S6

[260] Buzdar AU, Ibrahim NK, Francis D, et al. Significantly higher pathologic complete remission rate after neoadjuvant therapy with trastuzumab, paclitaxel, and epirubicin chemotherapy: results of a randomized trial in human epidermal growth factor receptor 2-positive operable breast cancer. J Clin Oncol 2005;23:3676-85

[261] Chang HR. Trastuzumab-based neoadjuvant therapy in patients with HER2-positive breast cancer. Cancer 2010;116:2856-67

[262] Gianni L, Eiermann W, Semiglazov V, et al. Neoadjuvant chemotherapy with trastuzumab followed by adjuvant trastuzumab versus neoadjuvant chemotherapy alone, in patients with HER2-positive locally advanced breast cancer (the NOAH trial): a randomised controlled superiority trial with a parallel HER2-negative cohort. Lancet 2010;375:377-84

[263] Pierga JY, Delaloge S, Espié M, et al. A multicenter randomized phase II study of sequential epirubicin/cyclophosphamide followed by docetaxel with or without celecoxib or trastuzumab according to HER2 status, as primary chemotherapy for localized invasive breast cancer patients. Breast Cancer Res Treat 2010;122:429-37

[264] Steger G, Greil R, Jakesz R, et al. Final results of ABCSG-24, a randomized phase III study comparing epirubicin, docetaxel, and capecitabine (EDC) to epirubicin and docetaxel (ED) as neoadjuvant treatment for early breast cancer and comparing ED/EDC þ trastuzumab (T) to ED/EDC as neoadjuvant treatment for early HER-2 positive breast cancer. Cancer Res 2009;69(24 Suppl.). Abstract nr 1081

[265] Chang HR, Slamon D, Prati R, et al. A phase II study of neoadjuvantdocetaxel/carboplatinwithorwithout trastuzumabinlocally advanced breast cancer: response and cardiotoxicity. J Clin Oncol 2006;24(18S):10515

[266] Chang HR, Slamon D, Gornbein JA, Chung D. Preferential pathologic complete response (pCR) by triple-negative (-) breast cancer to neoadjuvant docetaxel (T) and carboplatin (C). J Clin Oncol 2008;26 (May 20 Suppl.; abstr 604)

[267] Buzdar AU, Valero V, Ibrahim NK, et al. Neoadjuvant therapy with paclitaxel followed by 5-fluorouracil, epirubicin, and cyclophosphamide chemotherapy and concurrent trastuzumab in human epidermal growth factor receptor 2-positive operable breast cancer: an update of the initial randomized study population and data of additional patients treated with the same regimen. Clin Cancer Res 2007;13:228-33

[268] Coleman RE, Marshall H, Cameron D, et al; AZURE Investigators. Breast-cancer adjuvant therapy with zoledronic acid. N Engl J Med. 2011;365:1396-405

[269] Gnant M, Mlineritsch B, Stoeger H, et al; Austrian Breast and Colorectal Cancer Study Group, Vienna, Austria. Adjuvant endocrine therapy plus zoledronic acid in premenopausal women with early-stage breast cancer: 62-month follow-up from the ABCSG-12 randomised trial. Lancet Oncol. 2011;12:631-41

[270] Valachis A, Nearchou A, Polyzos NP, Mauri D, Lind P. Adjuvant therapy with Zoledronic acid in primary breast cancer: a systematic review and meta-analysis. Presented at ESMO 36-ECCO 16 (abstract nr 5157)

[271] Ha TC, Li H. Meta-analysis of clodronate and breast cancer survival. Br J Cancer. 2007;96:1796-801

[272] Stewart LA, Parmar MKB. Meta-analysis of the literature or of individual patient data: is there a difference? Lancet 1993;341:418–422

[273] Duchateau L, Pignon JP, Bijnens L, et al. Individual patient-versus literature-based meta-analysis of survival data: time to event and event rate at a particular time can make a difference, an example based on head and neck cancer. Controlled Clinical Trials 2001;22:538–547

[274] Cummings SR, Browner WS, Bauer D, et al. Endogenous hormones and the risk of hip and vertebral fractures among older women. Study of Osteoporotic Fractures Research Group. N Engl J Med 1998;339:733–8

[275] Chen Z, Maricic M, Bassford TL, et al. Fracture risk among breast cancer survivors. Results from the Women's Health Initiative Observational Study. Arch Intern Med 2005;165:552–8.

