Risk assessment tools and adjuvant therapy for breast cancer

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Chapter 1

GENERAL INTRODUCTION

Iris Noordhoek

HISTORY OF ENDOCRINE THERAPY

The treatment of breast cancer has long since consisted of a combination of locoregional management and of systemic cytotoxic and endocrine management. Already in 1896, it was discovered that breast tumors could be sensitive to hormones and metastatic growth reduced by performing an oophorectomy.[1] However, only one third of patients derived clear benefit from this type of treatment.[2] The discovery of the estrogen and progesterone receptor (ER and PR) in 1958 allowed for better selection of patients that would benefit from hormone depletion.[3] When hormones bind to these receptors, a cascade of events is triggered, resulting in cell division and tumor growth.[4] Breast cancer cells that do not express hormone receptors receive growth signals via other pathways, and are not affected by the presence or absence of hormones.

In 1960, oral tamoxifen, a selective estrogen receptor modulator, was introduced as an alternative to surgical ovarian ablation.[5] Tamoxifen has an antagonistic action in breast tissue, preventing estrogen from binding to the receptor and blocking the signaling cascade.[6] Several trials in patients with ER positive breast cancer demonstrated that using tamoxifen decreased the risk of developing breast cancer recurrences. Later, a large meta-analysis by the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) established that using tamoxifen for five years significantly decreased breast cancer recurrence and mortality.[7]

During the early 2000's, another type of drug became available to inhibit the growth of hormone receptor positive tumors; the aromatase inhibitor (AI).[8] In postmenopausal women, the only source of estrogen comes from the conversion of androgen to estrogen through the enzyme aromatase.[9] When this conversion is inhibited, there is no estrogen available to bind to the hormone receptors, and the tumor will not receive the signal for cell division. Several trials were conducted to compare different types of endocrine therapy (ET) for postmenopausal patients. Another meta-analyses by the EBCTCG showed that five years of AI monotherapy yields similar results as two to three years of tamoxifen followed by two to three years of an AI. Five years of tamoxifen monotherapy was proven an inferior treatment.[10]

EPIDEMIOLOGY OF BREAST CANCER

Breast cancer is still the most common type of cancer amongst women, though the distribution over different age categories changed significantly.[11,12] The proportion of women that are postmenopausal at diagnosis has increased from 74% in 1989 to 80% in 2019, and patients aged 70 years or over currently represent a third of all breast cancer patients (**figure 1**).[11] Nowadays, the systemic treatment strategy for postmenopausal patients with breast cancer is determined by clinical, histopathological and genetic parameters, and hormone receptors are still the most important of these biomarkers. Approximately 85% of all postmenopausal patients have breast tumors with ER or PR expression, or both.[13,14] Guidelines recommend 5 years of AI treatment for these patients.[15]

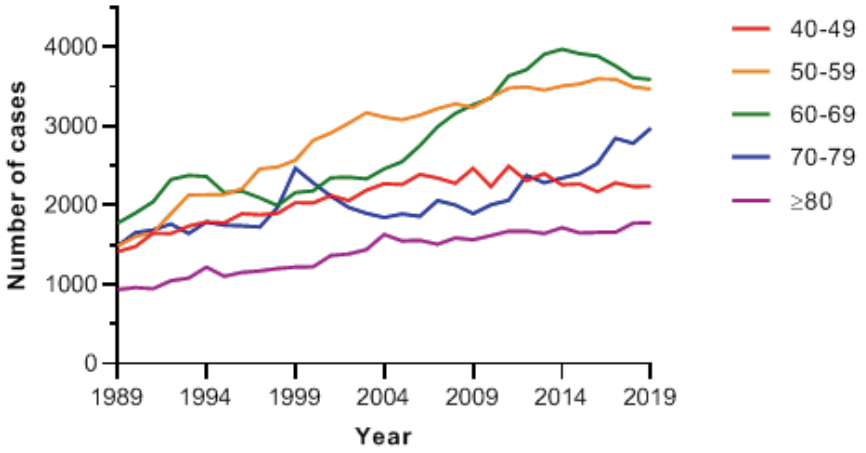

Figure 1: Incidence of invasive breast cancer in the Netherlands from 1989 to 2019 for five age categories. Data was obtained from the Dutch Cancer Registry (NKR).[11]

CHALLENGES IN THE TREATMENT OF BREAST CANCER

Much progress has been made in the past decades, and breast cancer survival has improved significantly. Nevertheless, several concerns in the treatment of hormone receptor positive breast cancer still remain.

Even after optimal locoregional management and adjuvant systemic therapy, breast cancer patients still have a lifetime risk of 20 to 41% of developing a recurrence, depending on tumor size and nodal status at diagnosis.[16] Apparently, endocrine therapy cannot prevent tumor recurrences in all patients with hormone receptor positive tumors and selection of patients that derive long term benefit from ET can still be improved.

Another concern is that hormone receptor positive breast cancer is characterized specifically by late recurrences. Two-thirds of recurrences happen after five years, i.e., after discontinuation of ET, and the risk of developing a recurrence continues to accumulate until 20 years after diagnosis.[16] Recent trials have demonstrated that extending ET beyond five years can reduce the risk of late distant recurrences (DR) and improve disease-free survival (DFS) in patients that have been treated with five years of tamoxifen monotherapy.[17,18] However, the effect of extending ET in patients treated with an AI are inconclusive, and as of yet, a clinically relevant benefit has not been demonstrated.[19-22] Combining these factors with the serious adverse events that accompany prolonged endocrine treatment, it would be irresponsible to treat all patients with extended ET. On the other hand, some patients do benefit from extended ET, and they must be identified to receive their optimal treatment.

Furthermore, the increasing age at which patients are diagnosed presents a problem. As mentioned, a third of all patients with breast cancer are aged 70 years or older at diagnosis, and in this growing population two specific age-related issues arise. As it takes time for a

recurrence to develop, and older patients have an increased probability to die of causes unrelated to their breast cancer, the risk of developing a recurrence is inversely correlated to age and the competing risk of other-cause mortality.[23] On top of that, older patients usually have more comorbidities and frailty than younger patients.[24] Therefore, they may experience more side effects and complications of cancer treatment and are at higher risk of hospitalization and long-term loss of quality of life.[25] Nevertheless, these age-specific factors are often not taken into account when determining the treatment strategy for older patients, which can lead to significant overtreatment of this population.[26,27]

PROGNOSTIC AND PREDICTIVE MODELS

Different types of models exist that can be used to help tailor adjuvant treatment to the individual patient. *Prognostic models* aim to identify patients that have an inherently worse disease prognosis and have a higher risk of developing recurrences. This risk assessment is then used to select patients for specific adjuvant treatment strategies. *Predictive models* are based on the idea that the patients with high risk of breast cancer recurrence are not necessarily the same patients who benefit from more extensive therapy, and aim to identify patients that derive the most benefit from therapy regardless of underlying prognosis.[28]

An obvious example of a predictive marker is the hormone receptor, as patients with ER negative breast cancer derive no benefit from ET at all. Breast tumors are deemed ER or PR positive when 1-10% or more of the cancer cells express these receptors. However, as has been established, some patients with ER positive breast cancer still have little benefit of ET. It is generally claimed that when tumors have a higher number of cells expressing ER or PR, they are more sensitive to and gain more effect from endocrine therapy (ET), though a consensus has not yet been reaches on the definition of high versus low levels of expression.[29] **Chapter 2** of this thesis investigates whether the quantitative assessment of hormone receptors is a better method to select patients for ET than the single cut-off value of 10%.

An example of a prognostic tool that is being used in clinical practice is the CTS5 (clinical treatment score post-5 years). The CTS5 aims to estimate the risk of late DR (i.e., after five years), provided that the patient was recurrence-free in the first five years after diagnosis. The CTS5 calculator generates a percentage that reflects the risk of developing a recurrence between five and ten years after diagnosis, and also categorizes patients as low, intermediate, or high risk. This risk estimation is based on readily available clinical and histopathological parameters.[30] **Chapter 3** of this thesis studies the utility and accuracy of the CTS5 to determine the risk of late DR and whether it can be used to predict benefit from extended ET.

A biomarker panel that was primarily developed as a predictive model is the Breast Cancer Index (BCI). BCI is a gene expression profile that examines the ratio between two genes, HOXB13 and IL17BR (H/I), that reflects activity of estrogen signaling pathways in breast tumors.[31] When this H/I ratio is high, estrogen signaling is upregulated, and the proliferation of the tumor is likely influenced by the availability of estrogens.[32] Previous

studies have established that BCI can identify patients that benefit of extended ET after five years of tamoxifen monotherapy. Whether BCI can also identify patients that benefit of extended ET after treatment with an AI, is described in **chapter 4** of this thesis.

Risk estimation in older patients is generally based on the same factors as in younger patients. Therefore, the age-related factors regarding recurrence risk and (adverse) effects of treatment, are often not taken into account when determining the treatment strategy.[26] Thus, instruments are needed that are validated specifically for the older population. The 70-gene signature test, or MammaPrint, is a genomic risk profile that is already established as an accurate prognostic model in younger breast cancer patients.[33] Previous studies showed that MammaPrint can be used to de-escalate the use of chemotherapy and ET in genomic low and ultralow risk patients, respectively. However, these trials did not include patients aged 70 years or older. In **chapter 5** of this thesis, the validity and accuracy of MammaPrint in older patients is examined.

ANOTHER APPROACH TO ADJUVANT THERAPY

Aside from determining which patients have most to gain from (extended) ET, other therapeutic agents might also assist in decreasing the risk of recurrences. Since hormone receptor positive breast cancer cells prefer osseous microenvironments, about 70% of breast cancer metastases are bone recurrences.[34,35] When metastatic cells infiltrate bone tissue, the equilibrium between osteoclasts and osteoblasts is disturbed.[36] The tumor cells stimulate the activity of osteoclasts, which increases bone resorption and the release of growth factors and cytokines. These instigate the proliferation and survival of tumor cells, creating a vicious cycle.[35]

Nitrogen-containing bisphosphonates also affect bone metabolism by inhibiting key enzymes of the intracellular mevalonate pathway. This decreases osteoclast-mediated bone resorption and osteoclast survival, causing an increase in bone density and a decreased release of cytokines and growth factors.[37] It is hypothesized that this makes bone a less attractive environment for metastatic breast cancer cells.[38]

Several trials have investigated the effect of (neo)adjuvant bisphosphonates on (breast) cancer recurrence. In 2015, a meta-analysis comparing patients treated with and without adjuvant bisphosphonates showed a reduction in breast cancer recurrence and mortality in the subgroup of women who were postmenopausal at the onset of treatment, but not in the premenopausal subgroup.[39] Thus far, the use of high-dose nitrogen-containing bisphosphonates has not been studied in exclusively postmenopausal patients.

The TEAM-IIB trial investigated the effect of daily oral ibandronate on the development of (bone) recurrences in postmenopausal patients with breast cancer, and its results are described in **chapter 6**.

Chapter 7 discusses and interprets the main results presented in this thesis and provides perspectives for the future.

REFERENCES

1. Beatson GT: On the Treatment of Inoperable Cases of Carcinoma of the Mamma: Suggestions for a New Method of Treatment, with Illustrative Cases. Lancet 2:104-107, 1896
2. Boyd S: On oophorectomy in cancer of the breast. Br Med J 2:1161-1167, 1900
3. Jensen EV, Jacobson HI: Fate of steroid estrogens in target tissues. Biological Activities of Steroids in Relation to Cancer:161-174, 1960
4. Russo J, Russo IH: The role of estrogen in the initiation of breast cancer. J Steroid Biochem Mol Biol 102:89-96, 2006
5. Quirke VM: Tamoxifen from Failed Contraceptive Pill to Best-Selling Breast Cancer Medicine: A Case-Study in Pharmaceutical Innovation. Front Pharmacol 8:620, 2017
6. Lewis JS, Jordan VC: Selective estrogen receptor modulators (SERMs): mechanisms of anticarcinogenesis and drug resistance. Mutat Res 591:247-63, 2005
7. EBCTCG: Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 365:1687-717, 2005
8. Buzdar A, Howell A: Advances in aromatase inhibition: clinical efficacy and tolerability in the treatment of breast cancer. Clin Cancer Res 7:2620-35, 2001
9. Cui J, Shen Y, Li R: Estrogen synthesis and signaling pathways during aging: from periphery to brain. Trends Mol Med 19:197-209, 2013
10. EBCTCG: Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. Lancet 386:1341-1352, 2015
11. Dutch Cancer Registry (NKR), IKNL. Obtained through https://www.iknl.nl/nkr-cijfers, accessed on April 4 2021.
12. Allemani C, Matsuda T, Di Carlo V, et al: Global surveillance of trends in cancer survival 2000-14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. Lancet 391:1023-1075, 2018
13. Osborne CK, Schiff R: Estrogen-receptor biology: continuing progress and therapeutic implications. J Clin Oncol 23:1616-22, 2005
14. Rosenberg PS, Barker KA, Anderson WF: Estrogen Receptor Status and the Future Burden of Invasive and In Situ Breast Cancers in the United States. J Natl Cancer Inst 107, 2015
15. Nationaal Borstkanker Overleg Nederland; https://www.oncoline.nl/borstkanker; accessed on April 8, 2021.
16. Pan H, Gray R, Braybrooke J, et al: 20-Year Risks of Breast-Cancer Recurrence after Stopping Endocrine Therapy at 5 Years. N Engl J Med 377:1836-1846, 2017
17. Davies C, Pan H, Godwin J, et al: Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. Lancet 381:805-16, 2013
18. Goss PE, Ingle JN, Pritchard KI, et al: Extending Aromatase-Inhibitor Adjuvant Therapy to 10 Years. N Engl J Med 375:209-19, 2016
19. Blok EJ, Kroep JR, Meershoek-Klein Kranenbarg E, et al: Optimal Duration of Extended Adjuvant Endocrine Therapy for Early Breast Cancer; Results of the IDEAL Trial (BOOG 2006-05). J Natl Cancer Inst 110, 2018
20. Gnant M, Steger G, Greil R, et al: Abstract GS3-01: A prospective randomized multi-center phase-III trial of additional 2 versus additional 5 years of anastrozole after initial 5 years of adjuvant endocrine therapy – results from 3,484 postmenopausal women in the ABCSG-16 trial. Cancer Res 78:GS3-01-GS3-01, 2018
21. Mamounas EP, Bandos H, Lembersky BC, et al: Use of letrozole after aromatase inhibitor-based therapy in postmenopausal breast cancer (NRG Oncology/NSABP B-42): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 20:88-99, 2019
22. Tjan-Heijnen VCG, van Hellemond IEG, Peer PGM, et al: Extended adjuvant aromatase inhibition after sequential endocrine therapy (DATA): a randomised, phase 3 trial. Lancet Oncol 18:1502-1511, 2017
23. de Boer AZ, van der Hulst HC, de Glas NA, et al: Impact of Older Age and Comorbidity on Locoregional and Distant Breast Cancer Recurrence: A Large Population-Based Study. Oncologist 25:e24-e30, 2020
24. Klepin H, Mohile S, Hurria A: Geriatric assessment in older patients with breast cancer. J Natl Compr Canc Netw 7:226-36, 2009
25. Lee SY, Seo JH: Current Strategies of Endocrine Therapy in Elderly Patients with Breast Cancer. Biomed Res Int 2018:808-820, 2018
26. Cardoso F, Kyriakides S, Ohno S, et al: Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 30:1194-1220, 2019
27. Derks MGM, Bastiaannet E, Kiderlen M, et al: Variation in treatment and survival of older patients with non-metastatic breast cancer in five European countries: a population-based cohort study from the EURECCA Breast Cancer Group. Br J Cancer 119:121-129, 2018
28. Sechidis K, Papangelou K, Metcalfe PD, et al: Distinguishing prognostic and predictive biomarkers: an information theoretic approach. Bioinformatics 34:3365-3376, 2018

29. Curigliano G, Burstein HJ, Winer EP, et al: De-escalating and escalating treatments for early-stage breast cancer: the St. Gallen International Expert Consensus Conference on the Primary Therapy of Early Breast Cancer 2017. Ann Oncol 28:1700-1712, 2017
30. Dowsett M, Sestak I, Regan MM, et al: Integration of Clinical Variables for the Prediction of Late Distant Recurrence in Patients With Estrogen Receptor-Positive Breast Cancer Treated With 5 Years of Endocrine Therapy: CTS5. J Clin Oncol 36:1941-1948, 2018
31. Sgroi DC, Sestak I, Cuzick J, et al: Prediction of late distant recurrence in patients with oestrogen-receptor-positive breast cancer: a prospective comparison of the breast-cancer index (BCI) assay, 21-gene recurrence score, and IHC4 in the TransATAC study population. Lancet Oncol 14:1067-1076, 2013
32. Sgroi DC, Carney E, Zarrella E, et al: Prediction of late disease recurrence and extended adjuvant letrozole benefit by the HOXB13/IL17BR biomarker. J Natl Cancer Inst 105:1036-42, 2013
33. Cardoso F, van't Veer LJ, Bogaerts J, et al: 70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer. N Engl J Med 375:717-29, 2016
34. Brockton NT, Gill SJ, Laborge SL, et al: The Breast Cancer to Bone (B2B) Metastases Research Program: a multi-disciplinary investigation of bone metastases from breast cancer. BMC Cancer 15:512, 2015
35. Coleman RE, Rubens RD: The clinical course of bone metastases from breast cancer. Br J Cancer 55:61-6, 1987
36. Coleman R, Gnant M: New results from the use of bisphosphonates in cancer patients. 3:213-218, 2009
37. Rogers MJ, Crockett JC, Coxon FP, et al: Biochemical and molecular mechanisms of action of bisphosphonates. Bone 49:34-41, 2011
38. van de Ven S, Kroep JR, Hamdy NA, et al: [Antitumour effects of bisphosphonates in breast cancer]. Ned Tijdschr Geneeskd 154:A1951, 2010
39. EBCTCG: Adjuvant bisphosphonate treatment in early breast cancer: meta-analyses of individual patient data from randomised trials. Lancet 386:1353-1361, 2015

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Chapter 2

HIGHER ER LOAD IS NOT ASSOCIATED WITH BETTER OUTCOME IN STAGE 1-3 BREAST CANCER: DESCRIPTIVE OVERVIEW OF QUANTITATIVE HR ANALYSIS IN OPERABLE BREAST CANCER

Iris Noordhoek, A. Florine de Groot, Danielle Cohen, Gerrit-Jan Liefers, Johanneke E.A. Portielje, Judith R. Kroep

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ABSTRACT

Purpose

In breast cancer, hormone receptor (HR) status is generally a qualitative measure; positive or negative. Quantitatively measured estrogen and progesterone receptors (ER and PR) are frequently proposed prognostic and predictive markers, some guidelines even provide different treatment options for patients with strong versus weak expression.

Aim

To evaluate quantitative HR load assessed by immunohistochemistry as a prognostic and predictive measure in stage 1-3 breast cancer.

Methods

We reviewed all the available literature on quantitatively measured HRs using immuno-histochemistry.

Results

All included studies (n = 19) comprised a cohort of 30,754 patients. Only 2 out of 17 studies found a clear correlation between higher quantitative ER and better disease outcome. Only one trial examined quantitative ER both as prognostic and predictive marker and found no association between ER% and survival. Ten studies examined quantitative PR load, only two of those found a significant correlation between higher PR load and better disease outcome. Two trials examined quantitative PR both as prognostic and predictive marker, neither found any association between PR% and disease outcome.

Conclusions

There is no clear evidence for using quantitatively assessed ER and PR as prognostic nor predictive marker in patients with stage 1-3 breast cancer. We recommend only using a qualitative HR status in future guidelines and treatment considerations.

INTRODUCTION

Breast cancer is the most common type of cancer amongst women worldwide and the leading cause of cancer specific death for women in Europe.[1] The estrogen receptor (ER) and progesterone receptor (PR) expression are the oldest biomarkers in breast cancer.[2,3]

Different methods exist for determining the expression of hormone receptors (HRs). The tissue can be analyzed using enzyme immunoassays (EIA), in which the amount of HRs is expressed in fmol/mg, defining HR positive as 15 fmol/mg or more.[4,5] More recently however, immunohistochemistry (IHC) has been the preferred method of staining hormone receptors. The number of cells expressing HRs is counted, generating a percentage of positive cells.[6] Different cut-off levels are used to determine whether a tumor is considered HR positive. Usually, a tumor is considered HR positive when more than 10% of the tumor cells express HRs.[7,8]

Furthermore, nuclei can be grouped into categories of negative, weak, moderate and strong nuclear staining to generate a continuous histoscore ranging from 0 to 300, calculated by multiplying the sum of the percentage of weakly stained cells times 1, moderately stained cells times 2, and strongly stained cells times 3.[9] Tumors with a histoscore of 50 or more are usually considered HR positive.

Additionally, the Allred scoring system has been used, which is a semi-quantitative measure that takes into consideration the proportion of positive cells (scored on a scale of 0-5) and staining intensity (scored on a scale of 0-3). The sum of these produces a score between 0 and 8, and tumors with a score of 3 or more are usually considered HR positive.[10]

Another semi-quantitative measure is the ER immunoreactive score (IRS), which also relies on the proportion and intensity. This produces a score between 0 and 12, considering tumors with a score of 2 or higher HR positive.[11]

Already more than 15 years ago, IHC was proposed as the reference method by different boards and peer committees and a dichotomous, qualitative scale of HR expression (i.e. “positive” or “negative”) was unanimously adopted.[12,13] This method remains the gold standard for HR expression evaluation.[14]

Although it is generally claimed that tumors with strong ER and/or PR expression are more sensitive to endocrine therapy (ET), there is no clear definition of weak or strong ER and PR expression. The most recent guidelines as proposed by the St. Gallen International Expert Consensus Conference on the Primary Therapy of Early Breast Cancer in 2017, briefly mention high ER expression as a characteristic of a low risk tumor and vice versa, but fail to provide any definition or cut-off value to determine which tumors are in fact high in ER expression.[14] As there is no consensus on the value of quantitative HR expression analysis, it is not (yet) common practice to report on HR load in the clinical setting.

This systematic review gives an overview of the methods to quantitatively assess HR load, the predictive and prognostic value of determining the HR load, gives recommendations

for clinical practice and discusses future developments for HR analysis and endocrine treatment.

METHODS

Data searches and study selection

In order to obtain all relevant literature, the electronic databases PubMed, Embase and Web of Science were searched in March 2018. This search was updated in August 2018 and in January 2019. The following key words were used for the data search: breast neoplasm, estrogen, progesterone, and hormone receptor, quantitative expression, and endocrine treatment.

According to PRISMA guidelines for systematic reviews, two of the authors (IN and AFG) individually and independently screened the articles for predefined inclusion criteria.[15] These were stated as follows:

- The article was published in English in a peer reviewed journal;
- The article was a primary report of original data;
- The study concerned women diagnosed with stage 1 to 3 adenocarcinoma of the breast;
- The tumor's ER and/or PR expression was analyzed using IHC (the international gold standard);
- ER and/or PR expression was reported quantitatively (continuous) or semi-quantitatively (minimum of 3 groups);
- Within the subset of HR positive cases, the (semi-)quantitative measure of ER and/or PR was analyzed in association to the primary clinical endpoint.

Only studies that the reviewers reached a consensus on were included. If needed, a third reviewer was consulted. Due to the retrospective nature of most included studies, it was elected not to perform a formal risk of bias assessment. Each study was awarded a level of evidence according to the Oxford Centre of Evidence Based Medicine.[16]

Data extraction

All data from the included studies were analyzed and data regarding the following items were extracted: number of participating patients, method to determine HR expression, method of HR expression quantification, type and timing (adjuvant *versus* neoadjuvant) of systemic treatment, primary clinical endpoint and follow-up time, and association primary clinical endpoint to quantified HR expression. Due to the heterogeneity of the included studies, data was not pooled, and no meta-analyses were performed.

RESULTS

Characteristics of the included studies

In total, 777 unique articles were identified. After matching these to the inclusion criteria, 19 articles were included. The most common ground to exclude studies was not reporting ER and/or PR expression quantitatively (n=273) (**figure 1**). Combined, all included studies comprised a cohort of 30,754 patients.

Quantitative assessment of HR expression

Of the 19 included studies, six studies performed HR staining on whole-section slides of the tumor tissue, whereas nine studies first created TMAs, where several cores are taken of the tissue blocks. HR staining is then performed on these cores instead of on whole-section slides. In four studies, it was not specified how the staining was performed.

In five studies, a continuous quantitative measure (percentage or histoscore) was used to determine HR load, in four studies patients were divided in groups of negative, low and high expression and in nine studies patients were divided in four or more groups according to HR expression. In one study both a continuous and a semi-quantitative measure was used.

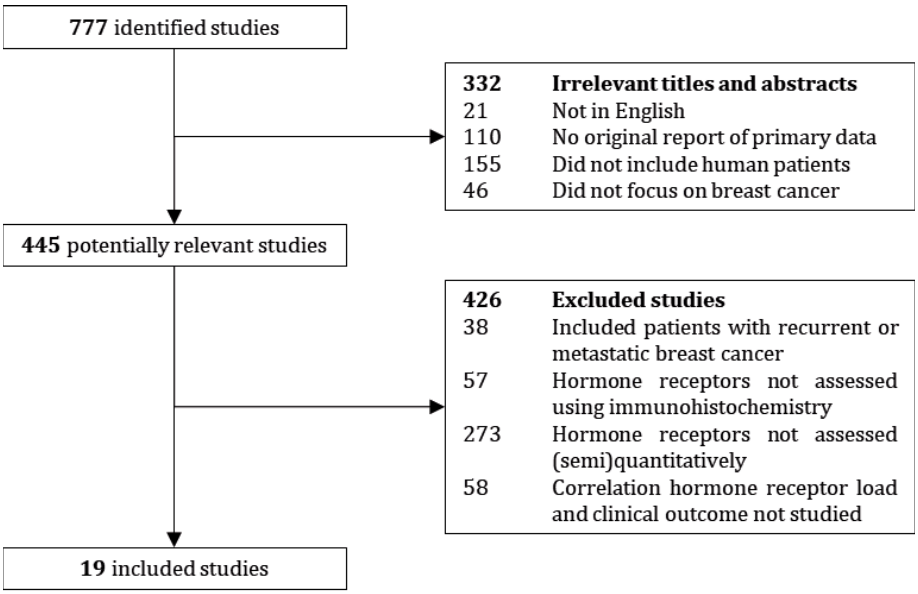

Figure 1: CONSORT diagram to account for excluded studies.

Reference	Study design	N	Pathologic methodology
Bartlett, 2011 [17]	Randomized trial	4325	Staining on TMA. Continuous histoscore (1-300).
Campbell, 2016 [18]	Cohort	503	Staining on TMA. Allred scoring system (negative *vs* low *vs* high).
Chae, 2011 [19]	Cohort	171	Staining on whole-section slides. Allred scoring system (negative *vs* low *vs* high).
Chapman, 2013 [20]	Randomized trial	345	Staining on TMA. Continuous score (0-100%).
Dowsett, 2008 [21]	Randomized trial	1856	Staining on TMA. Continuous histoscore (1-300).
Esslimani-Sahla, 2004 [22]	Case-control	50	Staining on whole-section slides. Continuous score (1-100%).
Harigopal, 2010 [23]	Randomized trial	1715	Staining on TMA. Continuous score (0-100%) and quartiles.
Hill, 2017 [24]	Case-control	1098	Staining on TMA. Visual score groups (1-59 *vs* 60-89 *vs* 90 *vs* 91-96 *vs* ≥97%).
Liu, 2010 [25]	Cohort	4046	Staining on TMA. Visual score groups (<1 *vs* 1-25 *vs* 26-75 *vs* ≥76%).
Ma, 2013 [26]	Case-control	1206	Staining on whole-section slides. Visual score groups (<1 *vs* 1-39 *vs* 40-59 *vs* 60-79 *vs* ≥80%).
Mazouni, 2010 [27]	Cohort	797	Staining method NS. Visual score groups (negative *vs* weak *vs* moderate *vs* high).
Morgan, 2011 [28]	Cohort	563	Staining on whole-section slides. Histoscore groups (1-50 *vs* 51-100 *vs* 101-200 *vs* ≥201).
Nordenskjöld, 2016 [29]	Randomized trial	449	Staining on TMA. Visual score groups (<1 *vs* 1-9 *vs* 10-24 *vs* 25-49 *vs* 50-74 *vs* 75-89 *vs* ≥90%).
Prabhu, 2014 [30]	Cohort	231	Staining on whole-section slides. Visual score groups (<1 *vs* 1-10 *vs* ≥11%).
Prat, 2013 [31]	Cohort	701	Staining method NS. Continuous histoscore (1-300).
Regierer, 2011 [11]	Cohort	3971	Staining method NS. IRS groups (negative *vs* weak *vs* moderate *vs* high).
Ryu, 2018 [32]	Cohort	4948	Staining method NS. Allred scoring system (negative *vs* low *vs* high).
Turbin, 2008 [33]	Cohort	3484	Staining on TMA. Visual score groups (<1 *vs* 1-24 *vs* 25-75 *vs* ≥76%).
Zhang, 2014 [34]	Cohort	295	Staining on whole-section slides. Visual score groups (<1 *vs* 1-10 *vs* 11-50 *vs* 51-70 *vs* ≥71%).

Table 1: Overview of methods used by the included articles.

Systemic treatment	Median FU, endpoint	ER load studied	PR load studied
ET: Tamoxifen followed by exemestane (n=2164) *vs* exemestane (n=2161). Chemotherapy: n=NS.	5 years Disease-free survival	Yes	Yes
ET: Tamoxifen (n=368) *vs* none (n=135). Chemotherapy: n=208.	5.7 years Disease-free survival	Yes	Yes
ET: Tamoxifen *vs* AI *vs* tamoxifen with GnRH-analogue *vs* tamoxifen followed by AI (n=NS). Chemotherapy: n=114.	4.3 years Disease-free survival	Yes	Yes
ET: Tamoxifen *vs* none (all n=NS). Chemotherapy: n=NS.	9.7 years Disease-free survival	Yes	Yes
ET: Tamoxifen (n=906) *vs* anastrozole (n=950). Chemotherapy: n=167.	5.7 years Disease-free survival	Yes	Yes
ET: Tamoxifen (n=50). Chemotherapy: n=0.	5 years Recurrence rate	Yes	Yes
ET: Tamoxifen *vs* none (all n=NS). Chemotherapy: n=1715.	7.2 years Disease-free survival	Yes	Yes
ET: n=NS. Chemotherapy: n=NS.	7.8 years Overall survival	Yes	No
ET: Tamoxifen (n=1,606) *vs* other (n=12) *vs* none (n=2,428). Chemotherapy: n=1,045.	10 years Breast cancer-specific survival	No	Yes
ET: n=NS. Chemotherapy: n=NS.	10 years Breast cancer-specific survival	Yes	No
ET: n=NS. Chemotherapy: n=NS.	6.3 years Overall survival	Yes	No
ET: Tamoxifen (n=563). Chemotherapy: n=0.	10 years Overall survival	Yes	No
ET: Tamoxifen (n=233) *vs* none (n=216). Chemotherapy: n=0.	18 years Recurrence rate	No	Yes
ET: Any (n=143) *vs* none (n=88). Chemotherapy: n=204.	2.4 years Disease-free survival	Yes	No
ET: Tamoxifen (n=701). Chemotherapy: n=0.	12.5 years Recurrence rate	Yes	Yes
ET: Any (n=2463) *vs* none (n=1508). Chemotherapy: n=1844.	5 years Recurrence-free survival	Yes	No
ET: Any (n=2463) *vs* none (n=1224). Data on ET missing: n=123. Chemotherapy: n=3646.	4.8 years Overall survival	Yes	No
ET: Tamoxifen (n=1385) *vs* none (n=2099). Chemotherapy: n=920.	12.5 years Breast cancer-specific survival	Yes	No
ET: Any (n=224) *vs* none (n=71). Chemotherapy: n=173.	5 years Overall survival	Yes	No

Table 1 continued

Systemic treatment of included patients

Of all included patients, 5,812 were treated with tamoxifen, 3,111 were treated with an aromatase inhibitor (AI), 2,164 were treated with a combination of tamoxifen and an AI, 6,614 were treated with unspecified ET and 7,769 patients did not receive any ET. For 5,284 patients, it was not specified whether they received ET or not (**figure 2**). Additionally, 10,036 patients were treated with chemotherapy. For 7,788 patients, it was not specified whether they received chemotherapy or not, 12,930 patients did not receive chemotherapy. Treatment with anti-HER2 medication was explicitly stated for only three patients.[30] **Table 1** provides detailed information on all included studies.

Overall association ER load and clinical outcome

In 17 of the 19 included studies, the ER load was analyzed, in a total of 26,259 patients with stage 1-3 breast cancer patients (**table 2**).[11,17-24,26-28,30-34] In 11 studies, HR negative patients were also included, all reported associations between the ER load and the primary outcome measure regard the subset of HR positive cases only. Disease-free survival (DFS) was used as primary outcome measure in seven studies, overall survival (OS) was used in five studies, recurrence in three studies and breast cancer specific mortality in two studies.

When studying ER load as a continuous measure (either using percentage or histoscore, n=6), a higher ER load was found statistically significantly associated with better clinical outcome in two studies, marginally significantly associated in one study, and three studies did not find a significant association between higher ER load and better clinical outcome.

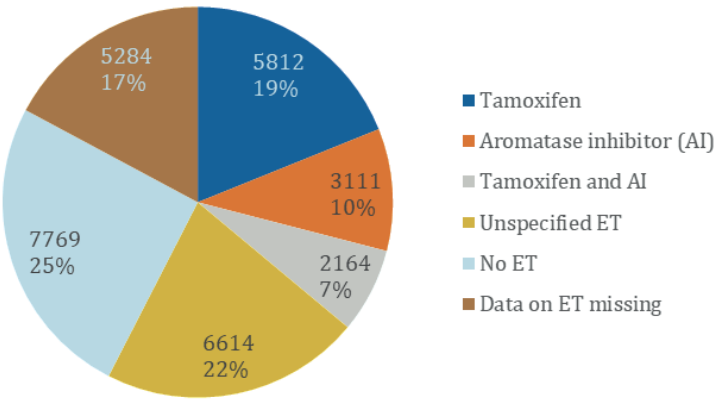

Figure 2: Distribution of types of endocrine therapy (ET) over patients.

When dividing patients in three groups, i.e. ER-negative, low ER and high ER expression (n=4), three studies did not find a significantly longer DFS for patients in the high ER expression group than patients in the low ER expression group, one study only found a marginally significant association between longer OS and higher ER expression.

When dividing patients in four or more groups based on ER expression (n=8), seven studies did not find a significantly better clinical outcome for patients with a higher ER expression and one study only found a marginally significant association between better clinical outcome and higher ER expression. These results are summarized in **table 2**.

Using ER load as a prognostic and/or predictive marker

There was only one randomized trial that compared patients with and without ET, which could be used to analyze the prognostic and predictive properties of the quantitative ER load without the risk of bias due to treatment indication. This study by Chapman et. al. (n=345) used a continuous quantitative measure, stained on TMAs and found no significant correlation between higher ER percentages and longer DFS in the overall study population (p=0.24).[20] They did not find any association between higher ER load and longer DFS in the subgroup that was randomized to receive no adjuvant ET and thus conclude that the quantitative ER load cannot be used as a prognostic marker. They also did not find any association between higher ER load and longer DFS in the subgroup of patients that was treated with adjuvant ET and therefore conclude that quantitative ER load is not an adequate predictive marker for sensitivity to ET, either.

Reference	Level of Evidence [16] Study design	N	Endpoint	Median FU (years)	Significant association
Bartlett, 2011 [17]	2b, randomized trial	4325	DFS	5	Yes
Esslimani-Sahla, 2004 [22]	3b, case-control	50	Recurrence	5	Yes
Dowsett, 2008 [21]	1b, randomized trial	1856	DFS	5.7	Marginally
Ma, 2013 [26]	3b, case-control	1206	BCSS	10	Marginally
Ryu, 2018 [32]	3b, cohort	4948	OS	4.8	Marginally
Campbell, 2016 [18]	2b, cohort	503	DFS	5.7	No
Chae, 2011 [19]	2c, cohort	171	DFS	4.3	No
Chapman, 2013 [20]	1b, randomized trial	345	DFS	9.7	No
Harigopal, 2010 [23]	2b, randomized trial	1715	DFS	7.2	No
Hill, 2017 [24]	3b, case-control	1098	OS	7.8	No
Mazouni, 2010 [27]	1b, cohort	797	OS	6.3	No
Morgan, 2011 [28]	3b, cohort	563	OS	10	No
Prahbu, 2014 [30]	2b, cohort	231	DFS	2.4	No
Prat, 2013 [31]	4, cohort	701	DRFS	12.5	No
Regierer, 2011 [11]	2b, cohort	3971	RFS	5	No
Turbin, 2008 [33]	2b, cohort	3484	BCSS	12.5	No
Zhang, 2014 [34]	3b, cohort	295	OS	5	No

Table 2: Overview of results of the included articles studying estrogen receptor (ER) load in 26,259 patients. In case of statistically significant associations, a higher ER load is associated with better clinical outcome.

Overall association PR load and clinical outcome

Of the 19 included studies, ten studies analyzed PR load in 14,161 early breast cancer patients (**table 3**).[17-23,25,29,31] In six studies, HR negative patients were also included, all reported associations between the PR load and the primary outcome measure regard the subset of HR positive cases only. DFS was used as primary outcome measure in six studies, three studies used recurrence as primary outcome and one study used breast cancer specific mortality.

When studying PR load as a continuous measure (n=6), a higher PR load was found to be significantly associated with better clinical outcome in two studies, a higher PR load was found marginally significantly associated with better clinical outcome in one study, and three studies did not find any association between PR load and clinical outcome.

When dividing patients in PR-negative, low PR and high PR expression groups (n=2), DFS was not significantly longer in the high PR expression group than in the low PR expression group. When dividing patients in four or more groups based on their PR expression (n=3), clinical outcome was not significantly better for a higher PR load. These results are summarized in **table 3**.

Using PR load as a prognostic and/or predictive marker

There were two randomized trials comparing patients with and without ET, which could be used to analyze the prognostic and predictive properties of the quantitative PR load without risk of bias.[20,29] Both studies randomized patients between tamoxifen or no adjuvant ET and used TMAs to stain the PR.

The study by Chapman et. al. (n=345) used a continuous quantitative measure and found no association between continuous higher PR percentage and longer DFS in the overall randomized study population (p=0.04; uncorrected for multiple testing). They did not find any association between higher PR load and longer DFS in the subgroup that received no adjuvant ET. They also did not find any association between higher PR load and longer DFS in the subgroup of patients that was treated with adjuvant ET.[20]

The study by Nordenskjöld et. al. (n=449) divided patients in seven groups based on the number of positive PR staining cells and did not find an association between the PR percentage groups and the occurrence of disease recurrences in the overall study population. They found no association between higher PR load and less disease recurrences within the subgroup of patients that did and did not receive ET, either.[29]

Thus, both studies concluded that quantitative PR load is not an adequate tool to determine the prognosis of early breast cancer patients, nor to predict sensitivity to ET.

Reference	Level of Evidence [16] Study design	N	Endpoint	Median FU (years)	Significant association
Bartlett, 2011 [17]	2b, randomized trial	4325	DFS	5	Yes
Dowsett, 2008 [21]	1b, randomized trial	1856	DFS	5.7	Yes
Prat, 2013 [31]	4, cohort	701	DRFS	12.5	Marginally
Campbell, 2016 [18]	2b, cohort	503	DFS	5.7	No
Chae, 2011 [19]	2c, cohort	171	DFS	4.3	No
Chapman, 2013 [20]	1b, randomized trial	345	DFS	9.7	No
Esslimani-Sahla, 2004 [22]	3b, case-control	50	Recurrence	5	No
Harigopal, 2010 [23]	2b, randomized trial	1715	DFS	7.2	No
Liu, 2010 [25]	2b, cohort	4046	BCSS	10	No
Nordenskjöld, 2016 [29]	2b, randomized trial	449	Recurrence	18	No

Table 3: Overview of results of the included articles studying progesterone receptor (PR) load in 14,161 patients. In case of statistically significant associations, a higher ER load is associated with better clinical outcome.

Interaction between ER and PR

Of the eight studies that examined both the ER and PR load, only two studied the interaction between ER and PR load. The study by Campbell et. al. found a statistically significant interaction between the quantitative ER and PR load, and only found a significant association between higher PR load and better outcome in those patients that also had a higher ER load.[18] The study by Harigopal et. al. found a moderate interaction between continuous quantitative ER and PR percentage (Pearson $r = 0.43$, $p<0.001$).[23]

This suggests that the quantitative PR load is not independently associated with outcome, but only in relation to the quantitative ER load.

DISCUSSION

Many efforts have been made to identify biomarkers or profiles in breast cancer patients capable of predicting sensitivity to endocrine treatment and the risk of recurrence after treatment is discontinued.[35,36] One of these methods is the quantitative assessment of ER and PR expression, i.e. the ER and PR load, instead of merely assigning tumors an ER and PR positive or negative status.[37]

This review concludes that in patients with an ER-positive tumor (defined as ER >10%), a higher ER load as assessed by IHC is not correlated to better outcome, and no evidence could be found for using quantitative ER load as a prognostic marker. In other words, patients with a higher ER load (e.g. 100%) do not inherently have a better prognosis than patients with a lower ER load (e.g. 20%). Furthermore, no evidence could be found for using quantitative ER load as a predictive marker, i.e. patients with a higher ER load do not have more benefit of ET than patients with a lower ER load.

This review also concludes that in patients with an HR-positive tumor, higher PR load does not seem to be correlated to better outcome. Based on the included studies, quantitative PR load is not a suitable prognostic marker; patients with a higher PR load do not inherently have a better prognosis than patients with a lower PR load, nor is it suitable as a predictive marker. Furthermore, PR load seems to be interacted with ER load and is therefore not recognized as an independent predictor.

One of the included studies, by Esslimani-Sahla, found an unusually high number of recurrences and only found an association between recurrence and ER load when examining ERβ, not when examining ERα.[22] As this is the only study that specifically examines ERβ, it is somewhat of an outlier, and its results should be interpreted with caution.

In this analysis, only studies that examined the HR expression using IHC were included. This method is the gold standard for determining HR status and other methods, such as EIA or mRNA expression profiles are not routinely used in clinical practice. Specifically, we have not focused on articles studying EIA to determine HR status, as this method is outdated and is not routinely used in the current clinical practice. This also ensures that the included studies create a homogenous cohort. Even still, different methods were used

to stain the HRs, such as staining on whole-section slides or on TMAs, though this did not seem to influence the outcome. Studies staining on TMAs were not less likely to find a correlation between HR load and outcome than studies staining on whole-section slides.

Studies also differed in their way of quantitatively measuring HR load; some studies used a continuous percentage or histoscore, some studies used groups of HR-negative, low HR expression and high HR expression and some divided patients in four or more groups based on Allred score or percentage. This does have an influence on the outcome. Studies were more likely to find a positive association between HR load and outcome if a continuous score was used. However, using a continuous quantitative measure to assess HR expression is questioned by several articles. Interobserver variability is high, and samples get assigned different HR percentages depending on the pathologist and the lab it was reviewed in.[38] Most importantly, staining breast cancer tissue using IHC does not allow for precise enough measurement of HR load to generate a continuous score and can only quantify into negative, weak positive and strong positive.[7,39,40] The problem with this approach is defining "weak" and "strong". A lack of generally accepted definition results in pathologists and papers choosing their own definition, making it difficult to compare multiple studies. Furthermore, and as mentioned previously, the St. Gallen Consensus makes a distinction between high and low ER expression but fails to provide any definition or cut-off value to determine which tumors are in fact high in ER expression.[14] The St. Gallen Consensus does not mention high and low PR expression at all.

Based on the results of this review, we propose using both ER and PR expression only as a qualitative measure; defining tumors with less than 1-10% of cells expressing this receptor as negative, and tumors with more than 1-10% of cells expressing the receptor as positive.[30,41] Using a continuous quantitative measure does not seem feasible without centralized, unambiguous and clear pathological measurement. The implications for the daily clinical practice of pathologists are that more detailed information on the HR status beyond "positive" or "negative" should no longer be provided, to prevent oncologists subconsciously or instinctively making different treatment decisions based on this information. Since there is no evidence for different treatment strategies, providing extra information is both unnecessary and undesirable.

Simultaneously, one can speculate whether there is any added value of measuring the PR status at all. It is generally accepted that there is no such thing as an ER negative/PR positive tumor.[42,43] Since the quantitative PR load is correlated to the quantitative ER load and PR load is inversely correlated to the histological grade of the tumor, the question arises whether PR status provides any additional prognostic information, when ER status, grade and potentially a proliferation factor such as ki-67 is known.[18,23] Likewise, when examining guidelines on adjuvant treatment, they do not propose different treatment strategies for tumors that are ER positive/PR positive compared to tumors that are ER positive/PR negative.[8,14] Therefore, if the PR status is unlikely to change the course of treatment, it could be considered wasteful and excessive to continue measuring it.[44,45] It might be worthwhile to focus future research on the independent contribution of PR status using multivariable models.

Gene expression profiling can be used to identify two inherently different entities within breast cancer, known as luminal-A and luminal-B. These are intrinsic molecular subtypes that reflect a different tumor biology and disease prognosis. Unfortunately, neither subtype is predictive for a better response to ET.[31,46-50] Moreover, the gene expression profiles used to differentiate between these subtypes are expensive and not universally available and the added value for daily clinical practice, in particular for which subgroup of patients, is still debated.[35] For these reasons, researchers have tried to approach the distinction between these molecular subtypes using IHC, which resulted in subtypes called luminal-A-like and luminal-B-like.[31] When defining IHC-based luminal-A-like tumors as HR positive, HER2 negative and ki-67 below 14%, approximately 81% to 85% of luminal-A tumors were correctly identified as luminal-A-like. However, approximately 35% to 52% of luminal-B tumors were incorrectly identified as luminal-A-like. When expanding the definition of luminal-A-like to HR positive, HER2 negative, ki-67 below 14% and PR above 20%, the specificity improves somewhat but not enough to accurately discriminate between the two subtypes.[31,48,50]

With these considerations, and the lack of prognostic and predictive value of IHC assessed quantitative ER and PR load as shown in this review, the distinction between IHC-based luminal-A-like and luminal-B-like tumors should not be used to tailor treatment decisions for women with HR positive stage 1-3 breast cancer.

Future perspectives

All in all, identifying the early breast cancer patients that could benefit most from ET remains a challenge, as more than half of all patients with an ER positive breast cancer will not respond to ET.[51] Considering the frequent and often severe side effects of ET, an improved upfront selection of likely responders may lower the treatment burden. Since quantitative measurement of HR does not seem an appropriate instrument for identifying these patients, the oncologic community is searching for much needed other means to predict response to ET. One potential method to identify patients is to measure the activity of the ER-pathway to distinguish in which patients the estrogen receptor is not only expressed, but also active and thus a suitable target for ET.[52] The use of predictive biomarkers in the neoadjuvant ET setting will be clearer after results of ongoing trials become available. Potentially, response to neoadjuvant therapy can be measured at a per patient level using postoperative pathology, bypassing the need for predictive markers altogether.

CONCLUSION

There is no clear evidence for using quantitative ER and PR load assessed by immunohistochemistry as a prognostic measure nor as a predictive marker for response to ET in patients with stage 1-3 breast cancer. Immunohistochemistry is the gold standard for measuring HR status but should only be used to distinguish HR negative and HR positive tumors. Gene expression profiles have prognostic value for women with ER positive disease, early response evaluation to neoadjuvant therapy holds promise in the prediction of long-term response to endocrine therapy.

REFERENCES

1. Allemani C, Matsuda T, Di Carlo V, et al: Global surveillance of trends in cancer survival 2000-14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. Lancet 391:1023-1075, 2018
2. Dekker TJ, Smit VT, Hooijer GK, et al: Reliability of core needle biopsy for determining ER and HER2 status in breast cancer. Ann Oncol 24:931-7, 2013
3. Dekker TJ, ter Borg S, Hooijer GK, et al: Quality assessment of estrogen receptor and progesterone receptor testing in breast cancer using a tissue microarray-based approach. Breast Cancer Res Treat 152:247-52, 2015
4. Heuson JC, Longeval E, Mattheiem WH, et al: Significance of quantitative assessment of estrogen receptors for endocrine therapy in advanced breast cancer. Cancer 39:1971-1977, 1977
5. McGuire WL: Steroid hormone receptors in breast cancer treatment. Recent Prog Horm Res 36:135-56, 1980
6. Khoshnoud MR, Löfdahl B, Fohlin H, et al: Immunohistochemistry compared to cytosol assays for determination of estrogen receptor and prediction of the long-term effect of adjuvant tamoxifen. Breast Cancer Res Treat 126:421-30, 2011
7. Hammond ME, Hayes DF, Wolff AC, et al: American society of clinical oncology/college of american pathologists guideline recommen-dations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J Oncol Pract 6:195-7, 2010
8. NBCA: Richtlijn mammacarcinoom: BEPALING VAN HORMOONRECEPTOREN EN HER2. Oncoline, 2017
9. Andersen J, Orntoft TF, Poulsen HS: Immunohistochemical demonstration of estrogen receptors (ER) in formalin-fixed, paraffin-embedded human breast cancer tissue by use of a monoclonal antibody to ER. J Histochem Cytochem 36:1553-60, 1988
10. Qureshi A, Pervez S: Allred scoring for ER reporting and it's impact in clearly distinguishing ER negative from ER positive breast cancers. J Pak Med Assoc 60:350-353, 2010
11. Regierer AC, Wolters R, Kurzeder C, et al: High estrogen receptor expression in early breast cancer: chemotherapy needed to improve RFS? Breast Cancer Res Treat 128:273-81, 2011
12. Bast RC, Jr., Ravdin P, Hayes DF, et al: 2000 update of recommendations for the use of tumor markers in breast and colorectal cancer: clinical practice guidelines of the American Society of Clinical Oncology. J Clin Oncol 19:1865-78, 2001
13. Goldhirsch A, Wood WC, Gelber RD, et al: Meeting highlights: updated international expert consensus on the primary therapy of early breast cancer. J Clin Oncol 21:3357-65, 2003
14. Curigliano G, Burstein HJ, Winer EP, et al: De-escalating and escalating treatments for early-stage breast cancer: the St. Gallen International Expert Consensus Conference on the Primary Therapy of Early Breast Cancer 2017. Ann Oncol 28:1700-1712, 2017
15. Moher D, Liberati A, Tetzlaff J, et al: Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLOS Med 6:e1000097, 2009
16. Oxford Centre for Evidence-based Medicine - Levels of Evidence. 2009
17. Bartlett JMS, Brookes CL, Robson T, et al: Estrogen receptor and progesterone receptor as predictive biomarkers of response to endocrine therapy: a prospectively powered pathology study in the Tamoxifen and Exemestane Adjuvant Multinational trial. J Clin Oncol 29:1531-1538, 2011
18. Campbell EJ, Tesson M, Doogan F, et al: The combined endocrine receptor in breast cancer, a novel approach to traditional hormone receptor interpretation and a better discriminator of outcome than ER and PR alone. Br J Cancer 115:967-973, 2016
19. Chae BJ, Bae JS, Yim HW, et al: Measurement of ER and PR status in breast cancer using the QuantiGene2.0 assay. Pathology 43:248-253, 2011
20. Chapman JAW, Nielsen TO, Ellis MJ, et al: Effect of continuous statistically standardized measures of estrogen and progesterone receptors on disease-free survival in NCIC CTG MA.12 Trial and BC Cohort. Breast Cancer Res 15:R71, 2013
21. Dowsett M, Allred C, Knox J, et al: Relationship between quantitative estrogen and progesterone receptor expression and human epidermal growth factor receptor 2 (HER-2) status with recurrence in the Arimidex, Tamoxifen, Alone or in Combination trial. J Clin Oncol 26:1059-65, 2008
22. Esslimani-Sahla M, Simony-Lafontaine J, Kramar A, et al: Estrogen receptor beta (ER beta) level but not its ER beta cx variant helps to predict tamoxifen resistance in breast cancer. Clin Cancer Res 10:5769-5776, 2004
23. Harigopal M, Barlow WE, Tedeschi G, et al: Multiplexed assessment of the Southwest Oncology Group-directed Intergroup Breast Cancer Trial S9313 by AQUA shows that both high and low levels of HER2 are associated with poor outcome. Am J Pathol 176:1639-1647, 2010
24. Hill DA, Barry M, Wiggins C, et al: Estrogen receptor quantitative measures and breast cancer survival. Breast Cancer Res Treat 166:855-864, 2017
25. Liu S, Chia SK, Mehl E, et al: Progesterone receptor is a significant factor associated with clinical outcomes and effect of adjuvant tamoxifen therapy in breast cancer patients. Breast Cancer Res Treat 119:53-61, 2010

26. Ma H, Lu Y, Marchbanks PA, et al: Quantitative measures of estrogen receptor expression in relation to breast cancer-specific mortality risk among white women and black women. Breast Cancer Res 15:R90, 2013
27. Mazouni C, Bonnier P, Goubar A, et al: Is quantitative oestrogen receptor expression useful in the evaluation of the clinical prognosis? Analysis of a homogeneous series of 797 patients with prospective determination of the ER status using simultaneous EIA and IHC. Eur J Cancer 46:2716-25, 2010
28. Morgan DA, Refalo NA, Cheung KL: Strength of ER-positivity in relation to survival in ER-positive breast cancer treated by adjuvant tamoxifen as sole systemic therapy. Breast 20:215-9, 2011
29. Nordenskjöld A, Fohlin H, Fornander T, et al: Progesterone receptor positivity is a predictor of long-term benefit from adjuvant tamoxifen treatment of estrogen receptor positive breast cancer. Breast Cancer Res Treat 160:313-322, 2016
30. Prabhu JS, Korlimarla A, Desai K, et al: A Majority of Low (1-10%) ER Positive Breast Cancers Behave Like Hormone Receptor Negative Tumors. J Cancer 5:156-65, 2014
31. Prat A, Cheang MC, Martín M, et al: Prognostic significance of progesterone receptor-positive tumor cells within immunohistochemically defined luminal A breast cancer. J Clin Oncol 31:203-9, 2013
32. Ryu JM, Choi HJ, Kim I, et al: Only estrogen receptor "positive" is not enough to predict the prognosis of breast cancer. Breast Cancer Res Treat 172:627-636, 2018
33. Turbin DA, Leung S, Cheang MCU, et al: Automated quantitative analysis of estrogen receptor expression in breast carcinoma does not differ from expert pathologist scoring: a tissue microarray study of 3,484 cases. Breast Cancer Res Treat 110:417-426, 2008
34. Zhang Z, Wang J, Skinner KA, et al: Pathological features and clinical outcomes of breast cancer according to levels of oestrogen receptor expression. Histopathology 65:508-516, 2014
35. Blok EJ, Bastiaannet E, van den Hout WB, et al: Systematic review of the clinical and economic value of gene expression profiles for invasive early breast cancer available in Europe. Cancer Treat Rev 62:74-90, 2018
36. Viale G, Ghioni M, Mastropasqua MG: Traditional molecular markers and response to adjuvant endocrine or trastuzumab-based therapies. Curr Opin Oncol 22:541-6, 2010
37. van de Water W, Fontein DB, van Nes JG, et al: Influence of semi-quantitative oestrogen receptor expression on adjuvant endocrine therapy efficacy in ductal and lobular breast cancer - a TEAM study analysis. Eur J Cancer 49:297-304, 2013
38. Reisenbichler ES, Lester SC, Richardson AL, et al: Interobserver concordance in implementing the 2010 ASCO/CAP recommendations for reporting ER in breast carcinomas: a demonstration of the difficulties of consistently reporting low levels of ER expression by manual quantification. Am J Clin Pathol 140:487-94, 2013
39. Nadji M: Quantitative immunohistochemistry of estrogen receptor in breast cancer: "much ado about nothing!". Appl Immunohistochem Mol Morphol 16:105-107, 2008
40. Schnitt SJ: Estrogen Receptor Testing of Breast Cancer in Current Clinical Practice: What's the Question? J Clin Oncol 24:1797-1799, 2006
41. Deyarmin B, Kane JL, Valente AL, et al: Effect of ASCO/CAP guidelines for determining ER status on molecular subtype. Ann Surg Oncol 20:87-93, 2013
42. Foley NM, Coll JM, Lowery AJ, et al: Re-Appraisal of Estrogen Receptor Negative/ Progesterone Receptor Positive (ER-/PR+) Breast Cancer Phenotype: True Subtype or Technical Artefact? Pathol Oncol Res 24:881-884, 2018
43. Kunc M, Biernat W, Senkus-Konefka E: Estrogen receptor-negative progesterone receptor-positive breast cancer: Nobody's land or just an artifact? Cancer Treat Rev 67:78-87, 2018
44. EBCTCG: Tamoxifen for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group. Lancet 351:1451-67, 1998
45. Olivotto IA, Truong PT, Speers CH, et al: Time to stop progesterone receptor testing in breast cancer management. J Clin Oncol 22:1769-70, 2004
46. Ellis MJ, Suman VJ, Hoog J, et al: Randomized phase II neoadjuvant comparison between letrozole, anastrozole, and exemestane for postmenopausal women with estrogen receptor-rich stage 2 to 3 breast cancer: clinical and biomarker outcomes and predictive value of the baseline PAM50-based intrinsic subtype--ACOSOG Z1031. J Clin Oncol 29:2342-9, 2011
47. Nielsen TO, Parker JS, Leung S, et al: A Comparison of PAM50 Intrinsic Subtyping with Immunohistochemistry and Clinical Prognostic Factors in Tamoxifen-Treated Estrogen Receptor–Positive Breast Cancer. Clin Cancer Res 16:5222-5232, 2010
48. Parker JS, Mullins M, Cheang MC, et al: Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol 27:1160-7, 2009
49. Prat A, Ellis MJ, Perou CM: Practical implications of gene-expression-based assays for breast oncologists. Nat Rev Clin Oncol 9:48-57, 2011
50. Viale G, de Snoo FA, Slaets L, et al: Immunohistochemical versus molecular (BluePrint and MammaPrint) subtyping of breast carcinoma. Outcome results from the EORTC 10041/BIG 3-04 MINDACT trial. Breast Cancer Res Treat 167:123-131, 2018
51. EBCTCG: Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. Lancet 386:1341-1352, 2015

52. Verhaegh W, van Ooijen H, Inda MA, et al: Selection of personalized patient therapy through the use of knowledge-based computational models that identify tumor-driving signal transduction pathways. Cancer Res 74:2936-45, 2014

Chapter 3

OVERESTIMATION OF LATE DISTANT RECURRENCES IN HIGH-RISK PATIENTS WITH ER-POSITIVE BREAST CANCER: VALIDITY AND ACCURACY OF THE CTS5 RISK SCORE IN THE TEAM AND IDEAL TRIALS

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ABSTRACT

Purpose

Most distant recurrences (DRs) in women with hormone receptor-positive breast cancer occur after five years from diagnosis. The Clinical Treatment Score post-5 years (CTS5) estimates DRs after five years of adjuvant endocrine therapy (ET). The aim of this study was to externally validate the CTS5 as a prognostic/predictive tool.