[276] Johnell O, Kanis JA, Oden A, et al. Predictive value of BMD for hip and other fractures. J Bone Miner Res 2005;20:1185–94

[277] Bruning PF, Pit MJ, de Jong-Bakker M, van den Ende A, Hart A, van Enk A. Bone mineral density after adjuvant chemotherapy for premenopausal breast cancer. Br J Cancer 1990;61:308–10.

[278] Parulekar WR, Day AG, Ottaway JA, et al. Incidence and prognostic impact of amenorrhea during adjuvant therapy in highrisk premenopausal breast cancer: analysis of a National Cancer Institute of Canada Clinical Trials Group Study—NCIC CTG MA.5. J Clin Oncol 2005;23:6002–8

[279] Bell R, Marshall H, Collinson M, et al. Reduction in fractures following adjuvant Zoledronic acid in stage II/III breast cancer - the AZURE trial (BIG 01/04). Presented at ESMO 36-ECCO 16 (abstract nr 5155)

[280] Coleman R, Woodward E, Brown J, et al. Safety of zoledronic acid and incidence of osteonecrosis of the jaw (ONJ) during adjuvant therapy in a randomised phase III trial (AZURE: BIG 01-04) for women with stage II/III breast cancer. Breast Cancer Res Treat. 2011;127:429-38

[281] Rosen LS, Gordon D, Kaminski M, et al. Zoledronic acid versus pamidronate in the treatment of skeletal metastases in patients with breast cancer or osteolytic lesions of multiple myeloma: a phase III, double-blind, comparative trial. Cancer J. 2001;7:377-387

[282] Xu B, Jiang Z, Shao Z, et al. Fulvestrant 250 mg versus anastrozole for Chinese patients with advanced breast cancer: results of a multicentre, double-blind, randomised phase III trial. Cancer Chemother Pharmacol 2011;67:223–230

[283] Addo S, Yates RA, Laight A. A phase I trial to assess the pharmacology of new oestrogen receptor antagonist fulvestrant on the endometrium in healthy postmenopausal volunteers. Br J Cancer 2002;87:1354–9

[284] Bergman L, Beelen ML, Gallee MP, et al. Risk and prognosis of endometrial cancer after tamoxifen for breast cancer. Comprehensive Cancer Centres' ALERT Group. Assessment of liver and endometrial cancer risk following tamoxifen. Lancet 2000;356:881–7

[285] Bergh J, Jönsson PE, Lidbrink E, et al. First results from FACT – An open-label, randomized phase III study investigating loading dose of fulvestrant combined with anastrozole versus anastrozole at first relapse in hormone receptor positive breast cancer. Presented at the 32nd Annual San Antonio Breast Cancer Symposium (December 9-13, 2009, San Antonio, TX),. abstr 23

[286] Robertson JF, Steger GG, Neven P, et al. Activity of fulvestrant in HER2-overexpressing advanced breast cancer. Ann Oncol. 2010;21:1246-53

[287] Robertson JF, Nicholson RI, Bundred NJ, et al: Comparison of the short-term biological effects of 7alpha-[9-(4,4,5,5,5-pentafluoropentylsulfinyl)-nonyl]estra-1,3,5, (10)-triene-3,17beta-diol (Faslodex) versus tamoxifen in postmenopausal women with primary breast cancer. Cancer Res 2001;61:6739-6746

[288] Di Leo A, Jerusalem G, Petruzelka L, et al. Results of the CONFIRM phase III trial comparing fulvestrant 250 mg with fulvestrant 500 mg in postmenopausal women with estrogen receptor-positive advanced breast cancer. J Clin Oncol. 2010;28:4594-600