Methods

The CTS5 categorizes patients who have been disease free for five years into low, intermediate, and high risk and calculates an absolute risk for developing DRs between five and ten years. Discrimination and calibration were assessed using data from the TEAM and IDEAL trials. The predictive value of the CTS5 was tested with data from the IDEAL trial.

Results

A total of 5895 patients from the TEAM trial and 1591 patients from the IDEAL trial were included. When assessing the CTS5 discrimination, significantly more DRs were found at ten years after diagnosis in the CTS5 high- and intermediate-risk groups than in the low-risk group (hazard ratio 5.7 [95% CI 3.6 – 8.8] and 2.8 [95% CI 1.7 – 4.4], respectively). In low- and intermediate-risk patients, the CTS5-predicted DR rates were higher, although not statistically significantly so, than observed rates. However, in high-risk patients, the CTS5-predicted DR rates were significantly higher than observed rates (29% versus 19%, respectively; $p<0.001$). The CTS5 was not predictive for extended adjuvant ET duration.

Conclusion

The CTS5 score as applied to patients treated in the TEAM and IDEAL cohorts discriminates between risk categories but overestimates the risk of late DRs in high-risk patients. Therefore, the numerical risk assessment from the CTS5 calculator in its current form should be interpreted with caution when used in daily clinical practice, particularly in high-risk patients.

INTRODUCTION

The disease course of estrogen receptor (ER)–positive breast cancer can be influenced significantly by targeting ER with adjuvant endocrine therapy (ET).[1] Currently, the optimal ET regimen for postmenopausal patients consists of either five years of treatment with an aromatase inhibitor (AI) or two to three years of tamoxifen followed by two to three years of an AI.[2] Tamoxifen monotherapy for five years has been proven an inferior treatment.[3]

The majority of distant recurrences (DRs) occur after the first five years from diagnosis (i.e., after ET has been stopped).[4] Increasingly, attention is being drawn to the challenge of preventing late relapse in ER-positive breast cancer. Extending ET to a total of ten years has proven beneficial for a minority of patients, if the initial treatment consisted of tamoxifen monotherapy, both for extended treatment with tamoxifen and for switching to an AI.[5,6] However, studies so far have not shown a clinically relevant benefit of extended therapy if the initial treatment included an AI.[7-10] ET is accompanied by significant toxicity, and extended therapy should only be prescribed after carefully weighing harms and benefits.[7,11,12]

Over the past decades, the field of estimating disease recurrences has mostly shifted toward gene expression profiles. For example, gene expression profiling can be used to identify luminal A and luminal B tumors, two subtypes of ER-positive breast cancer that reflect a different tumor biology and disease prognosis. Neither subtype, however, is predictive for a better response to endocrine therapy.[13-18] Moreover, gene expression profiles are expensive and not universally available. The value of gene expression profiling in routine clinical practice and, more specifically, the identification of patient subgroups where they should be used, remains challenging.[19] With this rationale, the Clinical Treatment Score post-5 years (CTS5) has been described using data from the ATAC and BIG-1-98 trials.[20-22] The CTS5 is a prognostic tool that aims to estimate the risk for late DRs and categorizes postmenopausal patients into low-, intermediate-, and high-risk groups on the basis of commonly reported clinicopathologic parameters and is therefore cheap and easy to use.

Prognostic models, such as the CTS5, are important because they provide additional information about the disease course and can help to guide the optimal treatment strategy. A crucial aspect of implementing prognostic models into daily clinical practice is the external validation of these models because using unvalidated models could lead to inappropriate modifications of treatment regimens. The CTS5 was created with patient data from two trial cohorts and was validated using the combined patient cohorts of these two trials[20-22] and, therefore, requires further validation. This study aims to externally validate the CTS5 as a prognostic tool for late DRs and to assess it as a predictive tool for choosing the optimal duration of extended ET using patient data from two prospective randomized clinical trials: the Tamoxifen and Exemestane Adjuvant Multicenter (TEAM) trial[2] and the Investigation on the Duration of Extended Adjuvant Letrozole (IDEAL) trial.[7]

METHODS

Algorithm

The CTS5 categorizes postmenopausal patients with ER-positive breast cancer who are disease free after five years of standard ET into low- (< 5%), intermediate- (5% – 10%), and high- (> 10%) risk groups for developing a DR between five and ten years from diagnosis.[21] This categorization is based on the number of positive lymph nodes, grade, size and quadratic size of the tumor, and age at diagnosis. Consistent with the method used in the original CTS5 publication,[21] tumor size was capped at 30 mm. Nodal status was divided into five groups: 0, node negative; 1, 1 positive lymph node; 2, 2-3 positive nodes; 3, 4-9 positive nodes; and 4, > 9 positive nodes. The CTS5 score is calculated using the following equation:

$$CTS5\ 5 = 0.438 * nodes + 0.988 * (0.093 * size - 0.001 * size^2 + 0.375 * grade + 0.017 * age)$$

The cutoff values were established at < 3.13 for the low-risk group, 3.13 – 3.86 for the intermediate-risk group, and > 3.86 for the high-risk group.[21] The online CTS5 calculator provides both the risk category and an absolute risk of developing a late DR for individual patients.[23]

TEAM study cohort

TEAM is a phase III trial that randomly assigned postmenopausal patients with ER-positive breast cancer to either five years of exemestane or two to three years of tamoxifen followed by two to three years of exemestane. All patients were randomly assigned within one month after diagnosis. Details of the trial have been reported previously.[2,24] For the analyses of this study, all patients were included who were disease free at five years after random assignment and for whom all clinicopathologic data were available.

IDEAL study cohort

IDEAL is a phase III randomized trial that investigated the optimal duration of letrozole after standard ET in postmenopausal patients with ER-positive early breast cancer. Patients needed to be disease free after five years of standard ET before random assignment to either 2.5 or 5 years of extended treatment with letrozole. More than 90% of patients were randomly assigned within six months after stopping standard ET. No significant difference in disease-free survival was found between the treatment arms after a median follow-up of 6.6 years after random assignment. Details of the trial have been reported previously.[7] For this study, all patients were included for whom all clinicopathologic data were available.

Validation as prognostic tool

The CTS5 is validated as a prognostic tool if it is able to significantly differentiate patients into low-, intermediate-, and high-risk groups for developing DRs and if the predicted absolute risks for developing DRs correspond with the observed DR rates (i.e., is properly calibrated).

Validation as a predictive tool

The CTS5 is validated as a predictive tool for choosing between 2.5 years and 5 years extended ET if there is statistically significant interaction between the risk categories and the treatment arms of the IDEAL trial.

Statistical analyses

The primary end point for the CTS5 is a late DR, defined as the occurrence of a DR between five and ten years after diagnosis. For this study, this is translated to the occurrence of a DR at ten years after random assignment in the TEAM cohort and at five years after random assignment in the IDEAL cohort. Kaplan-Meier survival estimates and cumulative incidence survival estimates were used to determine the discriminative prognostic performance of CTS5. Hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) and P-values for Kaplan-Meier survival estimates were estimated from univariable Cox regression models. HRs and 95% CIs for cumulative incidence survival estimates were derived from Fine and Gray analyses.[25,26]

To examine the calibration, the observed DR rates were compared with the predicted number of DRs. To calculate the predicted late DR rates, six random patients from the TEAM cohort and six random patients from the IDEAL cohort were entered in the online CTS5 calculator.[23] The risk scores that were provided by the calculator for these patients were used to calculate the cumulative baseline hazard at five years used in the algorithm behind the online calculator, using the following equation:

$$\text{“Baseline hazard”} = (-\ln(1 - \text{“predicted risk”}))/(e^{\text{CTS5 score}})$$

These 12 baseline hazards were then averaged. This average baseline hazard (0.00223) was used to calculate the predicted risks for the other patients, using the following equation:

$$\text{“Predicted risk”} = 1 - (e^{\wedge}(-0.00223*(e^{\text{CTS5 score}})))$$

These calculated risk scores were then cross-checked with the risk scores provided by the online calculator for another twelve random patients, six from the TEAM cohort and six from the IDEAL cohort. These were identical. The observed DR rates were determined using Kaplan-Meier survival estimates and cumulative incidence survival estimates[25] to account for death as a competing risk. To examine the calibration, the observed DR rates were compared with the predicted number of DRs for ten equal deciles.

To test the predictive value of the CTS5 for extended ET, Cox regression was used to test for interaction between the risk categories and treatment arms of the IDEAL cohort. Treatment arm and risk category were used as coefficients in the Cox regression interaction model. $P < 0.05$ was considered statistically significant. Analyses were performed using SPSS version 23.0 (IBM Corporation, Chicago, IL) and R 3.5.2 (R Foundation for Statistical Computing, Vienna, Austria) statistical software.

Ethical standards

The study was conducted in compliance with the guidelines of the Declaration of Helsinki, International Conference on Harmonization and Good Clinical Practice. The TEAM trial was approved by the medical ethics committee of the Erasmus Medical Center Rotterdam (198.231/2001/7). The IDEAL trial was approved by the medical ethics committee of the Leiden University Medical Center (P06.217).

RESULTS

Patients in the study cohorts

The TEAM trial consists of 9,779 patients. Of those patients, 2,366 were excluded because they withdrew informed consent, experienced a relapse, died, or were lost to follow-up before five years from random assignment. Another 391 patients were excluded because they continued treatment in the IDEAL trial. For 1,127 of the remaining 7,022 patients, a CTS5 and accompanying risk prediction could not be calculated because of missing clinicopathologic parameters. Thus, the cohort used in this study consisted of 5,895 patients (hereafter called the TEAM cohort; **figure 1A**). For the TEAM cohort, the observed late DR rate at ten years after random assignment was 8.7%. The IDEAL trial consists of 1,824 patients. For 233 patients, a CTS5 and risk prediction could not be calculated, which left 1,591 patients for this analysis (hereafter called the IDEAL cohort; **figure 1B**). For the IDEAL cohort, the observed late DR rate at five years after random assignment was 6.8%.

Comparison of cohorts

The observed late DR rates were 7.0% for the ATAC cohort and 5.5% for the BIG-1-98 cohort used to train and test the CTS5.[21] In the TEAM cohort, 2,113 patients (35.8%) were categorized into the low-risk group, 2,159 (36.6%) into the intermediate-risk group, and 1,623 (27.5%) into the high-risk group. Patients from the TEAM cohort were comparable to patients in the ATAC and BIG-1-98 cohorts with regard to baseline characteristics, treatment strategy (use of chemotherapy and type of ET), and overall late DR rates (**table 1**).[20-22] Patients in the TEAM cohort had slightly larger tumors (43% of patients had T21 tumors vs. 32% and 35% in the ATAC and BIG-1-98 cohorts, respectively; $P < 0.001$), and more patients had N1 disease (40% vs. 32% and 40%, respectively; $P < 0.001$).

In the IDEAL cohort, 343 patients (21.6%) were categorized into the low-risk group, 649 (40.8%) into the intermediate-risk group, and 599 (37.6%) into the high-risk group. Patients in the IDEAL cohort had some baseline characteristics that would classify them at higher risk than the patients in the ATAC and BIG-1-98 cohorts, such as age at diagnosis and number of positive lymph nodes (**table 1**). This resulted in a different distribution of patients over the risk categories and is reflected in a significantly higher proportion of patients who received chemotherapy. Nevertheless, within the three CTS5 risk categories, there were no significant differences among the cohorts in baseline characteristics and risk factors apart from age. In addition, the overall late DR rates at ten years were comparable between the IDEAL cohort and the ATAC and BIG-1-98 cohorts.

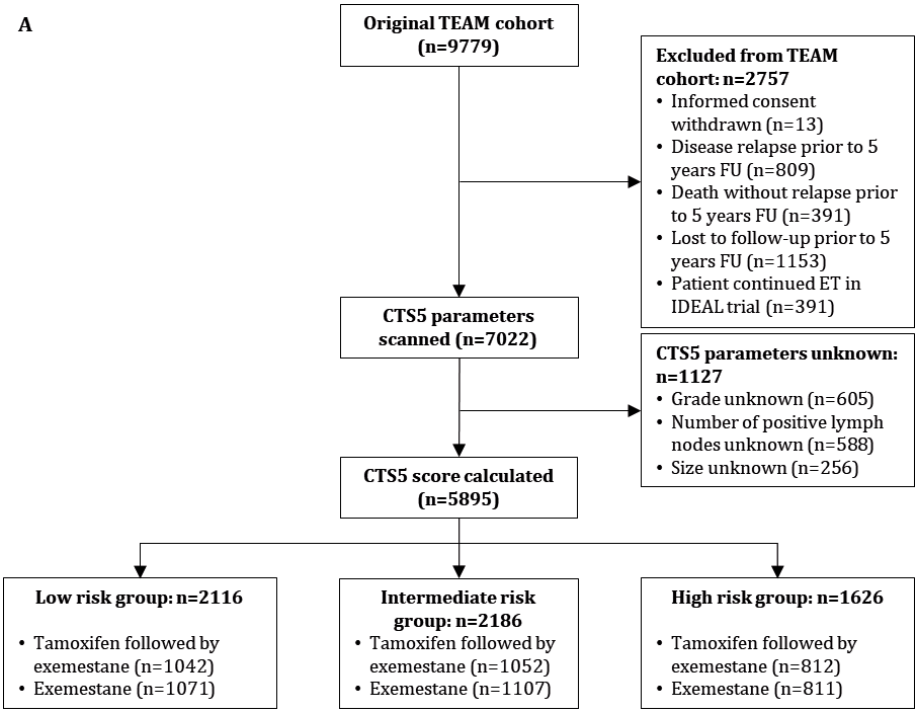


Figure 1: CONSORT diagram to account for missing patients in the TEAM cohort[2] (A) and in the IDEAL cohort[7] (B).

CTS5 = Clinical treatment score post-5 years.[21] DR = distant recurrence. ET = endocrine therapy.

The TEAM cohort	Total	Low-risk	Intermediate-risk	High-risk
Total	**5895**	**2113 (35.8)**	**2159 (36.6)**	**1623 (27.5)**
Age (years) Median	63	61	63	66
Interquartile range	57 – 70	56 – 66	58 – 70	59 – 74
Tumor size (mm) < 10	376 (6.4)	343 (16.2)	30 (1.4)	3 (0.2)
10 – 20	2968 (50.3)	1688 (79.9)	1037 (48.0)	243 (15.0)
> 20	2551 (43.3)	82 (3.9)	1092 (50.6)	1377 (84.8)
Histological grade I	1163 (19.7)	757 (35.8)	304 (14.1)	102 (6.3)
II	3176 (53.9)	1195 (56.6)	1221 (56.6)	760 (46.8)
III	1556 (26.4)	161 (7.6)	634 (29.4)	761 (46.9)
Positive lymph nodes 0	3501 (59.4)	1855 (87.8)	1359 (62.9)	287 (17.7)
1	1182 (20.1)	239 (11.3)	572 (26.5)	371 (22.9)
2 – 3	722 (12.2)	19 (0.9)	208 (9.6)	495 (30.5)
4 – 9	377 (6.4)	-	20 (0.9)	357 (22.0)
> 9	113 (1.9)	-	-	113 (7.0)
Prior chemotherapy yes	1929 (32.7)	398 (18.8)	789 (36.5)	742 (45.7)
Allocated ET 5 years EXE	2989 (50.7)	1071 (50.7)	1107 (51.3)	811 (50.0)
2-3 years TAM, 2-3 years EXE	2906 (49.3)	1042 (49.3)	1052 (48.7)	812 (50.0)

The IDEAL cohort	Total	Low-risk	Intermediate-risk	High-risk
Total	**1591**	**343 (21.6)**	**649 (40.8)**	**599 (37.6)**
Age (years) Median	55	52	55	56
Interquartile range	49 – 61	47 – 58	48 – 62	50 – 62
Tumor size (mm) < 10	56 (3.5)	47 (13.7)	8 (1.2)	1 (0.2)
10 – 20	607 (3.5)	251 (73.2)	267 (41.1)	89 (14.9)
> 20	928 (58.3)	45 (13.1)	374 (57.6)	509 (85.0)
Histological grade I	277 (17.4)	128 (37.3)	105 (16.2)	44 (7.3)
II	760 (47.8)	161 (46.9)	310 (47.8)	289 (48.2)
III	554 (34.8)	54 (15.7)	234 (36.1)	266 (44.4)
Positive lymph nodes 0	614 (38.6)	236 (68.8)	344 (53.0)	34 (5.7)
1	388 (24.4)	94 (27.4)	192 (29.6)	102 (17.0)
2 – 3	301 (18.9)	13 (3.8)	98 (15.1)	190 (31.7)
4 – 9	220 (13.8)	-	14 (2.2)	206 (34.4)
> 9	68 (4.3)	-	1 (0.2)	67 (11.2)
Prior chemotherapy yes	1085 (68.2)	230 (67.1)	403 (62.1)	452 (75.5)
Initial ET 5 years TAM	180 (11.3)	44 (12.8)	82 (12.6)	54 (9.0)
5 years AI	465 (29.2)	85 (24.8)	189 (29.1)	191 (31.9)
2-3 years TAM, 2-3 years AI	946 (59.5)	214 (62.4)	378 (58.2)	354 (59.1)
Allocated extended ET 2.5 years	785 (49.4)	172 (50.1)	336 (51.8)	278 (46.4)
5 years	805 (50.6)	171 (49.9)	313 (48.2)	321 (53.6)

Table 1: Overview of clinicopathologic and demographic characteristics of the patients in the TEAM and IDEAL cohorts. All values are N (%).

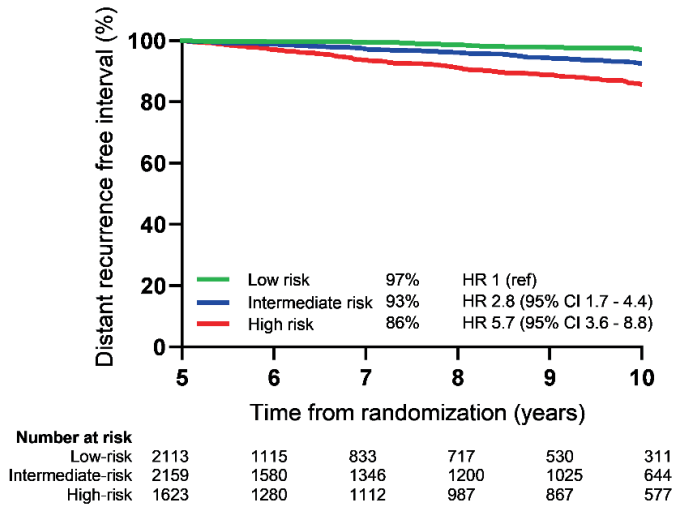
AI = aromatase inhibitor. ET = endocrine therapy. EXE = exemestane. TAM = tamoxifen.

Prognostic validation

Discrimination. In the TEAM cohort, the DR-free interval (DRFi) was 97% (95% CI 96% – 98%) in the low-risk group, 93% (95% CI 91% – 94%) in the intermediate-risk group, and 86% (95% CI 84% – 88%) in the high-risk group. The DRFi was significantly lower in the high-risk group (HR 5.7; 95% CI 3.6 – 8.8; log-rank P < 0.001) and the intermediate-risk group (HR 2.8; 95% CI 1.7 – 4.4; log-rank P < 0.001) compared with the low-risk group (**figure 2A**).

In the IDEAL cohort, the DRFi was 98% (95% CI 96% – 99%) in the low-risk group, 95% (95% CI 93% – 97%) in the intermediate-risk group, and 89% (95% CI 87% – 92%) in the high-risk group. The DRFi was significantly lower in the high-risk group (HR 4.8; 95% CI 2.3 – 10.2; log-rank P < 0.001) and intermediate-risk group (HR 2.2; 95% CI 1.0 – 4.8; log-rank P = 0.037) compared with the low-risk group as well (**figure 2B**).

A



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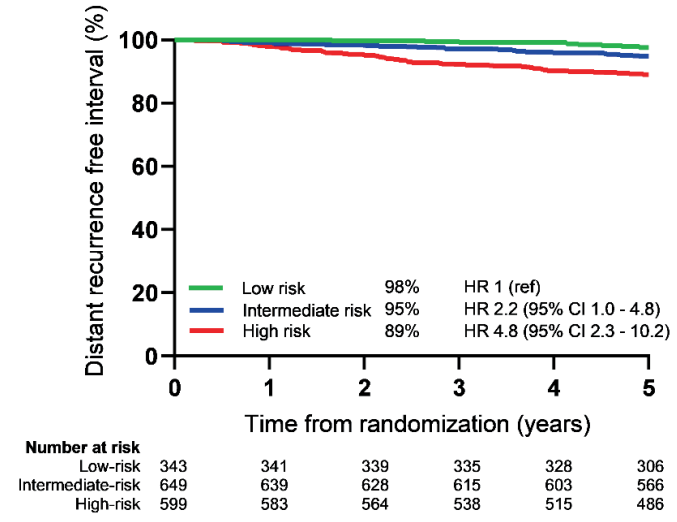

Figure 2: Kaplan-Meier survival estimates of observed late distant recurrence (DR) rates with accompanying risk table, indicating discriminatory prognostic value of the Clinical Treatment Score post-5 years (CTS5) for DRs in patient from the TEAM (A) and IDEAL (B) cohorts.

Calibration. In patients from the TEAM cohort with a predicted late DR risk up to 7%, the observed late DR rates corresponded with the predicted rates. However, in patients with higher predicted risks, the observed late DR rates were significantly lower than the predicted rates (**table 2**; **figure 3A**). In the highest decile, the absolute difference between the predicted DR rate and the observed rate was 10% (29.0% vs 19.3% [95% CI, 15.3 – 23.0], respectively; **figure 4A**).

In patients from the IDEAL cohort with predicted risk up to 8%, the predicted rate was higher than the observed rates, although not statistically significant. However, in patients with a higher predicted risk, the predicted rate was significantly higher than the observed rates (**table 2**; **figure 3B**). In the patients with the highest predicted risk, the predicted DR rate was 31.6%, while the observed rate was only 15.5% (95% CI, 9.6 – 21.0; **figure 4B**).

The observed late DR rates using the cumulative incidence method did not differ significantly from the rates obtained with the Kaplan-Meier method (data not shown).

The TEAM cohort	**Predicted** %	**Observed** % (95% CI)
Low-risk group	3.4	3.0 (1.6 – 4.3)
Intermediate-risk group	7.2	7.4 (5.8 – 86)
High-risk group	19.0	14.2 (12.1 – 16.3)
The IDEAL cohort	**Predicted** %	**Observed** % (95% CI)
Low-risk group	3.6	2.4 (0.8 – 4.1)
Intermediate-risk group	7.2	5.2 (3.5 – 6.9)
High-risk group	19.5	10.9 (8.4 – 13.4)

Table 2: Observed and predicted probability of late distant recurrences with accompanying 95% confidence intervals in the three risk categories in the TEAM and IDEAL cohorts.

CI = confidence interval.

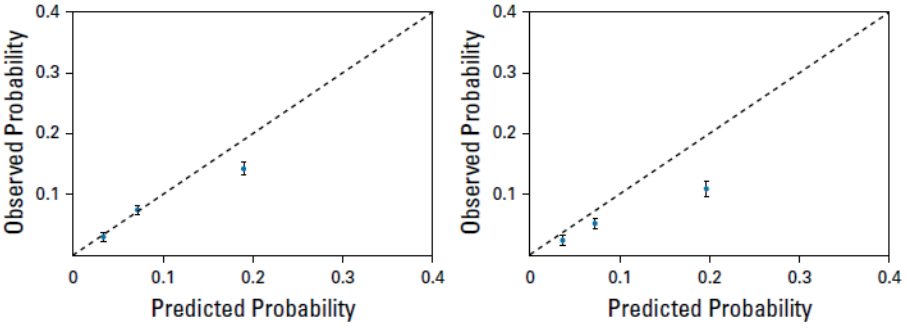

Figure 3: Observed and predicted probability of distant recurrences with accompanying 95% confidence intervals in the three risk categories in the TEAM (A) and IDEAL (B) cohorts.

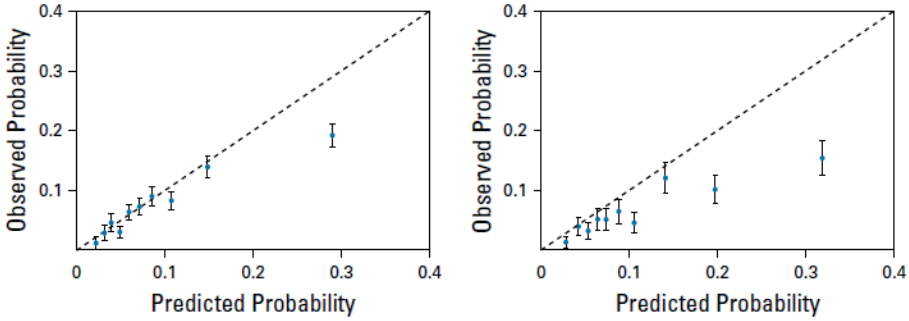

Figure 4: Observed and predicted probability of distant recurrences with accompanying 95% confidence intervals in ten equal deciles in the TEAM (A) and IDEAL (B) cohorts.

Predictive validation

In the IDEAL cohort, there was no statistically significant interaction between the risk categories and the treatment allocation (P = 0.5). No difference in observed DR rates was seen between 2.5 and 5 years of extended ET in the low-risk (HR 0.6; 95% CI 0.1 – 2.5), intermediate-risk (HR 1.7; 95% CI 0.8 – 3.3), or high-risk (HR 1.2; 95% CI 0.7 – 1.9) groups (**table 3**). Thus, there was no risk category in which the treatment allocation had a significant effect on the occurrence of late DR.

	2.5 years extended ET	5 years extended ET	P-value
Low-risk group	3.4	3.0 (1.6 – 4.3)	0.49
Intermediate-risk group	7.2	7.4 (5.8 – 86)	0.15
High-risk group	19.0	14.2 (12.1 – 16.3)	0.50

Table 3: Absolute late distant recurrence rates in the IDEAL cohort stratified by treatment arm and risk group.

P-values are derived from univariable Cox regression models.