[289] Robertson JF, Llombart-Cussac A, Rolski J, et al. Activity of fulvestrant 500 mg versus anastrozole 1 mg as first-line treatment for advanced breast cancer: results from the FIRST study. J Clin Oncol. 2009;27:4530-5

[290] US Food and Drug Administration [home page on the internet]. FASLODEX label and approval history: labelling revision document updated 09/09/2010. Document available from: http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/021344s007s012lbl.pdf

[291] Lau J, Ioannidis JP, Schmid CH. Summing up evidence: one answer is not always enough. Lancet 1998;351:123–7

[292] Athas WF, Adams-Cameron M, Hunt WC, Amir-Fazli A, Key CR. Travel distance to radiation therapy and receipt of radiotherapy following breast conserving surgery. J Natl Cancer Inst 2000;92:269–71

[293] Mikeljevic JS, Haward R, Johnston C, et al. Trends in postoperative radiotherapy delay and the effect on survival in breast cancer patients treated with conservation surgery. Br J Cancer. 2004;90:1343-8

[294] Livi L, Buonamici FB, Simontacchi G, et al. Accelerated partial breast irradiation with IMRT: new technical approach and interim analysis of acute toxicity in a phase III randomized clinical trial. Int J Radiat Oncol Biol Phys. 2010;77:509-15

[295] Smith TE, Lee D, Turner BC, Carter D, Haffty BG. True recurrence vs. new primary ipsilateral breast tumor relapse: an analysis of clinical and pathologic differences and their implications in natural history, prognoses, and therapeutic management. Int J Radiat Oncol Biol Phys. 2000;48:1281-9

[296] Holland R, Veling SH, Mravunac M, Hendriks JH. Histologic multifocality of Tis, T1-2 breast carcinomas. Implications for clinical trials of breast-conserving surgery. Cancer. 1985;56:979-90

[297] Offersen BV, Overgaard M, Kroman N, Overgaard J. Accelerated partial breast irradiation as part of breast conserving therapy of early breast carcinoma: a systematic review. Radiother Oncol. 2009;90:1-13

[298] Faverly DR, Hendriks JH, Holland R. Breast carcinomas of limited extent: frequency, radiologic-pathologic characteristics, and surgical margin requirements. Cancer 2001;91:647-59

[299] Schnall M. MR imaging evaluation of cancer extent: is there clinical relevance? Magn Reson Imaging Clin N Am. 2006;14:379-81

[300] Mannino M, Yarnold JR. Local relapse rates are falling after breast conserving surgery and systemic therapy for early breast cancer: can radiotherapy ever be safely withheld? Radiother Oncol. 2009;90:14-22

[301] Formenti SC, Truong MT, Goldberg JD, et al. Prone accelerated breast irradiation after breast-conserving surgery: preliminary clinical results and dose–volume histogram analysis. Int J Radiat Oncol Biol Phys 2004;60:493–504

[302] Vaidya JS, Joseph DJ, Tobias JS, et al. Targeted intraoperative radiotherapy versus whole breast radiotherapy for breast cancer (TARGIT-A trial): an international, prospective, randomised, non-inferiority phase 3 trial. Lancet. 2010;376:91-102

[303] Rothenberg BM, Aronson N, Ziegler KM, et al. Accelerated partial breast irradiation as sole radiotherapy after breast-conserving surgery for early stage breast cancer. Technol Eval Cent Asses Program 2007;22:1–34

[304] Daniele G, Marciano R, Tortora G. The role of bevacizumab in breast cancer. Eur J Cancer Suppl 2008;6:26–29

[305] Yoshiji H, Harris SR, Thorgeirsson UP. Vascular endothelial growth factor is essential for initial but not continued in vivo growth of human breast carcinoma cells. Cancer Res 1997;57:3924–3928