DISCUSSION

Considering the substantial risk of late DRs for patients with ER-positive breast cancer, even after five years of ET, and the potential benefit of extended ET, there is a demand for prognostic and predictive models.[4,27] The CTS5 was developed for this purpose. It aims to estimate the DR rate between five and ten years from diagnosis for postmenopausal patients with ER-positive breast cancer who remain disease free after five years of standard ET.[21]

As applied to the TEAM and IDEAL cohorts, the CTS5 is able to discriminate postmenopausal patients with ER-positive breast cancer into three risk categories with respect to late DR. In the two large cohorts of trial patients used in these analyses, the risk of late DR as predicted by the CTS5 corresponded to the observed DR rates in low-risk patients, but the CTS5 overestimated the observed risk of DR in patients with a higher

predicted risk. Furthermore, the CTS5 could not predict benefit of 5 years over 2.5 years of extended ET using data from the IDEAL cohort.

The discrimination of the CTS5 has also been tested and validated in one retrospective cohort of non-trial patients with ER-positive breast cancer; however, the calibration was not tested in that study.[28] Although our study is one of the first that aimed to externally validate it, the CTS5 is already being used in clinical practice through the online calculator.[23]

Patients in the TEAM cohort, who had ER-positive breast cancer and were disease free for five years on either exemestane monotherapy or tamoxifen followed by exemestane, were comparable to patients in both cohorts used to create the CTS5 with regard to most risk factors, treatment strategy, and overall DR rates. The proportional distribution of patients into the three risk categories was also similar.[20-22] Because there was no difference in the definition of DR, it was expected that the predicted late DR risk would not differ from the observed DR rates. This was confirmed in low- and intermediate-risk patients. However, in the high-risk patients, the predicted risk was significantly higher than the observed late DR rate (19% and 14%, respectively). Although 14% would still be categorized as high risk, a more valid numerical estimation can lead to more accurately tailored treatment advice.

Patients in the IDEAL cohort who had ER-positive breast cancer and were disease free for five years on any ET had comparable baseline characteristics within the risk categories to both CTS5 cohorts and had comparable overall DR rates. However, IDEAL patients differed from the CTS5 cohorts with regard to treatment strategy; patients in the IDEAL cohort were treated with extended ET for either 2.5 or 5 years. Preliminary results from the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) meta-analysis on extended ET showed that extending ET beyond the first five years may yield an absolute risk reduction of late DR of 1%-5% at ten years after diagnosis, with the effect size depending on the type of ET given during the first five years.[29]

When applying the CTS5 to the IDEAL cohort, the predicted DR rates were similar to the observed DR rates in low-risk patients, but in the highest risk decile, the CTS5 predicted a DR rate of 31.6%, while we observed a DR rate of 15.5% (95% CI, 9.6% to 21.0%). It is possible that the difference in treatment strategy led to a slight reduction in late DR rates (up to 5% according to the preliminary data of the EBCTCG), but extended treatment is unlikely to account for the 16% risk difference that was observed in this analysis.

A potential explanation for the difference in observed and predicted DR rates is the discrepancy in the years of diagnosis between the cohorts. While patients in the ATAC and BIG-1-98 cohorts were diagnosed between 1996 and 2003, patients in the TEAM and IDEAL cohorts were diagnosed between 2001 and 2006. Over this period, significant improvements in diagnostic accuracy (reliability of hormone receptor and human epidermal growth factor receptor 2 [HER2] status determination[30]) and systemic therapy (chemotherapy regimens and anti-HER2 medication) were made. Moreover, in the ATAC and BIG-1-98 cohorts, 20% and 24% of patients were treated with chemotherapy, while this was 33% and 68% in the TEAM and IDEAL cohorts, respectively. This could explain

the observation that the largest difference between observed and predicted late DR rates was seen in high-risk patients.

The most common reason for excluding patients from the TEAM and IDEAL trial cohorts to create the cohorts used in this study was unknown grade (**figure 1**). This was mostly due to the difficulty in scoring the histologic grade of lobular carcinomas.[31] Because there were no differences in late DR rates between the included patients and patients who were excluded on the basis of unknown grade (data not shown), this is not likely to bias the results of our analyses.

Furthermore, an aspect to keep in mind is the difference between prognostic and predictive tools.[32] Prognostic tools aim to distinguish patients with an inherently worse prognosis from those with a better prognosis, while predictive tools aim to distinguish those patients who will respond well to treatment from those who will not. Often, prognostic tools are not predictive and should not be used as such because patients with a worse prognosis are not necessarily the same patients who benefit from more extensive therapy.[33] In patients treated in the IDEAL trial, the CTS5 was not able to select patients who benefit from 5 years of extended ET as opposed to 2.5 years. Because of the design of the IDEAL trial, it was not possible to investigate whether the CTS5 is predictive of the type of extended ET (i.e., tamoxifen v AI v no extended ET).

Future prognostic and predictive models will most likely focus on genetic and/or functional profiles, and although these have shown promising results so far, accessibility and availability in daily clinical practice need to be prioritized as well.[19] This is one major advantage of the CTS5 because it is based on clinicopathologic parameters that are available for all patients and is cheap and easy to implement across all health care settings.

In conclusion, the CTS5 as applied to patients treated in the TEAM and IDEAL cohorts categorizes patients into low-, intermediate-, and high-risk groups. In low-risk patients, the predicted late DR risks correspond with the observed DR rates, but the CTS5 overestimates the risk of late DRs in high-risk patients from the TEAM and IDEAL cohorts. Using patient data from the IDEAL cohort, the CTS5 cannot be validated as a predictive tool for extended ET either. Especially in high-risk patients, an unrealistic assessment of the risk to develop a late DR could potentially lead to overtreatment. Therefore, the numerical risk assessment from the CTS5 calculator in its current form should be interpreted with caution when used in daily clinical practice, particularly when used in high-risk patients.

REFERENCES

1. EBCTCG: Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 365:1687-717, 2005
2. Derks MGM, Blok EJ, Seynaeve C, et al: Adjuvant tamoxifen and exemestane in women with postmenopausal early breast cancer (TEAM): 10-year follow-up of a multicentre, open-label, randomised, phase 3 trial. Lancet Oncol 18:1211-1220, 2017
3. EBCTCG: Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. Lancet 386:1341-1352, 2015
4. Pan H, Gray R, Braybrooke J, et al: 20-Year Risks of Breast-Cancer Recurrence after Stopping Endocrine Therapy at 5 Years. N Engl J Med 377:1836-1846, 2017
5. Davies C, Pan H, Godwin J, et al: Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. Lancet 381:805-16, 2013
6. Goss PE, Ingle JN, Martino S, et al: A randomized trial of letrozole in postmenopausal women after five years of tamoxifen therapy for early-stage breast cancer. N Engl J Med 349:1793-802, 2003
7. Blok EJ, Kroep JR, Meershoek-Klein Kranenbarg E, et al: Optimal Duration of Extended Adjuvant Endocrine Therapy for Early Breast Cancer; Results of the IDEAL Trial (BOOG 2006-05). J Natl Cancer Inst 110, 2018
8. Gnant M, Steger G, Greil R, et al: Abstract GS3-01: A prospective randomized multi-center phase-III trial of additional 2 versus additional 5 years of anastrozole after initial 5 years of adjuvant endocrine therapy – results from 3,484 postmenopausal women in the ABCSG-16 trial. Cancer Res 78:GS3-01-GS3-01, 2018
9. Mamounas EP, Bandos H, Lembersky BC, et al: Use of letrozole after aromatase inhibitor-based therapy in postmenopausal breast cancer (NRG Oncology/NSABP B-42): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 20:88-99, 2019
10. Tjan-Heijnen VCG, van Hellemond IEG, Peer PGM, et al: Extended adjuvant aromatase inhibition after sequential endocrine therapy (DATA): a randomised, phase 3 trial. Lancet Oncol 18:1502-1511, 2017
11. Blok EJ, Kroep JR, Meershoek-Klein Kranenbarg E, et al: Treatment decisions and the impact of adverse events before and during extended endocrine therapy in postmenopausal early breast cancer. Eur J Cancer 95:59-67, 2018
12. Niravath P, Rimawi MF, Osborne CK: Aromatase inhibitor adverse effects: are we sweeping them under the rug? J Clin Oncol 32:3779, 2014
13. Ellis MJ, Suman VJ, Hoog J, et al: Randomized phase II neoadjuvant comparison between letrozole, anastrozole, and exemestane for postmenopausal women with estrogen receptor-rich stage 2 to 3 breast cancer: clinical and biomarker outcomes and predictive value of the baseline PAM50-based intrinsic subtype--ACOSOG Z1031. J Clin Oncol 29:2342-9, 2011
14. Nielsen TO, Parker JS, Leung S, et al: A Comparison of PAM50 Intrinsic Subtyping with Immunohistochemistry and Clinical Prognostic Factors in Tamoxifen-Treated Estrogen Receptor–Positive Breast Cancer. Clin Cancer Res 16:5222-5232, 2010
15. Parker JS, Mullins M, Cheang MC, et al: Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol 27:1160-7, 2009
16. Prat A, Cheang MC, Martín M, et al: Prognostic significance of progesterone receptor-positive tumor cells within immunohistochemically defined luminal A breast cancer. J Clin Oncol 31:203-9, 2013
17. Prat A, Ellis MJ, Perou CM: Practical implications of gene-expression-based assays for breast oncologists. Nat Rev Clin Oncol 9:48-57, 2011
18. Viale G, de Snoo FA, Slaets L, et al: Immunohistochemical versus molecular (BluePrint and MammaPrint) subtyping of breast carcinoma. Outcome results from the EORTC 10041/BIG 3-04 MINDACT trial. Breast Cancer Res Treat 167:123-131, 2018
19. Blok EJ, Bastiaannet E, van den Hout WB, et al: Systematic review of the clinical and economic value of gene expression profiles for invasive early breast cancer available in Europe. Cancer Treat Rev 62:74-90, 2018
20. Cuzick J, Sestak I, Baum M, et al: Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 10-year analysis of the ATAC trial. Lancet Oncol 11:1135-41, 2010
21. Dowsett M, Sestak I, Regan MM, et al: Integration of Clinical Variables for the Prediction of Late Distant Recurrence in Patients With Estrogen Receptor-Positive Breast Cancer Treated With 5 Years of Endocrine Therapy: CTS5. J Clin Oncol 36:1941-1948, 2018
22. Regan MM, Neven P, Giobbie-Hurder A, et al: Assessment of letrozole and tamoxifen alone and in sequence for postmenopausal women with steroid hormone receptor-positive breast cancer: the BIG 1-98 randomised clinical trial at 8·1 years median follow-up. Lancet Oncol 12:1101-8, 2011
23. Queen Mary University of Loncon: CTS5 calculator. https://www.cts5-calculator.com
24. van de Velde CJH, Rea D, Seynaeve C, et al: Adjuvant tamoxifen and exemestane in early breast cancer (TEAM): a randomised phase 3 trial. Lancet 377:321-31, 2011
25. Andersen PK, Geskus RB, de Witte T, et al: Competing risks in epidemiology: possibilities and pitfalls. Int J Epidemiol 41:861-70, 2012

26. Fine JP, Gray RJ: A Proportional Hazards Model for the Subdistribution of a Competing Risk. J Am Stat Assoc 94:496-509, 1999
27. Davies C, Godwin J, Gray R, et al: Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet 378:771-84, 2011
28. Richman J, Ring A, Dowsett M, et al: Clinical validity of clinical treatment score 5 (CTS5) for estimating risk of late recurrence in unselected, non-trial patients with early oestrogen receptor-positive breast cancer. Breast Cancer Res Treat, 2020
29. Gray R: Abstract GS3-03: Effects of prolonging adjuvant aromatase inhibitor therapy beyond five years on recurrence and cause-specific mortality: An EBCTCG meta-analysis of individual patient data from 12 randomised trials including 24,912 women. Cancer Res 79:GS3-03-GS3-03, 2019
30. Wolff AC, Hammond MEH, Allison KH, et al: Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. J Clin Oncol 36:2105-2122, 2018
31. Barroso-Sousa R, Metzger-Filho O: Differences between invasive lobular and invasive ductal carcinoma of the breast: results and therapeutic implications. Ther Adv Med Oncol 8:261-6, 2016
32. Ballman KV: Biomarker: Predictive or Prognostic? J Clin Oncol 33:3968-71, 2015
33. Sechidis K, Papangelou K, Metcalfe PD, et al: Distinguishing prognostic and predictive biomarkers: an information theoretic approach. Bioinformatics 34:3365-3376, 2018

Chapter 4

BREAST CANCER INDEX (BCI) PREDICTS EXTENDED ENDOCRINE BENEFIT TO INDIVIDUALIZE SELECTION OF PATIENTS WITH HR+ EARLY-STAGE BREAST CANCER FOR 10 YEARS OF ENDOCRINE THERAPY

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ABSTRACT

Purpose

Individualized selection of patients with early-stage hormone receptor–positive (HR+) breast cancer for extended endocrine therapy (EET) is required to balance modest gains in outcome with toxicity of prolonged use. This study examined the Breast Cancer Index (BCI; HOXB13/IL17BR ratio [H/I]) as a predictive biomarker of EET benefit in patients from the Investigation on the Duration of Extended Adjuvant Letrozole (IDEAL) trial.

Experimental design

BCI was tested in primary tumor specimens from 908 patients randomized to receive 2.5 versus 5 years of extended letrozole. The primary endpoint was recurrence-free interval. Cox regression models and likelihood ratios tested the interaction between EET and BCI [H/I].

Results

BCI [H/I]-high significantly predicted benefit from extended letrozole in the overall cohort (hazard ratio [HR] 0.42, 95% CI 0.21 – 0.84; P = 0.011) and in the any aromatase inhibitor (AI) subset (HR 0.34, 95% CI 0.16 – 0.73; P = 0.004), whereas BCI [H/I]-low patients did not derive significant benefit (HR 0.95, 95% CI 0.58 – 1.56; P = 0.84 and HR 0.90, 95% CI 0.53 – 1.55; P = 0.71, respectively). Treatment to biomarker interaction was significant (P = 0.045 in the overall cohort, P = 0.025 in the any AI subset). BCI [H/I] identified approximately 50% of patients with clinically high-risk disease that did not benefit, and with clinically low-risk disease that derived significant benefit from an additional 2.5 years of EET.

Conclusions

BCI [H/I] predicted preferential benefit from 5 versus 2.5 years of EET and identified patients with improved outcome from completing 10 years of adjuvant endocrine therapy. These findings expand the clinical utility of BCI [H/I] to a broader range of patients and beyond prognostic risk factors as a predictive endocrine response biomarker for early-stage HR+ breast cancer.

INTRODUCTION

Whereas the role of primary adjuvant endocrine therapy for women diagnosed with early-stage hormone receptor–positive (HR+) breast cancer is well established, decisions regarding the optimal composition and duration of endocrine therapy in the extended setting have become increasingly complex. Large randomized trials have demonstrated that extension of endocrine therapy reduces the risk of late distant recurrence that persists in HR+ breast cancer for more than a decade after the initial five years of adjuvant endocrine therapy.[1-13] Results to date have been mixed, with modest benefits in disease-free survival (DFS) that were most apparent in patients who have received at least 5 years of tamoxifen prior to an aromatase inhibitor (AI).[4,7-10,14,15]

Antiestrogen therapy with AIs is the standard of care for postmenopausal patients; however, the treatment effects of extending AI therapy for early-stage breast cancer are inconclusive, particularly in patients treated with adjuvant AI monotherapy.[3,8,9,15,16] Notably, the modest absolute benefits ranging from approximately 1% to 4% observed with extending AI therapy for longer durations are accompanied by increased cardiovascular toxicity, bone fractures, and side effects that impair quality of life and compliance, underscoring a critical need to individualize patient selection.[1,5,8,9,17]

Current clinical practice recommendations favor up to ten years of an AI for postmenopausal women with moderate to high risk based on clinicopathologic features and prognostic biomarkers.[18] However, predictive biomarkers that stratify underlying patterns of tumor biology related to endocrine response to identify patients who are likely or unlikely to benefit from endocrine therapy would have a critical impact on patient selection for extended treatment.

The Breast Cancer Index (BCI) is a gene expression–based signature that consists of two functional biomarker panels, the HOXB13/IL17BR (H/I) ratio and the molecular grade index (MGI), that interrogate important estrogen signaling and proliferation pathways in breast cancer. The BCI prognostic score is an algorithmic combination of the H/I ratio and MGI and reports individualized risk of overall and late distant recurrence.[19-21] The predictive component of BCI (BCI [H/I]) is based on the H/I ratio and provides a categorical prediction of high versus low likelihood of benefit from extended endocrine therapy that is reported separately from the prognostic results.[19,21,22]

Previous clinical validation studies for prediction of extended endocrine benefit and outcome have demonstrated significant interaction between BCI [H/I] and extended endocrine therapy with letrozole or tamoxifen following initial tamoxifen treatment.[19,22] A key question regarding BCI [H/I] clinical utility is the biomarker effect in postmenopausal patients treated with a contemporary standard of care that includes an AI component as part of primary adjuvant treatment. In this study, the BCI [H/I] predictive performance was examined in patients treated in the IDEAL (Investigation on the Duration of Extended Letrozole) study, a randomized controlled trial designed to directly examine the potential benefit of extended durations of AI therapy.[1,16]

METHODS

Study design and patients

The IDEAL (BOOG 2006-05) trial is a prospective phase III study that randomized 1,824 patients with HR+ early-stage postmenopausal breast cancer to receive either 2.5 or 5 years of letrozole after completing five years of adjuvant therapy with either tamoxifen monotherapy, tamoxifen followed by an AI, or AI monotherapy.[1] While the study design allowed for completion of the initial five years of adjuvant endocrine therapy up to two years prior to randomization, 89% of patients were randomized within six months of completing primary adjuvant treatment.

This study is a prospective–retrospective translational study of patients enrolled in the IDEAL trial that specifically examined the predictive component of the BCI assay, the H/I ratio. Because both treatment arms were on therapy during the first 2.5 years, BCI study criteria included patients that were recurrence free from 2.5 years post-randomization, which was a secondary analysis in the parent IDEAL trial.[1] All patients with available tumor specimens were eligible and BCI [H/I] testing was conducted blinded to clinical outcome. Exclusion criteria included lack of invasive tumor, incorrect tumor specimen, insufficient or poor RNA signal, and patients with a follow-up time or had recurred less than 2.5 years after randomization. The translational study was based on an updated clinical database with a median FU of 9.3 years after randomization.

Statistical considerations

The primary objective of the study was to determine whether BCI [H/I]-high versus -low is predictive of extended endocrine benefit comparing an additional 2.5 years (7.5 years total endocrine therapy) versus 5 years (10 years total endocrine therapy) of letrozole treatment in patients that have completed five years of endocrine therapy. The key secondary objective was to determine the predictive performance of BCI [H/I] in the patient subset that received prior endocrine therapy that included an AI (primary AI subset). The IDEAL translational cohort (N = 908) showed a 4.9% absolute benefit in the reduction of 10-year risk of recurrence-free interval (RFI) events with 10 years versus 7.5 years of endocrine therapy (HR 0.69; 95%CI 0.47 – 1.03; P = 0.07). Powering assumptions included a 5% sample testing failure rate and that 40% of patients would be classified as BCI [H/I]-high based on previous studies.[19,22] At 80% power, it was estimated that a total of 768 patients would be required to detect the benefit in BCI [H/I]-high patients at the 5% significance level.

The primary endpoint was RFI, defined as the time from 2.5 years after randomization to first local, regional, or distant recurrence. Deaths before recurrence were considered as censoring events while new breast primaries (ipsilateral and contralateral disease) and other secondary cancers were not considered either as events or as censoring events. Secondary endpoints were disease-free interval (DFI) and DFS. DFI is defined as the time from 2.5 years after the random assignment to first local, regional, distant recurrence or new breast primary. DFS is defined as the time from 2.5 years after the random assignment

to first local, regional, distant recurrence, new primary breast tumor, or death from any cause as the first event.

Kaplan–Meier survival analysis and log-rank test were used to compare the two survival outcomes of the two treatment arms for all unselected patients and for patients in each of the BCI [H/I]-low and BCI [H/I]-high groups. The risk of RFI events at year 10 since randomization and 95% CIs were calculated to estimate the magnitude of absolute benefit between 10 and 7.5 years of endocrine therapy in each of the BCI [H/I] groups. Unadjusted HRs and 95% CIs were calculated from a Cox proportional hazards model to estimate the relative benefits within each BCI [H/I] group. The treatment to biomarker interaction was assessed using a likelihood ratio test based on comparing the full versus reduced Cox models, adjusting for age, tumor stage, grade, nodal stage, prior endocrine therapy, and prior chemotherapy. Analyses were prespecified in a statistical analysis plan prior to unblinding.

BCI molecular testing

BCI gene expression analysis by rt-PCR was performed using RNA extracted from formalin-fixed paraffin embedded (FFPE) tumor samples blinded to clinical outcome as described previously.[21] In addition to the five MGI genes, HOXB13, and IL17BR, gene expression analysis of ER, PR, andHER2 was also performed.[23] Briefly, manual macro-dissection was carried out on FFPE sections to enrich tumor content prior to RNA extraction. Total RNA was reverse transcribed, and the resulting cDNA was pre-amplified by PCR using the preamp Master Mix Kit (Thermo Fisher Scientific) and then subjected to TaqMan PCR analysis. Calculation of BCI was conducted utilizing a prespecified and validated assay cut-point to categorize patients as either BCI [H/I]-high or BCI [H/I]-low.[24]

Role of the funding source

This translational study was funded by Biotheranostics Inc. Novartis funded centralized biospecimen collection as part of the parent IDEAL trial. Tumor tissue processing, pathology review, and data collection were conducted by Leiden University Medical Center (LUMC, Leiden, the Netherlands). BCI assays were performed by Biotheranostics in a blinded manner, without knowledge of treatment assignment or clinical outcome. Study unblinding was led and conducted by LUMC. BCI data were merged with patient data at LUMC and access to merged data was limited to the study biostatisticians. The report was drafted in its entirety by the authors without benefit of paid assistance. Content of the final report was not subject to approval from the corporate sponsor. The corresponding author (G.J. Liefers) had final responsibility for the data submission for publication.

RESULTS

Patient characteristics

Of the 1,824 patients that participated in the IDEAL parent trial, archival tumor specimens were available for 1,047 patients (**figure 1**). Following pathology review, BCI testing was completed for 972 patients. Exclusion of 51 patients that were not recurrence-free for at least 2.5 years post-randomization resulted in a final translational cohort of 908 patients

comprising approximately 50% of the original study population with 454 patients in each arm. Patient tumors were 73% lymph node positive, 45% tumor stage 1, 48% tumor stage 2, 43% grade 2, 34% grade 3, and 9% HER2+. Regarding primary adjuvant endocrine treatment, 13% received five years of tamoxifen monotherapy, 27% received five years of AI monotherapy, 60% received sequenced therapy with tamoxifen followed by an AI, and 87% received primary adjuvant treatment with an AI (**table 1**). No statistical differences in clinicopathologic and treatment variables were observed between the translational cohort and the remaining patients not included in the study.

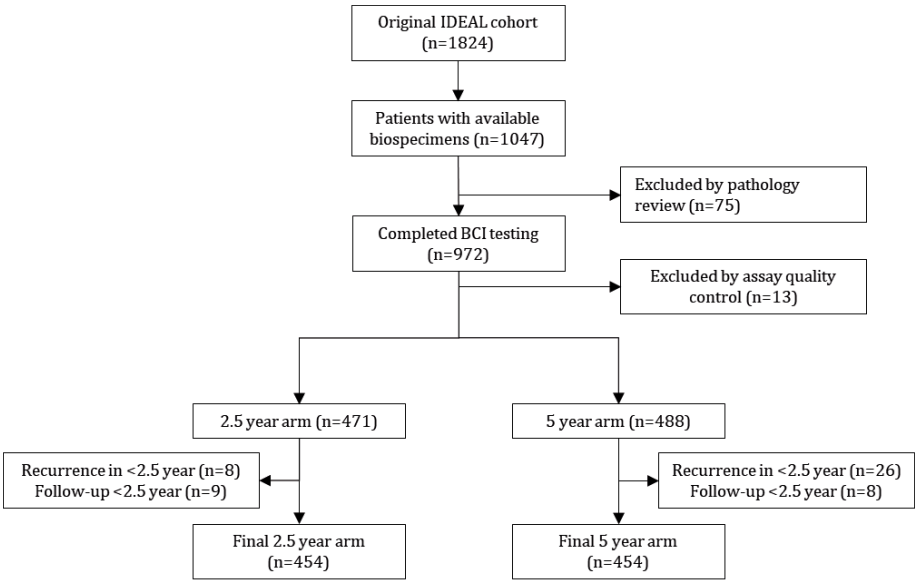

Figure 1: Modified REMARK diagram. The diagram shows biospecimen availability, biospecimen processing, and molecular testing, leading to a final analyzable cohort of 908 patients.

	Parent cohort (N = 1718)	Translational cohort (N = 908)	Remaining cohort (N = 810)	P-value
Age at surgery				0.877
> 50	564 (33)	296 (33)	264 (33)	
≥ 50	1154 (67)	612 (67)	541 (67)	
Tumor stage				0.198
pT1	802 (47)	406 (45)	396 (49)	
pT2	774 (45)	433 (48)	341 (42)	
pT3	89 (5)	49 (5)	40 (5)	
pT4	37 (2)	19 (2)	18 (2)	
Unknown	16 (1)	1 (0)	15 (2)	

Table 1: Clinicopathologic characteristics for patients who were recurrence free at 2.5 years after randomization in the parent trial, translational cohort, and those remaining patients not included in the study. All values are N (%).

P-values comparing the translational cohort versus the remaining patients not included in the study were calculated using Fisher exact tests.

	Parent cohort (N = 1718)	Translational cohort (N = 908)	Remaining cohort (N = 810)	P-value
Grade				0.077
1	268 (16)	135 (15)	133 (16)	
2	735 (43)	390 (43)	345 (43)	
3	536 (31)	311 (34)	225 (28)	
Unknown	179 (10)	72 (8)	107 (13)	
Nodal stage				0.544
pN0	461 (27)	241 (27)	220 (27)	
pN1	941 (55)	499 (55)	442 (55)	
pN2	234 (14)	131 (14)	103 (13)	
pN3	73 (4)	34 (4)	39 (5)	
Unknown	9 (1)	3 (0)	6 (1)	
Tumor type				0.447
Ductal	1340 (78)	720 (79)	620 (77)	
Mucinous	15 (1)	7 (1)	8 (1)	
Medullar	7 (0)	3 (0)	4 (0)	
Lobular	273 (16)	142 (16)	131 (16)	
Other	82 (5)	36 (4)	46 (6)	
Unknown	1 (0)	0 (0)	1 (0)	
Estrogen receptor				0.769
Positive	1670 (97)	881 (97)	789 (97)	
Negative	47 (3)	26 (3)	21 (3)	
Unknown	1 (0)	1 (0)	0 (0)	
Progesterone receptor				0.454
Positive	1333 (78)	720 (79)	613 (76)	
Negative	320 (19)	165 (18)	155 (19)	
Unknown	65 (4)	23 (3)	42 (5)	
HER2				0.659
Positive	163 (9)	79 (9)	84 (10)	
Negative	595 (35)	302 (33)	293 (36)	
Unknown	960 (56)	527 (58)	433 (54)	
Prior endocrine therapy				0.560
5 years TAM	211 (12)	114 (13)	97 (12)	
5 years AI	492 (29)	250 (27)	242 (30)	
TAM followed by AI	1015 (59)	544 (60)	471 (58)	
Prior chemotherapy				0.795
Yes	1176 (68)	619 (68)	557 (69)	
No	542 (32)	289 (32)	253 (31)	
Breast cancer events				0.783
Local recurrence	28 (12)	17 (14)	11 (10)	
Distant recurrence	150 (65)	79 (64)	71 (66)	
New breast primary	53 (23)	28 (22)	25 (23)	

Table 1 continued

HER = human epidermal growth factor receptor. TAM = tamoxifen. AI = aromatase inhibitor.

Within the translational cohort, the same number of patients (N = 454) received 5 years and 2.5 years of extended letrozole with 10.6% (95% CI 7.1 – 14.0) and 15.5% (95% CI 11.5 – 19.3) of patients having recurrences in the 5-year and 2.5-year arms, respectively. Overall, 75% of patients in the translational cohort completed 80% of their allocated treatment duration. Treatment arms of the translational cohort were also balanced,

recapitulating features of the parent trial, and confirming the translational cohort is essentially unbiased.

BCI [H/I] status predicts benefit from extended endocrine therapy

Analysis of the overall cohort (N = 908) showed that significant differences in outcome from randomized treatment of 2.5 years versus 5 years of letrozole were dependent on classification by BCI [H/I]. Significant benefit from 5 years of extended letrozole was demonstrated in the 47% (N = 429) of patients classified as BCI [H/I]-high (HR 0.42; 95% CI 0.21 – 0.84). The risk of recurrence was 5.9% (95% CI 2.3 – 9.3) and 15.7% (95% CI 9.5 – 21.5) for patients treated with 5 and 2.5 years of letrozole, respectively, demonstrating an absolute benefit of 9.8% for reduction of the risk of recurrence (P = 0.011; **figure 2A**, **figure 3**). In contrast, no statistically significant benefit from5 years versus 2.5 years of letrozole was observed in the 53% (N = 479) of patients classified as BCI [H/I]-low (HR 0.95; 95% CI 0.58 – 1.56). The risk of recurrence was 14.9% (95% CI 9.1 – 20.3) and 15.4% (95% CI 10.1 – 20.4) for patients treated with 5 and 2.5 years of letrozole, respectively (P = 0.835; **figure 2A**, **figure 3**). BCI [H/I] effects on extended endocrine benefit and outcome were consistent across recurrence endpoints of RFI and DFI as well as mortality based on DFS.

BCI [H/I]-status also significantly predicted benefit from extended letrozole in patients treated with primary adjuvant endocrine therapy that included an aromatase inhibitor (primary AI subset, N = 794). In a combined analysis of patients treated with either AI monotherapy or sequenced tamoxifen-AI, BCI [H/I]-high patients experienced a significant absolute benefit from 5 years versus 2.5 years of extended letrozole treatment of 11.8% (P = 0.004; HR 0.34; 95% CI 0.16 – 0.73) whereas BCI [H/I]-low patients had no statistically significant benefit (P = 0.712; HR 0.90; 95% CI 0.53 – 1.55; **figure 2B**). Importantly, treatment to biomarker interaction was significant in both the overall (P = 0.045) and primary AI (P = 0.025) cohorts, adjusting for age, tumor stage, grade, nodal stage, prior endocrine therapy, and prior chemotherapy.

BCI [H/I] prediction of extended endocrine benefit in clinical subsets

In patients with node-positive disease, the 46% (N = 307) that were classified as BCI [H/I]-high demonstrated a statistically significant benefit from 5 years versus 2.5 years of letrozole with a HR of 0.30 (95% CI 0.12 – 0.77) and absolute benefit of 10.8% (P = 0.008), whereas the 54% of node-positive patients (N = 357) classified as BCI [H/I]-low showed no significant benefit (HR 0.88; 95% CI 0.50 – 1.53; P = 0.644; **figure 4A**). Similar findings were observed in the node-negative subset (N = 241; **figure 4B**). Although statistical significance was not reached, possibly due to a limited sample size, a consistent trend in endocrine response was noted in the 50% of node-negative patients (N = 120) classified as BCI [H/I]-high demonstrating a HR of 0.74 (95% CI 0.25 – 2.13) and absolute benefit of 7.4% (P = 0.569). The 50% of node-negative patients (N = 121) classified as BCI [H/I]-low did not show benefit (HR 1.32; 95% CI 0.43 – 4.11; absolute benefit 4.2%; P = 0.626). In addition, a three-way test for statistical interaction evaluating the impact of nodal status on BCI biomarker effect did not show significance (P = 0.624), indicating BCI predictive activity is not dependent on nodal status.

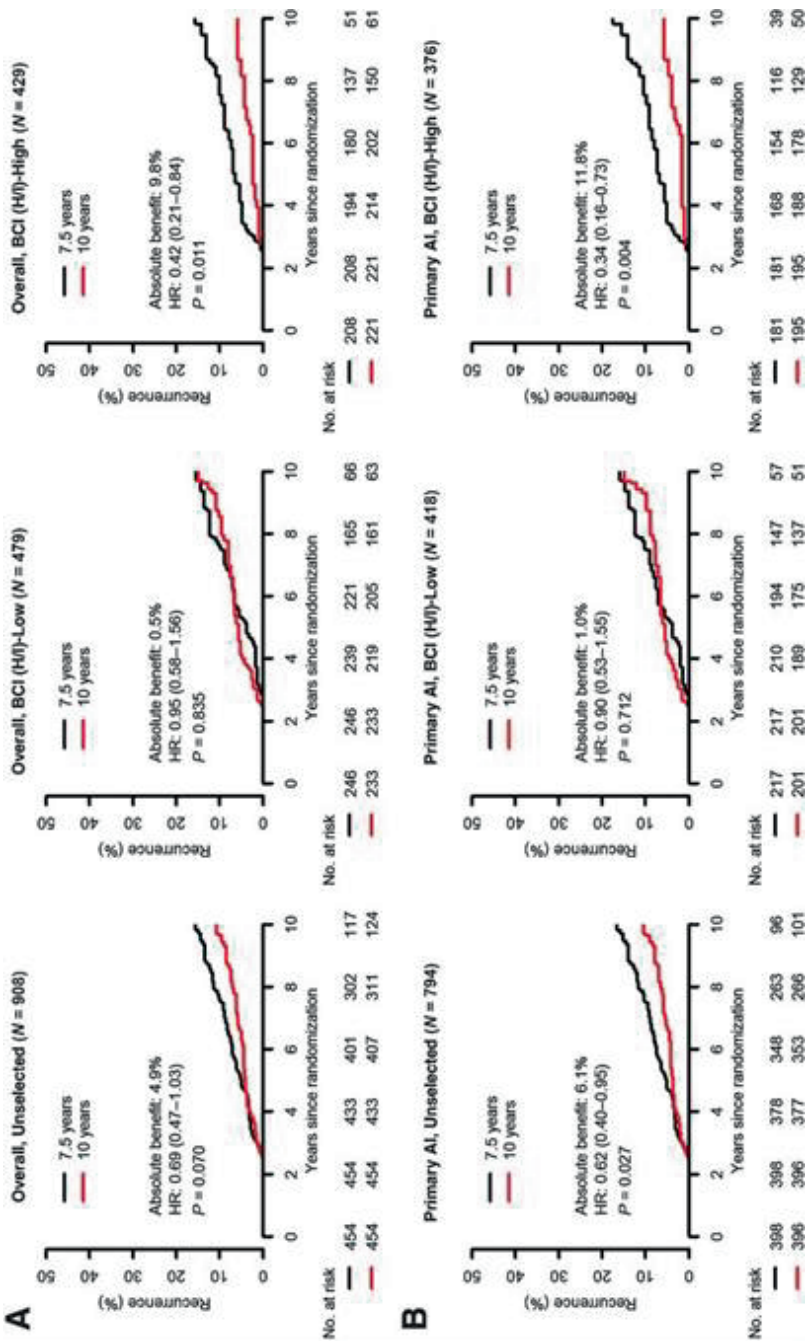

Figure 2: Kaplan-Meier analysis of risk of recurrence comparing 10 years versus 7.5 years of ET by BCI [H/I] groups in the overall cohort (A) and in the primary AI subset (B).

AI = aromatase inhibitor.

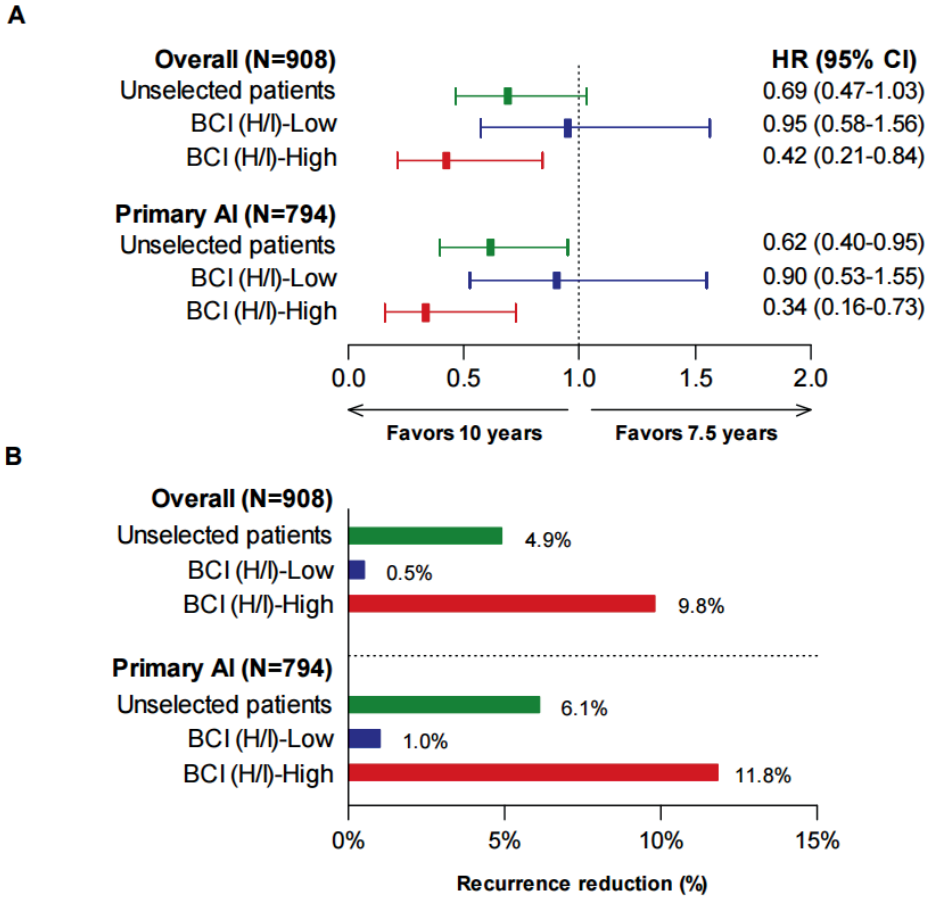

Figure 3: Forest plot of unadjusted hazard ratios of treatment effect (A) and bar graph of absolute recurrence risk reduction (B) by BCI [H/I] group in the overall cohort and the primary AI subset.

Analysis of HR point estimates for patients classified as BCI [H/I]-high were similar across all other clinical subsets by age, tumor stage, grade, prior endocrine therapy, and prior chemotherapy treatment, favoring 10 years of endocrine therapy, whereas those for BCI [H/I]-low patients were generally close to 1 (**figure 5**). One exception was observed in the subset of patients that did not receive prior chemotherapy. Of note, subset analysis of patients treated with primary adjuvant tamoxifen monotherapy was not calculated because of an imbalance in the number of node-positive patients across the two treatment arms, resulting in harm from longer treatment with endocrine therapy in the unselected patients. Overall, the predictive performance of BCI [H/I] was consistent across clinical subsets examined.

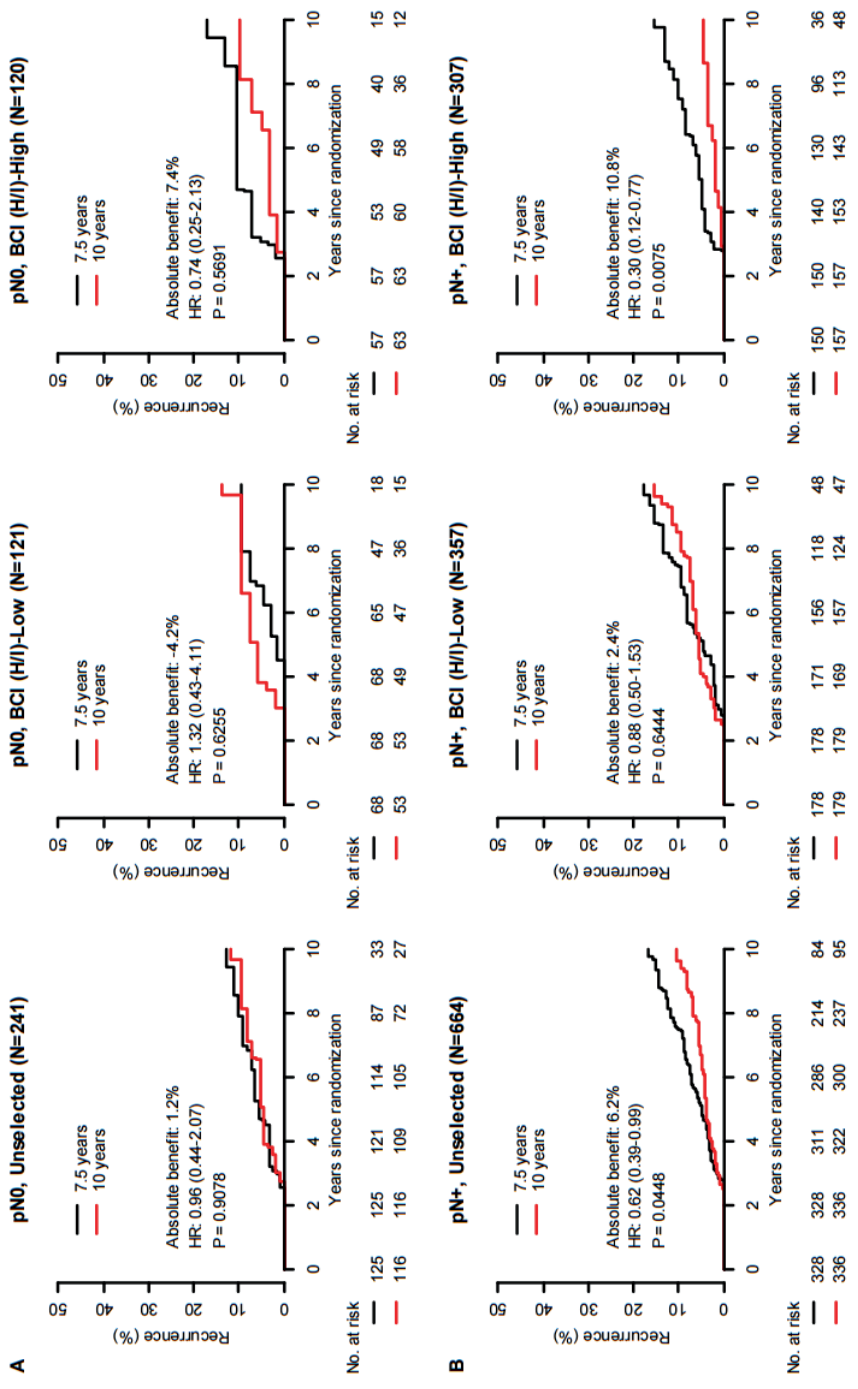

Figure 4: Kaplan-Meier analysis of risk of recurrence comparing 10 years versus 7.5 years of ET by BCI [H/I] group in node-negative (A) and node-positive (B) patients.

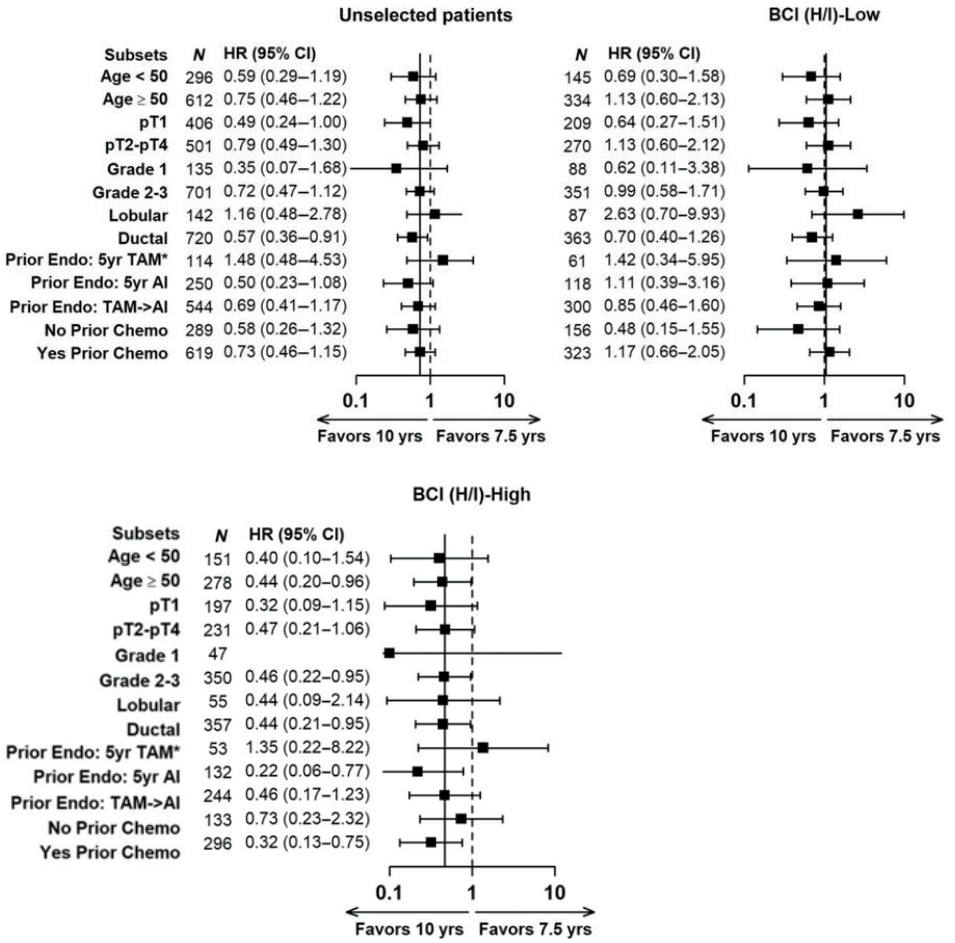

Figure 5: Forest plot of hazard ratios for comparing 10 years versus 7.5 years of ET by BCI [H/I] groups based on RFI in relevant clinical subsets.

BCI [H/I] prediction of extended endocrine benefit beyond clinicopathologic factors

In patients with clinically high-risk features (node-positive and tumor stage 2 or higher), the 46% (N = 162) that were classified as BCI [H/I]-high experienced a statistically significant benefit from 5 years versus 2.5 years of letrozole (HR 0.32; 95% CI 0.10 – 0.98; absolute benefit 12.5%; P = 0.035), whereas the 54% of clinically high-risk patients classified as BCI [H/I]-low (N = 191) did not show significant benefit (P = 0.742; HR 1.13; 95% CI 0.55 – 2.31; **figure 6A**).

Conversely, the 48% of patients (N = 220) in a clinically low-risk subset (tumor stage 1 or grade 1) that were classified as BCI [H/I]-high demonstrated a statistically significant benefit from 5 years versus 2.5 years of letrozole (HR 0.23; 95% CI 0.07 – 0.81) and absolute benefit of 11.9% (P = 0.013), whereas the 52% of clinically low-risk patients

(N = 239) classified as BCI [H/I]-low did not show significant benefit (HR 0.62; 95% CI 0.27 – 1.38; P = 0.235; **figure 6B**).

Distribution of BCI [H/I]-levels in relation to molecular ER, PR, and HER2 expression in the different primary endocrine treatment groups did not demonstrate any strong correlations. Weak negative correlations were observed between BCI [H/I] levels and ER and PR and weak positive correlations between BCI [H/I] and HER2.

DISCUSSION

Clinical trials evaluating extended endocrine therapy to reduce the ongoing residual risk of recurrence that is characteristic of HR+ disease have demonstrated modest benefits with increased morbidity, requiring patient selection to become highly individualized because of diverse prognosis, disease heterogeneity, biological characteristics of the tumor, and patient tolerability.[1-13] To date, the net benefit of extended endocrine therapy based on tumor biology has incorporated individualized assessment of risk but has lacked individualized assessment of endocrine responsiveness to predict the likelihood to benefit from longer durations of endocrine treatment.

Findings from this study further establish the predictive ability of BCI by H/I status to classify patients who demonstrate a high or low degree of endocrine responsiveness with categorical differences in outcome from extended endocrine therapy.

Similar relative improvements in outcome based on RFI by BCI [H/I] category were generally observed in all clinical and pathologic factors examined. One notable exception was observed in the subset analyzed that did not receive prior chemotherapy wherein patients classified as BCI [H/I]-low showed a response to extended endocrine therapy, and the relative response in BCI [H/I]-high patients was less pronounced. The basis of this inconsistency compared with the overall study findings is unclear and may be arbitrary in nature. Consistent with previous data BCI [H/I] expression did not show a strong correlation with canonical endocrine biomarkers, ER and PR.[22,25,26] In addition, multiple translational studies in the ATAC, BIG 1-98, and TEAM trials have reproducibly demonstrated that quantitative ER and PR expression levels in patients with HR+ breast cancer do not predict benefit from endocrine therapy, suggesting that BCI [H/I] predictive effects are predicated on distinct biological mechanisms that are not directly coupled to ER/PR expression levels.[27-29]

Whereas previous studies have established the ability of BCI [H/I] to predict endocrine response in patients treated with tamoxifen in the first five years, the novel finding from this study is the direct validation of BCI [H/I] in patients treated with an AI in the primary adjuvant setting. Importantly, these data align BCI [H/I] to the standard of care for antiestrogen therapy in patients with HR+ early-stage breast cancer that are post-menopausal at diagnosis, which comprise the majority of patients.[30] BCI validation studies consistently evaluated a total of ten years of endocrine therapy; however, the IDEAL study design differed in that it compared a treatment differential of an additional 2.5 years of

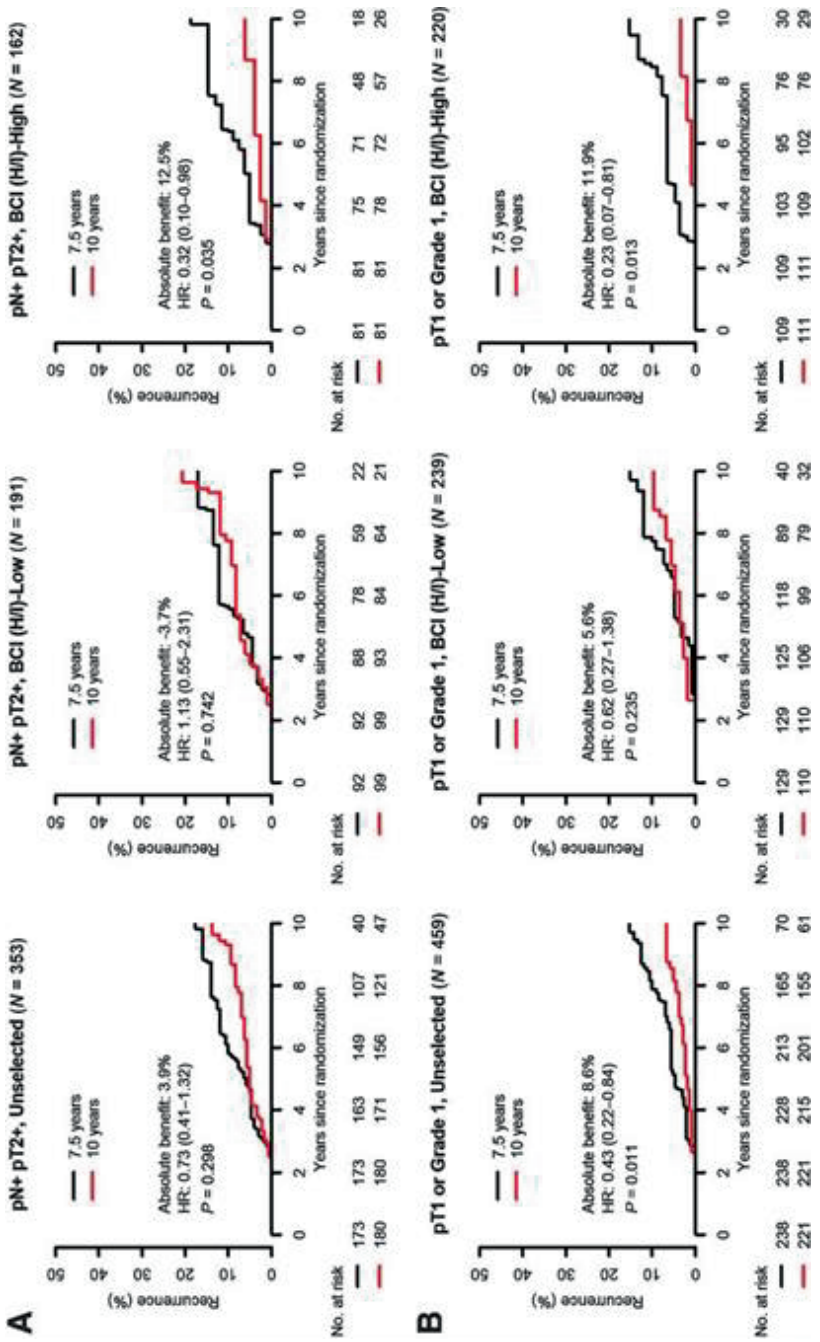

Figure 6: Kaplan-Meier analysis of risk of recurrence comparing 10 years versus 7.5 years of ET by BCI [H/I] group in clinically high risk (A) and clinically low risk (B) patients.

endocrine therapy versus an additional 5 years of endocrine therapy as was investigated in both the MA.17 and aTTom trials.[1,4,6] In comparison with the MA.17 cohort, the patient population evaluated in this study contained a relatively higher proportion of patients with node-positive and grade 3 tumors, whereas the Trans-aTTom study reported on node-positive patients only. BCI biomarker effects across these studies were associated with an approximately two- to threefold improvement in outcome based on the absolute benefit observed in BCI [H/I]-high disease relative to that observed in unselected patients. An important point to note is that results determined from population-based approaches (Trans-aTTom, IDEAL) versus case-controlled methods (MA.17) are regarded as a more accurate estimation of the BCI [H/I] biomarker effect on absolute benefit.[19,22]

Another interesting observation from this analysis is that unlike previous BCI [H/I] results from studies comparing five years versus ten years of therapy, BCI [H/I]-high and -low patients in the 2.5-year arm displayed relatively similar rates of recurrence (approximately 15%) due to the effect of the initial 2.5 years of therapy in the BCI [H/I]-high group. Overall, the data presented herein add to the growing body of evidence demonstrating significant treatment to biomarker interaction between BCI [H/I] and endocrine therapy in a variety of treatment scenarios, and in a manner that is agnostic to antiestrogen approach whether through prevention of ER action or estrogen synthesis. The underlying tumor biology interrogated by BCI [H/I] expression levels may distinguish tumors predominantly driven by estrogen signaling from those with reduced hormone receptor dependence and therefore less responsive to antiestrogen approaches.

Advances in adjuvant endocrine therapy for postmenopausal women include more widespread use of AIs in the adjuvant setting; however, data on the safety and efficacy of AI durations beyond five years of therapy are inconclusive.[3,5,8,9,15,16] Of particular concern are women who have received adjuvant AI monotherapy because longer treatment is associated with additional toxicity including adverse effects on cardiovascular and bone health. Risk stratification by clinicopathologic factors and prognostic biomarkers has been recommended in clinical practice guidelines to help guide patient selection.[18] The American Society of Clinical Oncology guidelines recommend AI treatment for up to a total of ten years for higher risk, node-negative patients, including women with tumor stage 2 or 3 and tumor stage 1c tumors with higher risk prognostic factors, and five years of adjuvant endocrine therapy in total including an AI as sufficient for women with tumor stage 1a and 1b or tumor stage 1c tumors with lower risk prognostic factors. Recommendations also include extending AI-based therapy for up to a total of ten years of adjuvant endocrine treatment for all women with node-positive breast cancer.[18]

However, not all patients with elevated intrinsic risk of recurrence such as those with larger tumors and nodal involvement will benefit equally from extended endocrine therapy. Indeed, findings from this study clearly show that BCI [H/I] identifies 54% of clinically high-risk patients with limited benefit from extended endocrine therapy. Conversely, patient selection based on risk assessment would also assume that improvements in outcome are proportional to level of risk, and that patients with lower risk clinicopathologic features would derive less benefit from extended endocrine therapy.

However, subset analysis of patients with tumor stage 1 or grade 1 tumors demonstrates a similar magnitude in extended endocrine benefit by BCI [H/I] classification as observed in higher risk patients. Moreover, a meta-analysis from the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) demonstrated that HR+ patients across all clinical stages, including those with stage 1 disease, have a significant ongoing risk of late distant recurrence, indicating that risk factors alone are inadequate to individualize patient selection for extended endocrine therapy.[12] These data highlight the distinct clinical utility of BCI [H/I] to provide increased resolution beyond prognostic factors to better individualize the risk-benefit considerations for extended therapy by providing insight into endocrine-responsive biology.