[306] Relf M, LeJeune S, Scott PA et al. Expression of the angiogenic factors vascular endothelial cell growth factor, acidic and basic fibroblast growth factor, tumor growth factor b-1, platelet-derived endothelial cell growth factor, placenta growth factor and pleiotrophin in human primary breast cancer and its relation to angiogenesis. Cancer Res 1997;57:963–969

[307] D'Agostino RB Sr. Changing end points in breast-cancer drug approval—the Avastin story. N Engl J Med. 2011;365:e2

[308] US Food and Drug Administration: FDA Briefing Document Oncology Drug Advisory Committee Meeting, July 20, 2010. http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/OncologicDrugsAdvisoryCommittee/UCM219224.pdf

[309] Kümler I, Nielsen DL. Trials of bevacizumab in breast cancer - a safety review. Expert Opin Drug Saf. 2011 Jun 11

[310] Dedes KJ, Matter-Walstra K, Schwenkglenks M, Pestalozzi BC, Fink D, Brauchli P, Szucs TD. Bevacizumab in combination with paclitaxel for HER-2 negative metastatic breast cancer: an economic evaluation. Eur J Cancer. 2009;45:1397-406

[311] Ioannidis JP, Trikalinos TA. The appropriateness of asymmetry tests for publication bias in meta-analyses: a large survey. CMAJ 2007;176:1091–1096

[312] Lazaridis G, Pentheroudakis G, Pavlidis N. Integrating trastuzumab in the neoadjuvant treatment of primary breast cancer: accumulating evidence of efficacy, synergy and safety. Crit Rev Oncol Hematol 2008;66:31-41

[313] Untch M, Rezai M, Loibl S, et al. Neoadjuvant treatment with trastuzumab in HER2-positive breast cancer: results from the GeparQuattro study. J Clin Oncol 2010;28:2024-31

[314] von Minckwitz G, Untch M, Nüesch E, et al. Impact of treatment characteristics on response of different breast cancer phenotypes: pooled analysis of the German neo-adjuvant chemotherapy trials. Breast Cancer Res Treat 2011;125:145-56

[315] Liao N, Zhang GC, Li XR, Yao M, Wang K, Zu J. Efficacy and safety assessment of neoadjuvant paclitaxel and trastuzumab in Chinese women with Her-2 positive operable breast cancer. Zhonghua Zhong Liu Za Zhi 2010;32:544-7

[316] Carlson RW, Allred DC, Anderson BO, et al. Breast cancer. Clinical practice guidelines in oncology. J Natl Compr Canc Netw 2009;7:122-92

[317] Tagliabue E, Agresti R, Carcangiu ML, et al. Role of HER2 in wound-induced breast carcinoma proliferation. Lancet 2003;362:527-33

[318] Russell SD, Blackwell KL, Lawrence J, et al. Independent adjudication of symptomatic heart failure with the use of doxorubicin and cyclophosphamide followed by trastuzumab adjuvant therapy: a combined review of cardiac data from the National Surgical Adjuvant breast and Bowel Project B-31 and the North Central Cancer Treatment Group N9831 clinical trials. J Clin Oncol 2010;28:3416-21

[319] Procter M, Suter TM, de Azambuja E, et al. Longer-term assessment of trastuzumab-related cardiac adverse events in the Herceptin Adjuvant (HERA) trial. J Clin Oncol 2010;28:3422-8

[320] Kaufmann M, Hortobagyi GN, Goldhirsch A, et al. Recommendations from an international expert panel on the use of neoadjuvant (primary) systemic treatment of operable breast cancer: an update. J Clin Oncol 2006;24:1940-9

[321] RAPID: Randomized Trial of Accelerated Partial Breast Irradiation. http://clinicaltrials.gov/ct2/show/NCT00282035?term=partial+breast+irradiation+randomized&rank=1 Accessed: 22 Januari 2012

[322] Standard or Hypofractionated Radiotherapy Versus Accelerated Partial Breast Irradiation (APBI)for Breast Cancer (SHARE).

http://clinicaltrials.gov/ct2/show/NCT01247233?term=partial+breast+irradiation+randomized&rank=8 Accessed: 22 Januari 2012