Aside from BCI, several genomic classifiers have been used in clinical practice. The 21-gene recurrence score is predictive for adjuvant chemotherapy benefit, as was more recently demonstrated in the TAILORx trial, but is not predictive of endocrine therapy benefit.[31] In a head-to-head comparison in the Trans-ATAC cohort, BCI, EPclin and ROR each had significant ability to prognosticate late (>5 years) distant recurrence.[32] In addition, the Clinical Treatment Score post-5 years (CTS5), which integrates clinicopathologic factors, was also shown to prognosticate late distant recurrence risk.[33] In a direct comparison between CTS5 and BCI prognostic scores, BCI was able to further stratify those with the intermediate CTS5 risk category into separate risk groups with clinically distinct rates of distant recurrences (12.5% for BCI-high vs. 0% for BCI-low; P = 0.140) indicating that BCI risk stratification provided improved prognostic resolution beyond CTS5.[34] However, these are all prognostic biomarkers. Among currently available genomic signatures, BCI [H/I] is the only assay validated to predict the likelihood of benefit from endocrine therapy. Notably, in a recent analysis of the TEAM and IDEAL clinical study cohorts, CTS5 showed no predictive ability in determining benefit from extended endocrine therapy, including patients that were classified as high-risk (P for interaction = 0.5).[35]

Recently completed studies have concluded that 7 to 7.5 years endocrine therapy in total may be sufficient for many HR+ patients.[1,3,13] However, a critical finding of this study is that BCI [H/I] identified approximately 50% of postmenopausal patients that derived a significant benefit from completing ten years of endocrine therapy (five years of extended AI therapy) versus stopping at 7.5 years (2.5 years of extended letrozole). The clinical implications of these results are that there is a substantial number of women who may derive additional benefit from longer durations of endocrine treatment based on BCI [H/I] status, and that BCI could serve a critical role in the identification of patients who are likely to experience a significant reduction in risk and improved outcomes from prolonging AI treatment to ten years.

Limitations of the study include its retrospective nature and that it was conducted in a parent trial with an open-label design. The study also examined a relatively high-risk early-stage breast cancer population of 73% node-positive patients, with a majority having received chemotherapy. Although BCI [H/I] prediction of endocrine response in node-negative disease has been validated in patients treated with primary adjuvant therapy, additional studies with adequate power to assess BCI [H/I] predictive ability in the

extended endocrine setting are of interest.[21] Strengths of the study include that the translational analysis was prospectively defined in a randomized study with an updated 9.3 years of median follow-up. In addition, the translational cohort contained approximately 50% of the parent trial population, which represents a larger proportion of patients than in previous BCI validation studies.

CONCLUSION

Findings from this study demonstrate significant prediction of extended endocrine benefit based on BCI [H/I] classification in patients treated with contemporary standards of care for primary adjuvant endocrine therapy. In conjunction with previous data from MA.17[19] and Trans-aTTom[22], BCI predictive performance is established across a comprehensive range of treatment scenarios involving tamoxifen and AIs. Clarifying the magnitude and level of efficacy of extended endocrine therapy with approaches that provide additive and distinct information is important to ensure that overtreatment and undertreatment do not occur. BCI may provide the rationale as a standardized molecular tool that measures preferential response and magnitude of benefit to help individualize patient selection for extending endocrine therapy to ten years.

REFERENCES

1. Blok EJ, Kroep JR, Meershoek-Klein Kranenbarg E, et al: Optimal Duration of Extended Adjuvant Endocrine Therapy for Early Breast Cancer; Results of the IDEAL Trial (BOOG 2006-05). J Natl Cancer Inst 110, 2018
2. Davies C, Pan H, Godwin J, et al: Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. Lancet 381:805-16, 2013
3. Gnant M, Steger G, Greil R, et al: Abstract GS3-01: A prospective randomized multi-center phase-III trial of additional 2 versus additional 5 years of anastrozole after initial 5 years of adjuvant endocrine therapy – results from 3,484 postmenopausal women in the ABCSG-16 trial. Cancer Res 78:GS3-01-GS3-01, 2018
4. Goss PE, Ingle JN, Martino S, et al: A randomized trial of letrozole in postmenopausal women after five years of tamoxifen therapy for early-stage breast cancer. N Engl J Med 349:1793-802, 2003
5. Goss PE, Ingle JN, Pritchard KI, et al: Extending Aromatase-Inhibitor Adjuvant Therapy to 10 Years. N Engl J Med 375:209-19, 2016
6. Gray RG, Rea D, Handley K, et al: aTTom: Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years in 6,953 women with early breast cancer. J Clin Oncol 31:5, 2013
7. Jakesz R, Greil R, Gnant M, et al: 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 99:1845-53, 2007
8. Mamounas EP, Bandos H, Lembersky BC, et al: Abstract S1-05: A randomized, double-blinded, placebo-controlled clinical trial of extended adjuvant endocrine therapy (tx) with letrozole (L) in postmenopausal women with hormone-receptor (+) breast cancer (BC) who have completed previous adjuvant tx with an aromatase inhibitor (AI): Results from NRG Oncology/NSABP B-42. Cancer Res 77:S1-05-S1-05, 2017
9. Mamounas EP, Bandos H, Lembersky BC, et al: Use of letrozole after aromatase inhibitor-based therapy in postmenopausal breast cancer (NRG Oncology/NSABP B-42): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 20:88-99, 2019
10. 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 26:1965-71, 2008
11. Munzone E, Colleoni M: Optimal management of luminal breast cancer: how much endocrine therapy is long enough? Ther Adv Med Oncol 10:1758835918777437, 2018
12. Pan H, Gray R, Braybrooke J, et al: 20-Year Risks of Breast-Cancer Recurrence after Stopping Endocrine Therapy at 5 Years. N Engl J Med 377:1836-1846, 2017
13. Tjan-Heijnen VCG, van Hellemond IEG, Peer PGM, et al: Extended adjuvant aromatase inhibition after sequential endocrine therapy (DATA): a randomised, phase 3 trial. Lancet Oncol 18:1502-1511, 2017
14. Goss PE, Ingle JN, Martino S, et al: Randomized trial of letrozole following tamoxifen as extended adjuvant therapy in receptor-positive breast cancer: updated findings from NCIC CTG MA.17. J Natl Cancer Inst 97:1262-71, 2005
15. Gray R: Abstract GS3-03: Effects of prolonging adjuvant aromatase inhibitor therapy beyond five years on recurrence and cause-specific mortality: An EBCTCG meta-analysis of individual patient data from 12 randomised trials including 24,912 women. Cancer Res 79:GS3-03-GS3-03, 2019
16. Blok EJ, Kroep JR, Meershoek-Klein Kranenbarg E, et al: Relevant factors for the optimal duration of extended endocrine therapy in early breast cancer. Breast Cancer Res Treat 168:413-420, 2018
17. Blok EJ, Kroep JR, Meershoek-Klein Kranenbarg E, et al: Treatment decisions and the impact of adverse events before and during extended endocrine therapy in postmenopausal early breast cancer. Eur J Cancer 95:59-67, 2018
18. Burstein HJ, Lacchetti C, Anderson H, et al: Adjuvant Endocrine Therapy for Women With Hormone Receptor-Positive Breast Cancer: ASCO Clinical Practice Guideline Focused Update. J Clin Oncol 37:423-438, 2019
19. Sgroi DC, Carney E, Zarrella E, et al: Prediction of late disease recurrence and extended adjuvant letrozole benefit by the HOXB13/IL17BR biomarker. J Natl Cancer Inst 105:1036-42, 2013
20. Sgroi DC, Sestak I, Cuzick J, et al: Prediction of late distant recurrence in patients with oestrogen-receptor-positive breast cancer: a prospective comparison of the breast-cancer index (BCI) assay, 21-gene recurrence score, and IHC4 in the TransATAC study population. Lancet Oncol 14:1067-1076, 2013
21. Zhang Y, Schnabel CA, Schroeder BE, et al: Breast cancer index identifies early-stage estrogen receptor-positive breast cancer patients at risk for early- and late-distant recurrence. Clin Cancer Res 19:4196-205, 2013
22. Bartlett JMS, Sgroi DC, Treuner K, et al: Breast Cancer Index and prediction of benefit from extended endocrine therapy in breast cancer patients treated in the Adjuvant Tamoxifen-To Offer More? (aTTom) trial. Ann Oncol 30:1776-1783, 2019
23. Ma XJ, Salunga R, Dahiya S, et al: A five-gene molecular grade index and HOXB13:IL17BR are complementary prognostic factors in early stage breast cancer. Clin Cancer Res 14:2601-8, 2008

24. Ma XJ, Hilsenbeck SG, Wang W, et al: The HOXB13:IL17BR expression index is a prognostic factor in early-stage breast cancer. J Clin Oncol 24:4611-9, 2006
25. Zhang Y, Schroeder BE, Jerevall Jannok PL, et al: Abstract P3-06-06: Prediction of benefit from endocrine therapy in ER+ early stage breast cancer: Correlative studies of the breast cancer index HoxB13/IL17BR (H/I) ratio, ER, PR, and HER2 expression in the randomized Stockholm trial. Cancer Res 75:P3-06-06-P3-06-06, 2015
26. Zhang Y, Sestak I, Cuzick JM, et al: Correlation of Breast Cancer Index HOXB13/IL17BR (H/I), ER, PR and HER2 and prediction of relative endocrine benefit from tamoxifen and anastrozole in HR+ breast cancer: A TransATAC study. J Clin Oncol 33:526-526, 2015
27. Bartlett JMS, Brookes CL, Robson T, et al: Estrogen receptor and progesterone receptor as predictive biomarkers of response to endocrine therapy: a prospectively powered pathology study in the Tamoxifen and Exemestane Adjuvant Multinational trial. J Clin Oncol 29:1531-1538, 2011
28. Dowsett M, Allred C, Knox J, et al: Relationship between quantitative estrogen and progesterone receptor expression and human epidermal growth factor receptor 2 (HER-2) status with recurrence in the Arimidex, Tamoxifen, Alone or in Combination trial. J Clin Oncol 26:1059-65, 2008
29. Viale G, Regan MM, Maiorano E, et al: Prognostic and predictive value of centrally reviewed expression of estrogen and progesterone receptors in a randomized trial comparing letrozole and tamoxifen adjuvant therapy for postmenopausal early breast cancer: BIG 1-98. J Clin Oncol 25:3846-52, 2007
30. Howlader N, Noone AM, Krapcho M, et al: SEER Cancer Statistics Review, 1975-2017, National Cancer Institute. Bethesda, MD. https://seer.cancer.gov/csr/1975_2017/, 2019
31. Sparano JA, Gray RJ, Makower DF, et al: Adjuvant Chemotherapy Guided by a 21-Gene Expression Assay in Breast Cancer. N Engl J Med 379:111-121, 2018
32. Sestak I, Buus R, Cuzick J, et al: Comparison of the Performance of 6 Prognostic Signatures for Estrogen Receptor-Positive Breast Cancer: A Secondary Analysis of a Randomized Clinical Trial. JAMA Oncol 4:545-553, 2018
33. Dowsett M, Sestak I, Regan MM, et al: Integration of Clinical Variables for the Prediction of Late Distant Recurrence in Patients With Estrogen Receptor-Positive Breast Cancer Treated With 5 Years of Endocrine Therapy: CTS5. J Clin Oncol 36:1941-1948, 2018
34. Sgroi D, Zhang Y, Schnabel CA: Comparative performance of Breast Cancer Index (BCIN+) and CTS5 in hormone receptor-positive (HR+) lymph node-positive (N+) breast cancer patients with one to three positive nodes (N1). J Clin Oncol 37:555, 2019
35. Noordhoek I, Blok EJ, Meershoek-Klein Kranenbarg E, et al: Overestimation of Late Distant Recurrences in High-Risk Patients With ER-Positive Breast Cancer: Validity and Accuracy of the CTS5 Risk Score in the TEAM and IDEAL Trials. J Clin Oncol 38:3273-3281, 2020

Chapter 5

VALIDATION OF THE 70-GENE SIGNATURE TEST (MAMMAPRINT) TO IDENTIFY BREAST CANCER PATIENTS AGED ≥70 YEARS WITH ULTRALOW RISK OF DISTANT RECURRENCE

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ABSTRACT

Introduction

When risk estimation in older patients with hormone receptor positive breast cancer (HR+BC) is based on the same factors as in younger patients, age-related factors regarding recurrence risk and other-cause mortality are not considered. Genomic risk assessment could help identify patients with ultralow risk BC who can forgo adjuvant treatment. However, assessment tools should be validated specifically for older patients. This study aims to determine whether the 70-gene signature test (MammaPrint) can identify patients with HR+BC aged ≥70 years with ultralow risk for distant recurrence.

Materials and methods

Inclusion criteria: ≥70 years; invasive HR+BC; T1-2N0-3M0. Exclusion criteria: HER2+BC; neoadjuvant therapy. MammaPrint assays were performed following standardized protocols. Clinical risk was determined with St. Gallen risk classification.

Primary endpoint was 10-year cumulative incidence rate of distant recurrence in relation to genomic risk. Subdistribution hazard ratios (sHR) were estimated from Fine and Gray analyses. Multivariate analyses were adjusted for adjuvant endocrine therapy and clinical risk.

Results

This study included 418 patients, median age 78 years (interquartile range [IQR] 73-83). Sixty percent of patients were treated with endocrine therapy. MammaPrint classified 50 patients as MammaPrint-ultralow, 224 patients as MammaPrint-low, and 144 patients as MammaPrint-high risk. Regarding clinical risk, 50 patients were classified low, 237 intermediate, and 131 high. Discordance was observed between clinical and genomic risk in 14 MammaPrint-ultralow risk patients who were high clinical risk, and 84 patients who were MammaPrint-high risk, but low or intermediate clinical risk. Median follow-up was 9.2 years (IQR 7.9-10.5).

The 10-year distant recurrence rate was 17% (95% confidence interval [CI] 11-23) in MammaPrint-high risk patients, 8% (4-12) in MammaPrint-low (HR 0.46; 95%CI 0.25-0.84), and 2% (0-6) in MammaPrint-ultralow risk patients (HR 0.11; 95%CI 0.02-0.81). After adjustment for clinical risk and endocrine therapy, MammaPrint-high risk patients still had significantly higher 10-year distant recurrence rate than MammaPrint-low (sHR 0.49; 95%CI 0.26-0.90) and MammaPrint-ultralow patients (sHR 0.12; 95%CI 0.02-0.85). Of the 14 MammaPrint-ultralow, high clinical risk patients none developed a distant recurrence.

Discussion

These data add to the evidence validating MammaPrint's ultralow risk threshold. Even in high clinical risk patients, MammaPrint-ultralow risk patients remained recurrence-free ten years after diagnosis. These findings justify future studies into using MammaPrint to individualize adjuvant treatment in older patients.

INTRODUCTION

As the general population ages, breast cancer is increasingly becoming a disease of older women. A third of all patients diagnosed with breast cancer are aged ≥70 years, and in this growing population two specific age-related issues arise.[1]

Firstly, with higher age, the proportion of hormone receptor positive (HR+) tumors increases, and these tumors are characterized by late recurrences. Two-thirds of distant recurrences present after five years, and the recurrence risk accumulates until 20 years after diagnosis.[2,3] However, in older patients, the recurrence risk is inversely correlated to their age and the competing risk of other-cause mortality.[4] Secondly, older patients are usually more frail than younger patients, may experience more adverse events of cancer treatment, and are at higher risk of hospitalization and long-term loss of quality of life.[5,6]

Nevertheless, most older women with breast cancer are treated according to standard treatment guidelines.[7] Age-specific factors are often not taken into account, which may lead to significant overtreatment in this population.[8] Thus, new tools are needed, that accurately estimate recurrence risk in older patients with breast cancer.

The 70-gene signature test (MammaPrint, MP) is a genomic risk profile based on microarray gene expression. Previous studies showed that MammaPrint may be used to de-escalate the use of chemotherapy and endocrine therapy (ET) in genomic low and ultralow risk patients, respectively.[9-11] However, these trials did not routinely include patients aged ≥70 years. This study aims to determine if MammaPrint can be used to accurately estimate recurrence risk within the older population, where breast cancer is increasingly common.

METHODS

Details on the FOCUS cohort (Female breast cancer in the elderly: Optimizing Clinical guidelines USing clinic-pathological and molecular data) have been described previously.[12] Briefly, it is a retrospective population-based observational cohort, which included all consecutive women aged ≥65 years and diagnosed with breast cancer between 1997 and 2004 in the West region of the Comprehensive Cancer Center of the Netherlands. The FOCUS cohort was approved by the scientific committee of the Netherlands Comprehensive Cancer Registry, waiving the need for informed consent because all data has been anonymized.

Data on patient, tumor and treatment characteristics, adverse events, recurrences, and death were recorded from medical charts. Tumor samples were collected from biopsies and excised material from surgery. Sections were formalin-fixed paraffin-embedded (FFPE) and stored in the research laboratory of the department of Pathology, Leiden University Medical Center.

A subset of patients from this cohort was analyzed to examine the prognostic ability of MammaPrint in older women with HR+ breast cancer.

Inclusion criteria for this analysis were: aged ≥70 years; invasive breast cancer; T1-2N0-3M0; any histological tumor grade. Exclusion criteria were: HR negative; human epidermal growth factor receptor 2 positive (HER2+); neoadjuvant therapy. Tumor specimens had to be available for genomic profiling.

FFPE tumor samples of eligible patients were analyzed according to standardized protocols and blinded to clinical characteristics. Samples were categorized as MP-ultralow risk (MammaPrint Index [MPI] ≥0.355), MP-low risk (0 < MPI <0.355), or MP-high risk (MPI ≤ 0) of developing distant recurrences. These thresholds have been previously developed and validated.[9,10]

Determination of patients' clinical risk was based on the St. Gallen risk classification.[13] Briefly, patients were low risk if they had T1a-bN0 grade 1-2, or T1cN0 grade 1 disease. Patients were intermediate risk with T1cN0 grade 2 disease, T2N0, T1N1, or grade 3 disease, and high risk with T2N1 or N2 disease (**figure 1**).

The primary endpoint was 10-year cumulative incidence rate of distant recurrences (DR). Secondary endpoints were 10-year overall mortality rate, defined as death of any cause, and multivariate analyses adjusted for clinical risk, and use of adjuvant ET.

Statistical analyses

For comparison of baseline characteristics between risk groups, Fisher's exact tests were used. Cumulative incidences of DR were estimated using competing risk survival analyses, in which DR were the event of interest, and death before recurrence was considered a competing event. The association between genomic risk and DR rate was assessed by performing univariate and multivariate Fine and Gray competing risk regression analyses, and the effects were expressed as subdistribution hazard ratios (sHR) with corresponding 95% confidence intervals (CI).[14] The Fine and Gray analysis is a proportional hazards model that is analogous to the Cox proportional hazard model, except that it models a hazard function from a cumulative incidence function instead of Kaplan-Meier survival estimates.

Overall mortality was estimated from Kaplan-Meier survival analyses, and P-values were derived from log-rank tests. Hazard ratios (HRs) and corresponding 95% CI were estimated from univariate and multivariate Cox regression models. Two models were used for multivariate analyses; model 1 was adjusted for clinical risk, and model 2 was also adjusted for hormone receptor status and use of adjuvant ET. P-values less than 0.05 were considered statistically significant. Analyses were performed using SPSS version 24.0 and STATA/SE version 16.

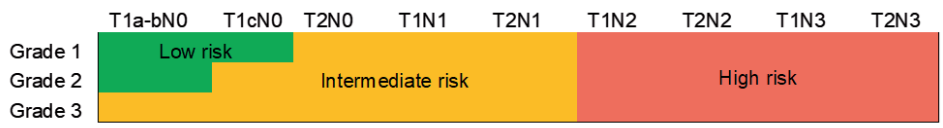

Figure 1: Clinical risk classification based on the St. Gallen criteria

RESULTS

Between 1997 and 2004, 2,095 women aged ≥65 years were diagnosed with any stage breast cancer in the West region of the Comprehensive Cancer Center of the Netherlands and were included in the FOCUS cohort. Of those patients, 1,195 were excluded from this study because they were younger than 70 years, had T3 or T4 tumors, had metastatic disease at diagnosis, had HR– or HER2+ tumors, or had received neoadjuvant therapy. Of the 900 eligible patients, 482 did not have sufficient tumor material for genomic testing, and thus the 418 remaining patients were included in this study (**figure 2**). No significant differences were observed in age, hormone receptor expression, and adjuvant treatment strategy between the 418 included patients and the 482 excluded but eligible patients. Excluded patients did have significantly smaller tumors, lower histological grade, less lymph node involvement, and more often did not undergo surgery than included patients (**table 1**). Specifically, the smaller tumor size and less extensive surgery may explain why these patients did not have sufficient tumor material for genomic testing.

Baseline characteristics of the included patients are described in **table 2**. Median age was 78 years (interquartile range [IQR] 73-83). Local treatment was a mastectomy in 272 patients (65%), breast conserving surgery (BCS) with radiotherapy in 99 patients (24%) and BCS without radiotherapy in 32 (8%). Fifteen patients (3%) did not undergo any surgery. Most patients (N=252; 60%) were treated with adjuvant ET, only 22 patients (5%) received adjuvant chemotherapy, and 164 patients (39%) did not receive any systemic treatment. Approximately 39% of patients aged 70-75 years (N=58/149) had screening-detected cancers. National guidelines in the Netherlands discourage routine breast cancer screening in women aged ≥76 years.[15] Median follow-up was 9.2 years (IQR 7.9 – 10.5).

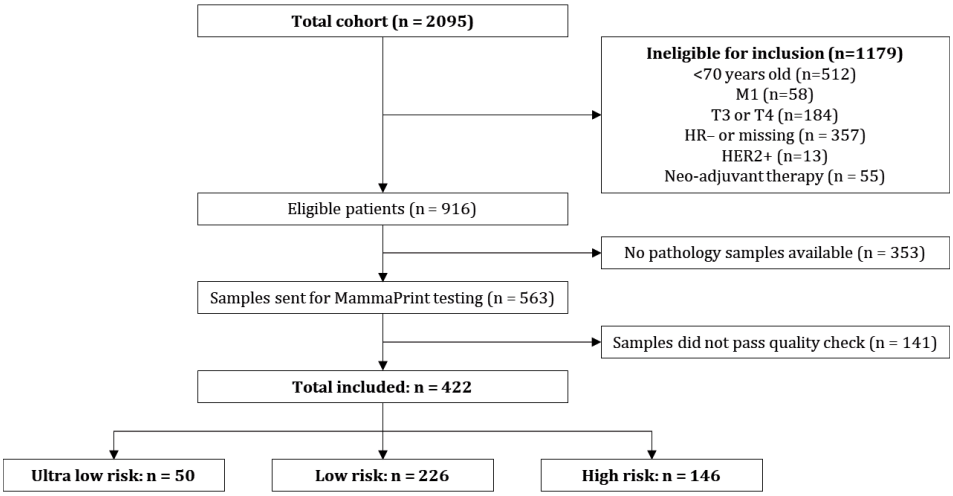

Figure 2: CONSORT diagram.

HR– = hormone receptor negative. HER2+ = human epidermal growth factor receptor 2 positive.

	Eligible (n=900)[a]	Excluded (n =482)[b]	Included (n =418)[c]	P
Age (years)				0.585
70-74	280 (31)	149 (31)	131 (31)	
75-79	251 (28)	134 (28)	117 (28)	
80-84	204 (23)	116 (24)	88 (21)	
85-89	121 (13)	64 (13)	57 (14)	
≥90	44 (5)	19 (4)	25 (6)	
Year of diagnosis				0.378
1997-2000	406 (45)	224 (46)	182 (44)	
2001-2004	494 (55)	258 (54)	236 (56)	
Cancer detection method				0.290
Screening-detected	141 (16)	80 (17)	61 (15)	
Symptom-detected	522 (58)	270 (56)	252 (60)	
Unknown	237 (26)	132 (27)	105 (25)	
Tumor stage				<0.001
T1	430 (48)	249 (52)	181 (43)	
T2	468 (52)	232 (48)	236 (56)	
Unknown	2 (0)	1 (0)	1 (0)	
Nodal status				<0.001
N0/N0(i+)	575 (64)	337 (70)	238 (57)	
N1	234 (26)	101 (21)	133 (32)	
N2	48 (5)	22 (5)	26 (6)	
N3	21 (2)	9 (2)	12 (3)	
Unknown	22 (2)	13 (3)	9 (2)	
Histological tumor grade				0.045
1	140 (16)	85 (18)	55 (13)	
2	302 (34)	147 (30)	155 (37)	
3	166 (18)	81 (17)	85 (20)	
Unknown	292 (32)	169 (35)	123 (29)	
Histological subtype				0.031
Ductal	670 (74)	344 (71)	326 (78)	
Lobular	100 (11)	55 (11)	45 (11)	
Other	130 (14)	83 (17)	47 (11)	
Hormone receptor status				0.854
ER+/PR+	533 (59)	282 (59)	251 (60)	
ER+/PR-	209 (23)	111 (23)	98 (23)	
ER-/PR+	18 (2)	11 (2)	7 (2)	
Unknown	140 (16)	78 (16)	62 (15)	
Most extensive surgery				0.002
None	57 (6)	42 (9)	15 (3)	
Breast conserving with RT	230 (26)	131 (27)	99 (24)	
Breast conserving without RT	54 (6)	22 (5)	32 (8)	
Mastectomy	559 (62)	287 (59)	272 (65)	
Adjuvant chemotherapy				0.323
No	845 (94)	449 (93)	396 (95)	
Yes	55 (6)	33 (7)	22 (5)	
Adjuvant endocrine therapy				0.268
No	375 (42)	209 (43)	166 (40)	
Yes	525 (58)	273 (57)	252 (60)	

Table 1: Baseline characteristics of eligible patients from the FOCUS cohort

P-values are derived from Fisher's exact tests and Mann-Whitney U tests. All values are N (%).

ER = estrogen receptor. PR = progesterone receptor. RT = radiotherapy.

[a] All patients who conform to the inclusion criteria.

[b] Eligible patients who had insufficient tumor material available for MammaPrint testing, and were therefore excluded.

[c] Eligible patients who are included in the present study.

	Included patients (n=418)	MP-high (n=144)	MP-low (n=224)	MP-ultralow (n=50)	P
Age (years)					0.103
70-74	131 (31)	46 (32)	70 (31)	15 (30)	
75-79	117 (28)	46 (32)	57 (25)	14 (28)	
80-84	88 (21)	19 (13)	60 (27)	9 (18)	
85-89	57 (14)	22 (15)	28 (13)	7 (14)	
≥90	25 (6)	11 (8)	9 (4)	5 (10)	
Year of diagnosis					0.236
1997-2000	182 (44)	70 (49)	94 (42)	18 (36)	
2001-2004	236 (56)	74 (51)	130 (58)	32 (64)	
Cancer detection method					0.094
Screening-detected	61 (15)	13 (9)	40 (18)	8 (16)	
Symptom-detected	252 (60)	89 (62)	130 (58)	33 (66)	
Unknown	105 (25)	42 (29)	54 (24)	9 (18)	
Tumor stage					0.009
T1	181 (43)	53 (37)	111 (50)	17 (34)	
T2	236 (56)	90 (63)	113 (50)	33 (66)	
Unknown	1 (0)	1 (1)	0	0	
Nodal status					0.006
N0/N0(i+)	238 (57)	65 (45)	140 (63)	33 (66)	
N1	133 (32)	62 (43)	56 (25)	15 (30)	
N2	26 (6)	9 (6)	16 (7)	1 (2)	
N3	12 (3)	5 (3)	7 (3)	0	
Unknown	9 (2)	4 (3)	5 (2)	1 (2)	
Histological tumor grade					<0.001
1	55 (13)	5 (3)	39 (17)	11 (22)	
2	155 (37)	42 (29)	92 (41)	21 (42)	
3	85 (20)	53 (37)	31 (14)	1 (2)	
Unknown	123 (29)	44 (31)	62 (28)	17 (34)	
Histological subtype					0.267
Ductal	326 (78)	120 (83)	170 (76)	36 (72)	
Lobular	45 (11)	10 (7)	29 (13)	6 (12)	
Other	47 (11)	14 (10)	25 (11)	8 (16)	
Hormone receptor status					0.014
ER+/PR+	251 (60)	71 (49)	145 (65)	35 (70)	
ER+/PR-	98 (23)	45 (31)	46 (21)	7 (14)	
ER-/PR+	7 (2)	5 (3)	1 (0)	1 (2)	
Unknown	62 (15)	23 (16)	32 (14)	7 (14)	
Most extensive surgery					0.222
None	15 (3)	6 (4)	5 (2)	4 (8)	
Breast conserving with RT	99 (24)	28 (19)	62 (28)	9 (18)	
Breast conserving without RT	32 (8)	13 (9)	16 (7)	3 (6)	
Mastectomy	272 (65)	97 (68)	141 (63)	34 (68)	
Adjuvant chemotherapy					0.421
No	396 (95)	135 (94)	215 (96)	46 (92)	
Yes	22 (5)	9 (6)	9 (4)	4 (8)	
Adjuvant endocrine therapy					0.001
No	166 (40)	40 (28)	102 (46)	24 (48)	
Yes	252 (60)	104 (72)	122 (54)	26 (52)	

Table 2: Baseline characteristics of included patients from the FOCUS cohort

P-values are derived from Fisher's exact tests and Mann-Whitney U tests. All values are N (%).

ER = estrogen receptor. PR = progesterone receptor. RT = radiotherapy.

MammaPrint classified 144 patients (34%) as MP-high risk, 224 patients (54%) as MP-low risk, and 50 patients (12%) as MP-ultralow risk. Patients with MP-ultralow risk tumors were less often treated with adjuvant ET than patients with MP-low and MP-high risk tumors (52% vs 54% vs 72%, respectively). The distribution of MammaPrint risk categories was similar in screening-detected versus symptom-detected tumors and also to what is reported in the literature (**table 2**).[16]

Ten years after diagnosis, 17% (95%CI 11–23) of MP-high risk patients developed DR. The 10-year DR rate in MP-low risk patients was 8% (4–12; HR 0.46, 95%CI 0.25–0.84), and 2% (0–6; HR 0.11, 95%CI 0.02–0.81) in MP-ultralow risk patients (**table 3**; **figure 2A**). These differences remained statistically significant in multivariate analyses adjusted for clinical risk and use of adjuvant ET (**table 3**). Death without recurrence occurred at similar rates throughout the genomic risk groups. Overall mortality was significantly higher for MP-high risk patients (68%) than for MP-low and MP-ultralow risk patients (52% and 49%, respectively; P=0.001; **figure 2B**).

When applying the St. Gallen risk classification, 50 patients were deemed clinically low risk, 237 were clinically intermediate risk, and 131 were clinically high risk. Discordance was observed between clinical and genomic risk in 14 MP-ultralow risk patients who were deemed clinically high risk, and 84 patients who were MP-high risk, but clinically low or intermediate risk.

Among clinically high risk patients, MP-high risk patients had a higher 10-year DR rate (22% [95%CI 12–32]) than MP-low risk (9% [2–16]; HR 0.39, 95%CI 0.14–1.10) or MP-ultralow risk patients (0%, HR 0; **figure 2C**). In clinically low and intermediate risk patients, the 10-year DR rate was 13% (95%CI 6–20) in MP-high risk patients, 8% (4–12; HR 0.57, 95%CI 0.26–1.28) in MP-low risk patients, and 3% (0–8; HR 0.20, 95%CI 0.03–1.55) in MP-ultralow risk patients (**table 4**; **figure 2D**).

	MP-low risk		MP-ultralow risk	
	HR (95% CI)[a]	**HR (95% CI)[b]**	**HR (95% CI)[a]**	**HR (95% CI)[b]**
Any recurrence	0.46 (0.27 - 0.78)	0.49 (0.28 - 0.84)	0.17 (0.04 - 0.69)	0.17 (0.04 - 0.71)
Locoregional recurrence	0.48 (0.20 - 1.14)	0.57 (0.24 - 1.38)	0.24 (0.03 - 1.87)	0.30 (0.04 - 2.21)
Distant recurrence	0.49 (0.26 - 0.90)	0.49 (0.26 - 0.93)	0.12 (0.02 - 0.85)	0.12 (0.02 - 0.82)
Death without recurrence	0.87 (0.63 - 1.21)	0.84 (0.60 - 1.19)	1.10 (0.67 - 1.79)	1.05 (0.63 - 1.74)
Overall mortality	0.63 (0.48 - 0.84)	0.61 (0.46 - 0.82)	0.63 (0.40 - 0.99)	0.61 (0.39 - 0.97)

Table 3: Multivariate analysis of primary and secondary endpoints in all patients.

The MP-high risk group is used as reference. Subdistribution hazard ratios (HR) for recurrence rates and corresponding 95% confidence intervals (CI) are derived from Fine and Gray analyses. HR for mortality are derived from Cox regression models.

[a] Model 1 is adjusted for clinical risk based on St. Gallen criteria.

[b] Model 2 is adjusted for hormone receptor status and use of adjuvant endocrine therapy.

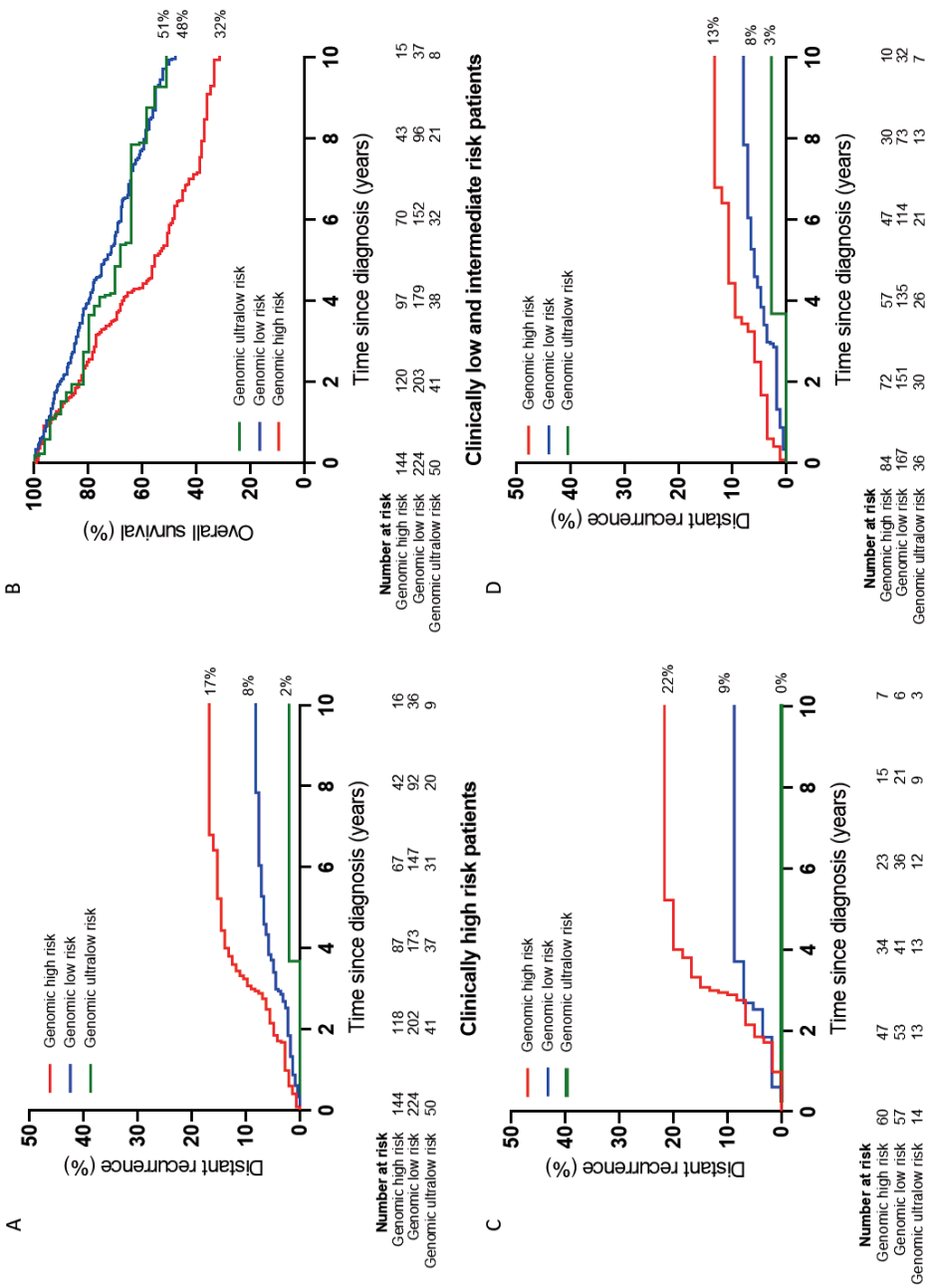

Figure 2: Distant recurrences (A) and overall survival (B) in all patients, distant recurrences in clinically high (C) and clinically low and intermediate risk (D) patients.

	MP-high risk		MP-low risk			MP-ultralow risk		
	N events	10-year rate (95% CI)	N events	10-year rate (95% CI)	(s)HR (95% CI)	N events	10-year rate (95% CI)	(s)HR (95% CI)
All patients	144		224			50		
Distant recurrence	24	17% (11-23)	18	8% (4-12)	0.46 (0.25-0.84)	1	2% (0-6)	0.11 (0.02-0.81)
Death without recurrence	69	46% (37-55)	85	42% (34-50)	0.87 (0.63-1.20)	22	46% (31-61)	1.09 (0.67-1.78)
Overall mortality	93	68% (59-77)	101	52% (44-60)	0.62 (0.47-0.81)	22	49% (33-65)	0.63 (0.40-0.98)
Clinically low, intermediate risk patients	84		167			36		
Distant recurrence	11	13% (6-20)	13	8% (4-12)	0.57 (0.26-1.28)	1	3% (0-8)	0.20 (0.03-1.55)
Death without recurrence	40	46% (34-58)	61	39% (31-47)	0.87 (0.58-1.32)	19	54% (36-72)	1.39 (0.78-2.48)
Overall mortality	51	68% (56-80)	72	48% (39-57)	0.64 (0.45-0.91)	19	59% (41-77)	0.85 (0.50-1.42)
Clinically high risk patients	60		57			14		
Distant recurrence	13	22% (12-32)	5	9% (2-16)	0.39 (0.14-1.10)	0	0%	0
Death without recurrence	29	45% (32-58)	24	57% (38-76)	0.89 (0.52-1.54)	3	21% (0-43)	0.56 (0.20-1.58)
Overall mortality	42	70% (58-82)	29	69% (51-87)	0.62 (0.39-0.99)	3	21% (0-43)	0.28 (0.10-0.79)

Table 4: Primary and secondary endpoints in all patients and stratified for clinical risk.

Recurrence rates are cumulative incidences estimated from competing risk analyses. Subdistribution hazard ratios (sHR) and corresponding 95% confidence intervals (CI) are estimated from Fine and Gray analyses. Mortality is estimated from Kaplan-Meier analyses, and HR with corresponding 95%CI are estimated from Cox regression models. Bold HR represent statistical significance.

DISCUSSION

Genomic risk assessment tools like the 70-gene signature test can be used to accurately estimate DR risk in patients with breast cancer aged ≥70 years. MammaPrint-ultralow risk patients had excellent clinical outcome up to ten years after diagnosis, despite 48% not receiving any systemic therapy. Significantly more MP-high risk patients developed DR, even though 72% of MP-high risk patients did receive adjuvant ET. Multivariate analyses adjusted for ET usage still showed significantly lower 10-year DR rates for MP-ultralow risk patients.

This is the first study examining a gene-expression profile in the older population. Our data show that genomic ultralow risk patients had excellent long-term outcomes even if clinically high risk. This may be explained by the discontinuation of routine screening at the age of 75. With increasing age, breast cancer is more often diagnosed at a higher clinical stage as women participate at reduced rates in routine screening. [17] Therefore, larger tumors and more elaborate regional spread may not be signs of aggressive tumor biology, but rather of late diagnoses. Thus, using only clinical parameters to determine recurrence risk for older patients may result in inaccurate risk estimation. Our data show that the 70-gene signature test provides more accurate risk assessment, and it seems reasonable to

suggest that all older patients with ultralow risk breast cancer could forgo adjuvant endocrine therapy. This would apply to patients with a high risk of competing events, but also to those who are relatively fit, since the risk of developing a distant recurrence seems extremely low. Prospective randomized trials should examine whether MammaPrint can indeed be used to decide if these older patients can safely forgo adjuvant ET.

The use of other genetic profiling tools, such as the Breast Cancer Index, PAM50, Prosigna and EndoPredict as prognostic or predictive tools in the older patient population has not been validated as of yet, either.[18] The prognostic ability of the Oncotype Dx has been validated in older patients with HR+ breast cancer.[19] However, it remains unresolved whether Oncotype Dx can be used to influence decisions regarding chemo- and endocrine therapy.[20] Our study presents an important addition to the evidence of using genetic profiling in this specific patient population.

Limitations of this study are the retrospective nature of the FOCUS cohort and the small sample size. Adjuvant treatment was decided by the treating physician, which may have introduced confounding by indication. Furthermore, due to adhering to Dutch treatment guidelines, the use of endocrine therapy was low when compared to other European countries.[8] Consequently, caution should be used when extrapolating these results to patient populations outside of the Netherlands, and these results should be confirmed in prospective trials.

Nonetheless, this study presents unique and valuable results for geriatric oncology practice, as it is the first analysis of the 70-gene signature test in patients aged ≥70 years. Another clear strength of this study is the use of real-life data from a population based cohort, which provides more accurate representation of patients in clinical practice than a trial population.[21]

CONCLUSION

This analysis adds to the growing body of data demonstrating the validity of MammaPrint's ultralow risk threshold. Women with ultralow risk, regardless of clinical stage or grade, had an extremely low risk of recurrence. These data are especially relevant for clinicians working with older patients, who may be frailer and more susceptible to adverse effects of treatment.

REFERENCES

1. Dutch Cancer Registry (NKR), IKNL. Obtained through https://www.iknl.nl/nkr-cijfers, accessed on April 4 2021.
2. Al Zaman AS, Mughal SA, Al Zaman YS, et al: Correlation between hormone receptor status and age, and its prognostic implications in breast cancer patients in Bahrain. Saudi Med J 37:37-42, 2016
3. Pan H, Gray R, Braybrooke J, et al: 20-Year Risks of Breast-Cancer Recurrence after Stopping Endocrine Therapy at 5 Years. N Engl J Med 377:1836-1846, 2017
4. de Boer AZ, van der Hulst HC, de Glas NA, et al: Impact of Older Age and Comorbidity on Locoregional and Distant Breast Cancer Recurrence: A Large Population-Based Study. Oncologist 25:e24-e30, 2020
5. Klepin H, Mohile S, Hurria A: Geriatric assessment in older patients with breast cancer. J Natl Compr Canc Netw 7:226-36, 2009
6. Lee SY, Seo JH: Current Strategies of Endocrine Therapy in Elderly Patients with Breast Cancer. Biomed Res Int 2018:808-820, 2018
7. Cardoso F, Kyriakides S, Ohno S, et al: Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 30:1194-1220, 2019
8. Derks MGM, Bastiaannet E, Kiderlen M, et al: Variation in treatment and survival of older patients with non-metastatic breast cancer in five European countries: a population-based cohort study from the EURECCA Breast Cancer Group. Br J Cancer 119:121-129, 2018
9. Cardoso F, van't Veer LJ, Bogaerts J, et al: 70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer. N Engl J Med 375:717-29, 2016
10. Esserman LJ, Yau C, Thompson CK, et al: Use of Molecular Tools to Identify Patients With Indolent Breast Cancers With Ultralow Risk Over 2 Decades. JAMA Oncology 3:1503-1510, 2017
11. Lopes Cardozo JMN, Drukker CA, Rutgers EJT, et al: Outcome of Patients With an Ultralow-Risk 70-Gene Signature in the MINDACT Trial. J Clin Oncol 40:1335-1345, 2022
12. de Glas NA, Kiderlen M, Bastiaannet E, et al: Postoperative complications and survival of elderly breast cancer patients: a FOCUS study analysis. Breast Cancer Res Treat 138:561-9, 2013
13. Goldhirsch A, Wood WC, Gelber RD, et al: Progress and promise: highlights of the international expert consensus on the primary therapy of early breast cancer 2007. Ann Oncol 18:1133-44, 2007
14. Putter H, Schumacher M, van Houwelingen HC: On the relation between the cause-specific hazard and the subdistribution rate for competing risks data: The Fine-Gray model revisited. Biom J 62:790-807, 2020
15. National Institute for Public Health and the Environment (RIVM). Bevolkingsonderzoek borstkanker. . 2020
16. Lopes Cardozo JMN, Schmidt MK, van 't Veer LJ, et al: Combining method of detection and 70-gene signature for enhanced prognostication of breast cancer. Breast Cancer Res Treat 189:399-410, 2021
17. Cappellani A, Di Vita M, Zanghì A, et al: Prognostic factors in elderly patients with breast cancer. BMC Surg 13 Suppl 2:S2, 2013
18. Gordon-Craig S, Parks RM, Cheung KL: The Potential Use of Tumour-Based Prognostic and Predictive Tools in Older Women with Primary Breast Cancer: A Narrative Review. Oncol Ther 8:231-250, 2020
19. Zhou P, Zhang WW, Bao Y, et al: Chemotherapy and 21-gene recurrence score testing for older breast cancer patients: A competing-risks analysis. Breast 54:319-327, 2020
20. Barni S, Cognetti F, Petrelli F: Is the oncotype DX test useful in elderly breast cancer patients: a subgroup analysis of real-life Italian PONDx study. Breast Cancer Res Treat 191:477-480, 2022
21. van de Water W, Bastiaannet E, van de Velde CJH, et al: Inclusion and Analysis of Older Adults in RCTs. J Gen Intern Med 26:831-831, 2011

Chapter 6

DAILY ORAL IBANDRONATE WITH ADJUVANT ENDOCRINE THERAPY IN POST-MENOPAUSAL WOMEN WITH HORMONE RECEPTOR POSITIVE BREAST CANCER: RANDOMIZED PHASE 3 TEAM-IIB TRIAL (BOOG 2006-04)

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ABSTRACT

Purpose

For postmenopausal patients with breast cancer, previous subgroup analyses have shown a modest benefit from adjuvant bisphosphonate treatment. However, the efficacy of oral nitrogen-containing bisphosphonates such as ibandronate is unclear in this setting. TEAM-IIB investigates adjuvant ibandronate in postmenopausal women with estrogen receptor–positive (ER+) breast cancer.

Methods

TEAM-IIB is a randomized open-label multicenter phase III study. Postmenopausal women with stage I-III ER+ breast cancer and an indication for adjuvant endocrine therapy (ET) were randomly assigned 1:1 to five years of ET with or without oral ibandronate 50mg once daily for three years. Major ineligibility criteria were bilateral breast cancer, active gastroesophageal problems, and health conditions that might interfere with study treatment. Primary end point was disease-free survival (DFS), analyzed in the intention-to-treat population.

Results

Between February 1, 2007, and May 27, 2014, 1,116 patients were enrolled, 565 to ET with ibandronate (ibandronate arm) and 551 to ET alone (control arm). Median follow-up was 8.5 years. DFS was not significantly different between the ibandronate and control arms (HR 0.97; 95% CI 0.76 – 1.24; log-rank P = 0.811). Three years after random assignment, DFS was 94% in the ibandronate arm and 91% in the control arm. Five years after random assignment, this was 89% and 86%, respectively. In the ibandronate arm, 97/565 (17%) of patients stopped ibandronate early because of adverse events. Significantly more patients experienced GI issues, mainly dyspepsia, in the ibandronate arm than in the control arm (89 [16%] and 54 [10%], respectively; P < 0.003). Eleven patients in the ibandronate arm developed osteonecrosis of the jaw.

Conclusion

In postmenopausal women with ER+ breast cancer, adjuvant ibandronate 50mg once daily does not improve DFS and should not be recommended as part of standard treatment regimens.

INTRODUCTION

Metastatic spread of breast cancer is still the leading cause of cancer-related mortality in women.[1] Because hormone receptor–positive breast cancer cells prefer an osseous microenvironment, about 70% of breast cancer metastases are bone recurrences.[2,3] Nitrogen-containing bisphosphonates such as ibandronate affect bone metabolism by inhibiting key enzymes of the intracellular mevalonate pathway.[4] This decreases osteoclast-mediated bone resorption and osteoclast survival, causing an increase in bone density and a decreased release of cytokines and growth factors.[5] Preclinical studies suggest a direct antitumor effect by inhibition of tumor proliferation, induction of apoptosis, and enhanced immunosurveillance.[6] However, the exact anticancer mechanism of bisphosphonates is still unclear.

Several trials have investigated the effect of (neo)adjuvant bisphosphonates on cancer recurrence.[7-9] In 2015, a meta-analysis of 26 trials comparing patients treated with and without adjuvant bisphosphonates showed a reduction in breast cancer recurrence and mortality in the subgroup of women who were postmenopausal at the onset of treatment, but not in the premenopausal subgroup.[10] Thus far, the use of nitrogen-containing bisphosphonates has not been studied in exclusively postmenopausal patients.

The randomized TEAM-IIB trial investigates the addition of daily oral ibandronate to adjuvant endocrine therapy (ET) in postmenopausal women with estrogen receptor–positive (ER+) breast cancer. The registered dose of ibandronate to reduce skeletal events in the metastatic setting was used (50mg once daily). This paper describes the results of the TEAM-IIB trial, including safety and toxicity.

METHODS

TEAM-IIB is a randomized, open-label multicenter clinical phase III trial, conducted in 37 hospitals in the Netherlands. The study protocol was approved by the Medical Ethics Committee of the Netherlands Cancer Institute.

Participants

Eligible patients were postmenopausal and diagnosed with invasive stage I-III ER+ breast cancer, defined as estrogen receptor ≥ 10% (estrogen receptor–positive) and/or progesterone receptor ≥ 10% (progesterone receptor–positive). Patients had completed locoregional treatment and (neo)adjuvant chemotherapy following national guidelines, and had an indication for adjuvant ET. Postmenopausal status was defined as age ≥ 50 years and amenorrhea for > 1 year at diagnosis, or bilateral surgical oophorectomy and no use of hormone replacement therapy. In case of doubt, postmenopausal status was confirmed biochemically.

Exclusion criteria were bilateral breast cancer, prior invasive breast cancer in the past 15 years, a history of bone disease with potential interference of bone metabolism, active dental or gastroesophageal problems, a creatinine clearance of < 30 mL/min, and other

conditions that might interfere with the study treatment or determination of causality of adverse events (AEs).

Random assignment

All patients provided written informed consent for inclusion in the study. Patients were randomly assigned by a computer in a 1:1 ratio. Stratification was performed according to Pocock's minimization strategy[11] by center, age (< 50 versus 50-59 versus 60-69 versus ≥ 70 years), human epidermal growth factor receptor 2 status (positive [+] v negative [–]), hormone receptor status (estrogen receptor [ER]+ progesterone receptor [PR]+ versus ER+PR– versus ER–PR+), tumor grade (1 versus 2 versus 3 versus Gx), tumor size (T1 versus T2 versus T3 versus T4a-c), nodal status (pN0 versus pN0/i+ versus pN1[mi] versus pN1 versus pN2 versus pN3 versus pNx), neoadjuvant endocrine therapy (none versus 3 months versus 6 months), time between surgery and random assignment (< 3 months versus 3-6 months versus > 6 months), and (neo)adjuvant chemotherapy (yes versus no). There was no masking in this open-label trial.

Procedures

Included patients were randomly assigned to either ET (control arm) or ET combined with ibandronate (ibandronate arm). For ET, the study protocol followed the guidelines of the National Breast cancer Organization of the Netherlands, meaning all patients with human epidermal growth factor receptor 2–negative (HER2–) disease were to be prescribed tamoxifen 20mg once daily for 2-3 years, followed by exemestane 25mg once daily for 2-3 years, for a total of at least 5 years.[12] Patients with HER2+ breast cancer, or patients who received neoadjuvant exemestane in the TEAM-IIA trial, were treated with exemestane monotherapy for 5 years.[13] Extended use of ET was given according to the Dutch national guidelines. In the ibandronate arm, patients additionally received oral ibandronate 50mg once daily for 3 years.

All patients were followed until at least 10 years after random assignment. Patients diagnosed with osteonecrosis of the jaw (ONJ) during follow-up had to stop ibandronate immediately. Patients with a history of osteoporosis, defined by a T-score of < –2.5 by dual-energy X-ray absorptiometry (DEXA) scan, were allowed to participate, and if randomly assigned to the control arm, they were allowed to continue their own bisphosphonate for 3 years or switch to ibandronate 150mg once a month combined with calcium and vitamin D. A sensitivity analysis was predefined in the study protocol to assess any diluting effect.

DEXA scans were recommended for all patients starting with exemestane treatment at baseline and 3 years after starting treatment. Detailed information about follow-up intervals and assessments at each visit is described in the study protocol.

An independent data monitoring committee regularly reviewed the progress and safety of the trial. The independent data monitoring committee also reviewed the interim analysis, which was performed after enrollment of 100 patients.

Outcomes

The primary endpoint was 3-year disease-free survival (DFS), which included any breast cancer recurrence, second primary breast cancer, ductal carcinoma in situ, or death of any cause as event. DFS was calculated between random assignment and the occurrence of an event or end of follow-up, whichever came first.[14]

Secondary end points were 5-year DFS, overall survival (OS), and recurrence-free interval (RFi), defined as the interval between random assignment and any breast cancer recurrence, excluding contralateral breast cancer and ductal carcinoma in situ. Other end points were cumulative incidence rates of locoregional recurrence, distant recurrence, bone metastases, and visceral metastases.

All end points are measured from the date of random assignment until date of (competing) event or date of last follow-up moment. For measuring adherence, start and stop dates were collected and registered for ibandronate as well as endocrine therapy. Stopping ibandronate early was defined as stopping 3 months or longer before the planned stop date, thereby having a total ibandronate treatment duration of 2.75 years or less.

AEs were assessed during the first 3 years after random assignment, using the Common Terminology Criteria for Adverse Events version 3.0 for collection and version 4.03 for analysis.

Statistical analysis

Initially, a 91% and 94% 3-year DFS was assumed for the control and ibandronate arms, respectively. To achieve a power of 90% and an alpha of 5% for a two-sided log-rank test, 2,058 patients needed to be included. The trial protocol was amended in June 2009 because accrual was slower than expected. The power was decreased from 90% to 80%, the inclusion period was extended from 4 to 6 years, and on the basis of data from the TEAM trial, the assumed DFS was increased to 92% and 95% for the control and ibandronate arms, respectively.[15] This resulted in an adjusted sample size of 1,116 patients.

Primary and secondary end points were analyzed in the intention-to-treat (ITT) population, defined as all randomly assigned patients (ITT). A predefined sensitivity analysis was performed on the ITT population excluding patients with osteoporosis diagnosed by routine DEXA scans (ITT2). A predefined per-protocol (PP) analysis was performed on the ITT population excluding patients with major exclusion criteria violations (**figure 1**).

DFS and OS were estimated using Kaplan-Meier survival analyses, and treatment arms were compared using log-rank tests. RFi and other cumulative incidence rates were estimated using competing risk survival analyses.[16] HRs and corresponding 95% confidence intervals (CI) were estimated from Cox regression models. As predefined in the study protocol, Cox regression models were used to explore the influence of stratification and prognostic factors on DFS. Each factor was evaluated for inclusion in the multivariable model, and only factors significant at the 10% level were considered.

The proportional hazards assumption was assessed using the Schoenfeld residuals approach.[17] Since the proportional hazards assumption for randomized treatment was indeed violated for DFS, additional analyses were performed to address this issue. An interaction between treatment and time (using a categorical covariate distinguishing between the first time period, from random assignment to 3 years, and the second time period, starting at 3 years after random assignment until the end of follow-up) was added, which is an established method to estimate time-dependent treatment effects.[18] In univariate analyses, this is equivalent to separately estimating the HR over the second period by a landmark analysis, which included all patients that were event-free for DFS at 3 years after random assignment. Similar analyses were performed for the secondary end points.

Following the study protocol, chi-square tests were used to analyze differences in AEs between treatment groups in case the type of AE or the event frequency was judged to be clinically relevant.

Unplanned exploratory univariable subgroup analyses to test for heterogeneity of treatment effect were performed for DFS and RFi using prognostically relevant variables.

P-values smaller than .05 were considered statistically significant. Data lock for the current analyses was set at March 19, 2021. Analyses were performed using SPSS version 26.0. and R version 3.5.2. This study is registered with the Netherlands Trial Register, NL774.

RESULTS

Between February 1, 2007, and May 27, 2014 a total of 1,116 postmenopausal women were recruited and randomly assigned, of whom 551 to standard ET (the control arm) and 565 to daily oral ibandronate for 3 years combined with standard ET (the ibandronate arm; **figure 1**). For the large majority of patients, standard ET equates 5 years. Some patients received extended ET, following Dutch national treatment guidelines.

Baseline characteristics are summarized in **table 1**. The median age was 62 years (interquartile range, 56-67 years), 106/1,116 (10%) patients had HER21 breast cancer, and 623/1,116 (56%) patients received chemotherapy. In 452/ 1,116 (41%) patients, the treating physician opted for an ET regimen that differed from the study protocol, such as 5 years of tamoxifen monotherapy or a different aromatase inhibitor (AI) than exemestane. Median follow-up at data lock was 8.5 years (interquartile range, 7.1-10.0 years) after random assignment.

There was no significant difference in DFS between the ibandronate and control arms (HR 0.97; 95% CI 0.76 – 1.24; log-rank P = 0.811; **figure 2A**). Three years after randomization, there were 31 events in the ibandronate arm, resulting in a DFS of 94% (95% CI 92 – 96), and 51 events in the control arm (DFS 91% [88 – 93]). At 5 years after randomization, there were 62 events in the ibandronate arm (DFS 89% [86 – 91]) and 79 events in the control arm (DFS 86% [83 – 88]), and 8 years after randomization, 109 in the ibandronate arm (DFS 79% [75 – 82]) and 110 in the control arm (DFS 79% [75 – 82]; **table 2**).

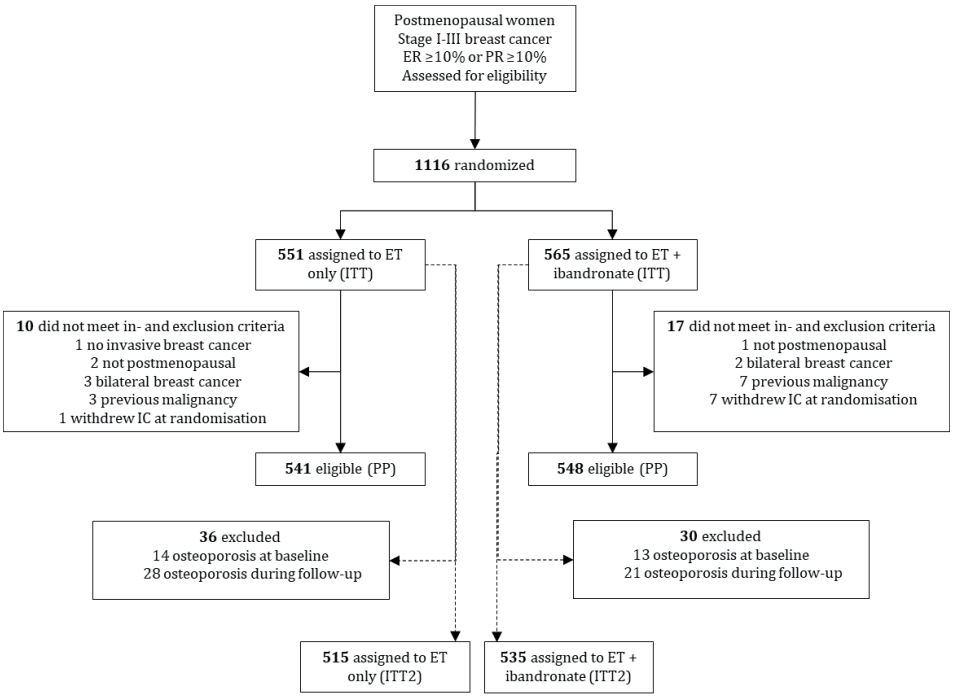

Figure 1: CONSORT diagram.

ER = estrogen receptor. PR = progesterone receptor. ET = endocrine therapy. ITT = intention-to-treat. IC = informed consent. PP = per-protocol.

The Schoenfeld residuals approach showed a violated proportional hazards assumption (P = .024). Therefore, a potential interaction between time and treatment effect was examined. When truncated at 3 years after random assignment, DFS was numerically higher in the ibandronate arm than in the control arm (HR 0.59; 95% CI 0.38 – 0.92). In a landmark analysis starting at 3 years after random assignment, DFS was lower in the ibandronate arm than in the control arm (HR 1.22; 95% CI 0.91 – 1.63; **table 3**; **figure 3**).

OS in the ibandronate arm was similar to the control arm, with 14 and 19 deaths at 3 years, 38 and 46 at 5 years, and 72 and 67 deaths at 8 years after random assignment, respectively (HR 1.10; 95% CI, 0.82 – 1.49; log-rank P = 0.517; **figure 2B**). There were no deaths reported to be related to ibandronate. Causes of death were similar between the treatment arms as well (**table 4**).

The cumulative incidence of breast cancer recurrences was similar in the ibandronate arm and the control arm, with 19 and 39 events at 3 years after random assignment, 37 and 56 at 5 years, and 65 and 75 events at 8 years after random assignment, respectively (**table 2**, **figure 2C**).

The risk of bone recurrences was not significantly reduced by using ibandronate (HR 0.83; 95% CI 0.55 – 1.25; **figure 2D**). The cumulative incidence of bone recurrences at 8 years after random assignment was 7% (95% CI 5 – 10, 36 events) and 8% (6 – 11, 43 events) in the ibandronate arm and control arm, respectively (**table 2**).

	All (n=1116)	Ibandronate (n=565)	Control (n=551)
Age (years)			
< 50	27 (4.2)	16 (2.8)	11 (2.0)
50 – 59	424 (38.0)	210 (37.2)	214 (38.8)
60 – 69	470 (42.1)	244 (43.2)	226 (41.0)
≥ 70	195 (17.5)	95 (16.8)	100 (18.1)
Tumor stage			
Tis	1 (0.1)	1 (0.2)	0 (0.0)
T1	634 (56.8)	325 (57.5)	309 (56.1)
T2	414 (37.1)	210 (37.2)	204 (37.0)
T3	43 (3.9)	16 (2.8)	27 (4.9)
T4	21 (1.9)	11 (1.9)	10 (1.8)
Unknown	3 (0.3)	2 (0.4)	1 (0.2)
Nodal status			
N0/N0(i+)	558 (50.0)	287 (50.8)	271 (49.2)
N1	428 (38.4)	213 (37.7)	215 (39.0)
N2	88 (7.9)	43 (7.6)	45 (8.2)
N3	38 (3.4)	19 (3.4)	19 (3.4)
Unknown	4 (0.3)	3 (0.5)	1 (0.2)
Histological grade			
1	157 (14.1)	66 (11.7)	91 (16.5)
2	631 (56.5)	330 (58.4)	301 (54.6)
3	290 (26.0)	148 (26.2)	142 (25.8)
Unknown	38 (3.4)	21 (3.7)	17 (3.1)
Histological subtype			
Ductal	861 (77.2)	441 (78.1)	420 (76.2)
Lobular	156 (14.0)	73 (12.9)	83 (15.1)
Other	96 (8.6)	49 (8.7)	47 (8.6)
Unknown	3 (0.2)	2 (0.4)	1 (0.2)
HER2 status			
Negative	1010 (90.5)	510 (90.3)	500 (90.7)
Positive	106 (9.5)	55 (9.7)	51 (9.3)
Hormone receptor status			
ER+/PR+	823 (73.7)	417 (73.8)	406 (73.7)
ER+/PR-	287 (25.7)	147 (26.0)	140 (25.4)
ER-/PR+	6 (0.5)	1 (0.2)	5 (0.9)
Chemotherapy			
Anthracycline	179 (16.0)	95 (16.8)	84 (15.2)
Anthracycline + taxane	422 (37.8)	213 (37.7)	209 (37.9)
Other	22 (2.0)	10 (1.8)	12 (2.2)
None	493 (44.2)	247 (43.7)	246 (44.6)
Anti-HER2 medication*			
Yes	75/106 (70.8)	42/55 (76.4)	33/51 (64.7)
No	31/106 (29.2)	13/55 (23.6)	18/51 (35.3)
Endocrine therapy			
AI only	189 (16.9)	91 (16.1)	98 (17.8)
TAM → AI	839 (75.2)	426 (75.4)	413 (75.0)
TAM only	65 (5.8)	36 (6.4)	29 (5.3)
Other	21 (1.9)	10 (1.8)	11 (2.0)
Unknown	2 (0.2)	2 (0.4)	0 (0.0)
Bisphosphonates at baseline			
Yes	11 (1.0)	2 (0.4)	9 (1.6)
No	1103 (98.8)	561 (99.3)	542 (98.4)
Unknown	2 (0.2)	2 (0.4)	0 (0.0)

Table 1: Baseline characteristics of the intention-to-treat population.

All values are N (%).

HER = human epidermal growth factor receptor. ER = estrogen receptor. PR = progesterone receptor. AI = aromatase inhibitor. TAM = tamoxifen.

* Out of number of patients that had HER2 expression.

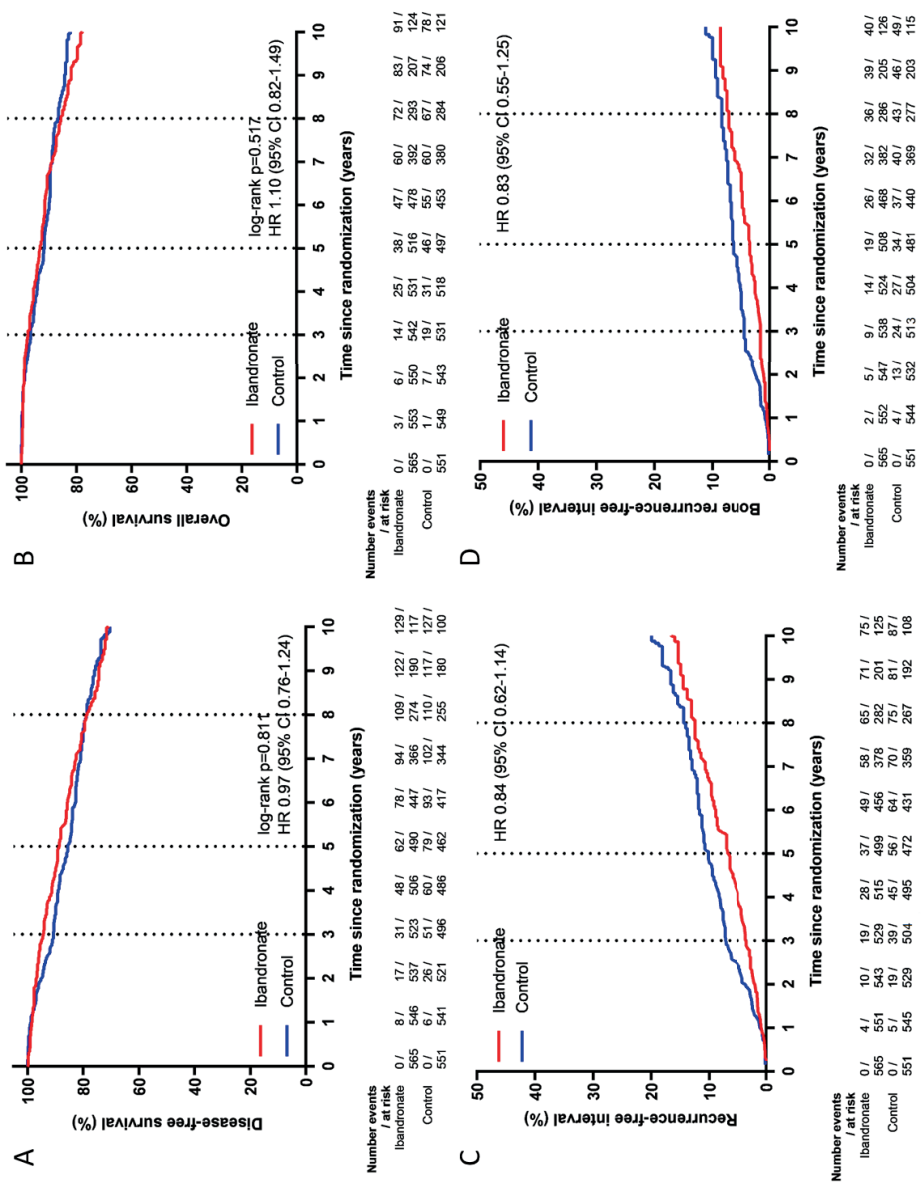

Figure 2: Kaplan-Meier estimates of disease-free survival (A) and overall survival (B), and cumulative incidence estimates of any recurrence (C) and bone recurrence (D) in the intention-to-treat population.

HR = hazard ratio. DI = confidence interval.

		Ibandronate (n=565)		Control (n=551)	
		N events/ N at risk	Point estimate (95% CI)	N events/ N at risk	Point estimate (95% CI)
Disease-free survival					
	3 year	31 / 523	94% (92-96)	51 / 496	91% (88-93)
	5 year	62 / 490	89% (86-91)	79 / 462	86% (83-88)
	8 year	109 / 274	79% (75-82)	110 / 225	79% (75-82)
Overall survival					
	3 year	14 / 542	98% (69-99)	19 / 531	97% (95-98)
	5 year	38 / 516	93% (91-95)	46 / 497	92% (89-94)
	8 year	72 / 293	86% (83-89)	67 / 284	87% (84-90)
Any recurrence					
	3 year	19 / 529	3% (2-5)	39 / 504	7% (5-10)
	5 year	37 / 499	7% (5-9)	56 / 472	10% (8-13)
	8 year	65 / 282	12% (10-16)	75 / 267	14% (12-18)
Locoregional recurrence					
	3 year	7 / 537	1% (1-3)	11 / 523	2% (1-4)
	5 year	13 / 506	2% (1-4)	18 / 485	3% (2-5)
	8 year	21 / 289	4% (3-6)	24 / 273	5% (3-7)
Distant recurrence					
	3 year	14 / 533	3% (2-4)	33 / 510	6% (4-8)
	5 year	30 / 504	5% (4-8)	46 / 479	8% (6-11)
	8 year	55 / 282	11% (8-14)	60 / 277	12% (9-15)
Bone recurrence					
	3 year	9 / 537	2% (1-3)	24 / 513	4% (3-6)
	5 year	19 / 508	3% (2-5)	34 / 481	6% (4-9)
	8 year	36 / 286	7% (5-10)	43 / 277	8% (6-11)
Bone as first event					
	3 year	7 / 537	1% (1-3)	15 / 513	3% (2-5)
	5 year	13 / 508	2% (1-4)	23 / 481	4% (3-6)
	8 year	25 / 286	5% (3-7)	29 / 277	6% (4-8)
Visceral recurrence					
	3 year	10 / 537	2% (1-3)	24 / 517	4% (3-6)
	5 year	25 / 509	4% (3-7)	32 / 488	6% (4-8)
	8 year	42 / 284	8% (6-11)	42 / 281	8% (6-11)

Table 2: Primary and secondary endpoints based on Kaplan-Meier survival estimates (disease-free and overall survival) and on cumulative incidence estimates (recurrences) in the intention-to-treat population.

	HR[a]	95% CI	HR[b]	95% CI
Disease-free survival				
Randomization – 3 years	0.590	0.378 – 0.922	0.982	0.627 – 1.539
3 years – end of FU	1.216	0.905 – 1.633	1.199	0.882 – 1.630
Overall	0.971	0.762 – 1.238	0.999	0.777 – 1.283
Overall survival				
Randomization – 3 years	0.726	0.364 – 1.447	1.000	0.498 – 2.008
3 years – end of FU	1.219	0.873 – 1.701	1.154	0.821 – 1.623
Overall	1.104	0.819 – 1.488	1.000	0.738 – 1.354
Any recurrence				
Randomization – 3 years	0.474	0.274 – 0.820	1.000	0.596 – 1.679
3 years – end of FU	1.122	0.767 – 1.641	1.130	0.758 – 1.686
Overall	0.837	0.616 – 1.136	0.925	0.668 – 1.280
Locoregional recurrence				
Randomization – 3 years	0.625	0.242 – 1.611	0.635	0.243 – 1.663
3 years – end of FU	1.006	0.537 – 1.885	1.066	0.541 – 2.101
Overall	0.868	0.516 – 1.459	0.876	0.508 – 1.511
Distant recurrence				
Randomization – 3 years	0.414	0.221 – 0.773	0.648	0.354 – 1.184
3 years – end of FU	1.250	0.814 – 1.919	1.275	0.815 – 1.995
Overall	0.857	0.609 – 1.206	1.000	0.707 – 1.415
Bone recurrence				
Randomization – 3 years	0.365	0.170 – 0.785	0.344	0.157 – 0.751
3 years – end of FU	1.265	0·752 – 2·127	1.228	0.710 – 2.123
Overall	0.826	0·547 – 1·248	0.956	0.622 – 1.469
Bone as first event				
Randomization – 3 years	0.454	0.185 – 1.114	0.440	0.175 – 1.104
3 years – end of FU	1.292	0.690 – 2.418	1.311	0.674 – 2.552
Overall	0.901	0.548 – 1.483	0.990	0.546 – 1.797
Visceral recurrence				
Randomization – 3 years	0.407	0.195 – 0.851	0.363	0.169 – 0.780
3 years – end of FU	1.395	0.838 – 2.324	1.380	0.818 – 2.329
Overall	0.912	0.610 – 1.364	0.875	0.579 – 1.322

Table 3: Primary and secondary endpoints truncated at 3 years after randomization and as landmark analyses starting at 3 years after randomization in the intention-to-treat population.

HR = hazard ratio. CI = confidence interval. FU = follow-up.

[a] Univariable Cox regression model.

[b] Multivariable Cox regression model. Included variables: age, hormone receptor status, time between surgery and randomization, BMI, radiotherapy, tumor size, nodal status, and type of surgery.

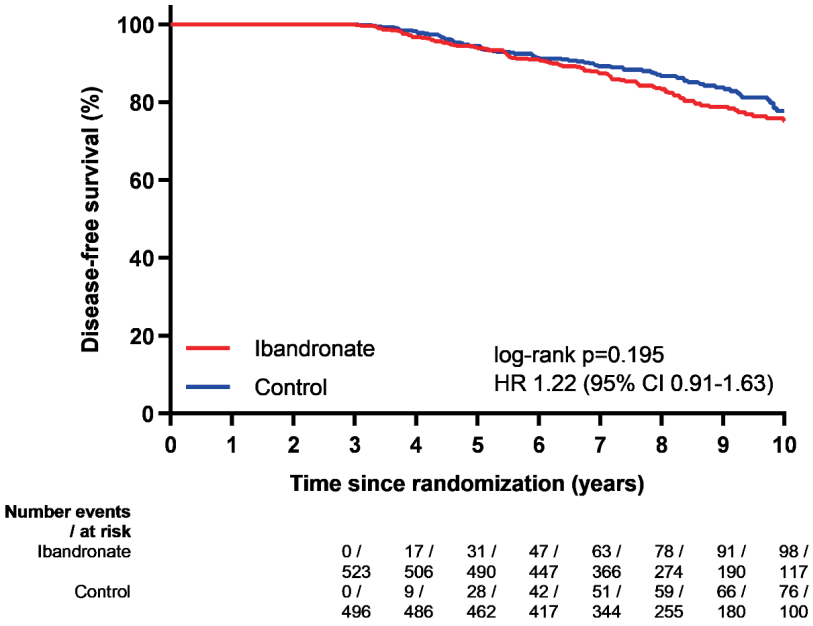

Figure 3: Landmark analysis starting at 3 years after randomization of disease-free survival in the intention-to-treat population.

	All (n=1116)	Ibandronate (n=565)	Control (n=551)
All breast cancer events	194	92	102
Recurrences	165	77	88
Local	35	16	19
Regional	29	12	17
Distant	132	62	70
Visceral	96	46	50
Bone	93	42	51
Bone as first event	62	30	32
New primary breast tumors	38	19	19
Carcinoma in situ	10	6	4
Invasive	28	13	15
Other primary cancers	89	48	41
Second primary without breast cancer	83	46	36
Angiosarcoma of the breast	2	1	1
Mortality	173	87	81
Breast cancer related	100	43	53
Second primary malignancy	32	17	15
Cardiac	13	8	5
Pulmonary	7	5	2
Other	12	8	4
Unknown	9	6	2
Disease-free survival events	261	128	129

Table 4: Number of events in the intention-to-treat population.

Of all patients who started treatment with ibandronate (n = 543), 163 (30%) patients stopped early. The main reason to stop early was AEs (n = 97/163 [60%]). Of these, 53/97 (55%) patients stopped within the first 6 months of study treatment (**figure 4A**). Of all patients who stopped ibandronate because of AEs, 31/97 (32%) stopped because of GI issues. Adherence to ET was similar in both treatment arms (**figure 4B**).

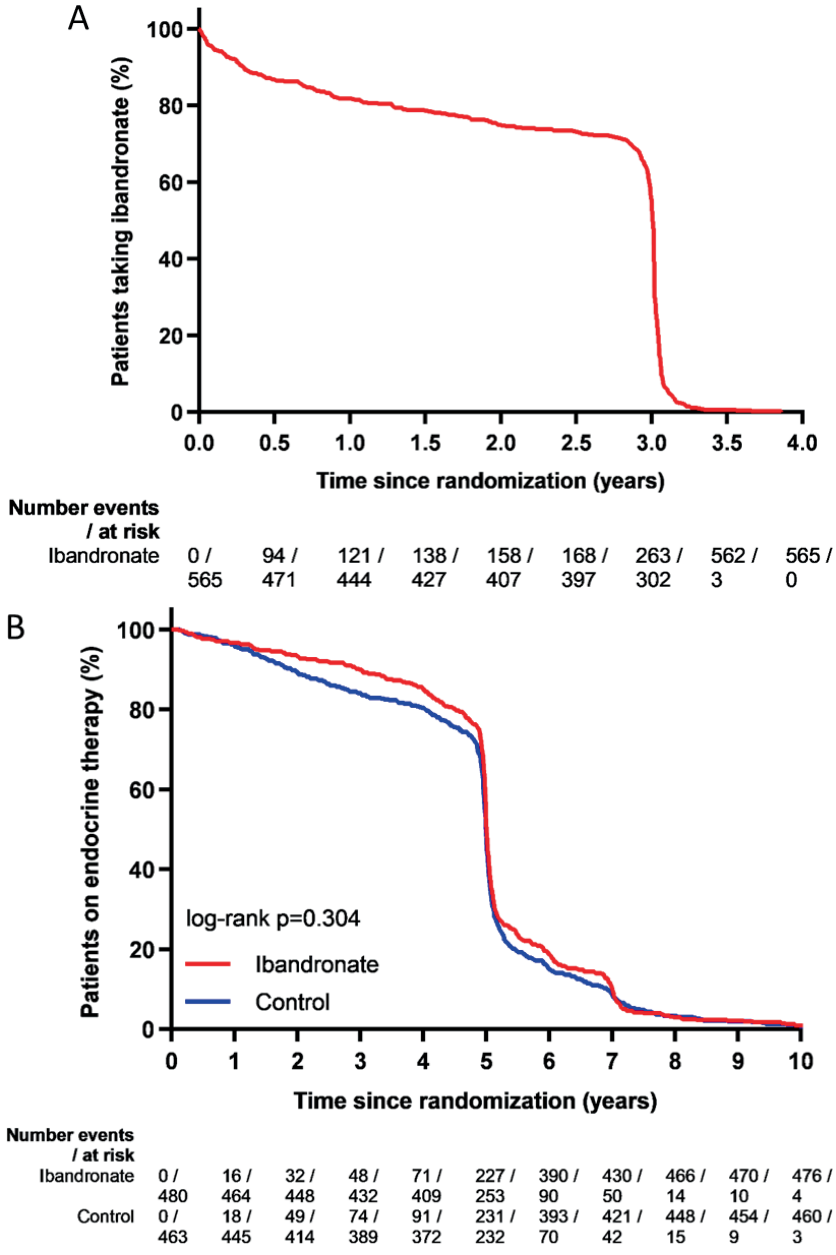

Figure 4: Patients taking ibandronate (A) and patients on adjuvant endocrine therapy (B).

AEs (all grades) that occurred in ≥ 5% of the patients are summarized in **table 5**. In total, 933/1,116 (84%) patients reported at least one AE, 473/565 (84%) in the ibandronate arm and 460/551 (84%) in the control arm. Of all AEs reported, the ibandronate arm reported a higher number of GI events, mainly dyspepsia, compared with the control arm (89 and 54 events, respectively; **table 6**). The number of patients who developed osteonecrosis was also significantly higher in the ibandronate arm compared with the control arm (12 and 1 events, respectively; P = 0.002). In the ibandronate arm, 11 of 12 events (92%) were classified as ONJ. Of the 12 patients in the ibandronate arm who developed osteonecrosis, nine cases occurred while the patient was on an AI, whereas three of the ONJ events occurred while the patient was on tamoxifen treatment. Osteoporosis and osteopenia occurred less frequently in the ibandronate arm compared with the control arm (36 and 62 events, respectively; **table 5**). Bone fractures occurred in 22 (3.9%) patients in the ibandronate arm and 26 (4.7%) patients in the control arm.

A predefined sensitivity analysis (ITT2) was performed to assess any diluting effect of including patients with osteoporosis. DEXA scans were performed in 431/565 patients in the ibandronate arm and in 434/551 patients in the control arm. Thirty-six patients in the control arm and 30 patients in the ibandronate arm had a history of or were diagnosed with osteoporosis during the study, and they were excluded from the ITT2 analyses (**figure 1**). The ITT2 analysis showed similar results to those obtained with the ITT analyses (data not shown).

A predefined PP population was also analyzed. In total, 10 and 17 patients in the control and ibandronate arms, respectively, did not meet all inclusion and exclusion criteria, and

	All (n=1116)	Ibandronate (n=565)	Control (n=551)	P-value
Hot flashes	381 (34.1)	184 (32.6)	197 (35.8)	0.262
Arthralgia	269 (24.1)	145 (25.7)	124 (22.5)	0.217
Fatigue	196 (17.6)	93 (16.5)	103 (18.7)	0.327
Depression	105 (9.4)	45 (8.0)	30 (10.9)	0.094
Pain in extremity	102 (9.1)	54 (9.6)	48 (8.7)	0.624
Osteoporosis or osteopenia	98 (8.8)	36 (6.4)	62 (11.3)	0.004
Nausea	88 (7.9)	48 (8.5)	40 (7.3)	0.444
Decreased range of joint motion	87 (7.8)	42 (7.4)	45 (8.2)	0.648
Lymphedema	83 (7.4)	35 (6.2)	48 (8.7)	0.109
Back pain	74 (6.6)	33 (5.8)	41 (7.4)	0.283
Peripheral sensory neuropathy	71 (6.4)	35 (6.2)	36 (6.5)	0.817
Dizziness	63 (5.6)	30 (5.3)	33 (6.0)	0.623
Alopecia	52 (4.7)	23 (4.1)	29 (5.3)	0.345
Myalgia	50 (4.5)	29 (5.1)	21 (3.8)	0.286
Maculo-papular rash	50 (4.5)	19 (3.4)	31 (5.6)	0.068
Dyspepsia	48 (4.3)	37 (6.5)	11 (2.0)	<0.001

Table 5: Incidence of adverse events, all grades and occurring in ≥ 5% of patients, between randomization and 3.25 years.

All values are N (%). P-values are derived from Pearson's chi-squared tests.

were excluded from the PP population (**figure 1**). This analysis also showed results consistent with the ITT analyses (data not shown).

Unplanned univariable analyses of prognostically relevant subgroups are shown in **figure 5**. For DFS, heterogeneity of treatment effect was observed for histologic tumor grade (P-value for interaction = 0.049; **figure 5A**). For RFi, no heterogeneity of treatment effect was observed (**figure 5B**).

	All (n=1116)		Ibandronate (n=565)		Control (n=551)	
	Grade 1-2	Grade 3-5	Grade 1-2	Grade 3-5	Grade 1-2	Grade 3-5
Cardiac disorders	6 (0.5)	6 (0.5)	1 (0.2)	4 (0.7)	5 (0.9)	2 (0.4)
Endocrine disorders	4 (0.4)	0 (0.0)	3 (0.5)	0 (0.0)	1 (0.2)	0 (0.0)
Eye disorders	4 (0.4)	6 (0.5)	1 (0.2)	3 (0.5)	3 (0.5)	3 (0.5)
Gastrointestinal disorders	131 (11.7)	12 (1.1)	85 (15.0)	4 (0.7)	46 (8.3)	8 (1.5)
General disorders and site conditions	35 (3.1)	5 (0.4)	14 (2.5)	2 (0.4)	21 (3.8)	3 (0.5)
Hepatobiliary disorders	0 (0.0)	4 (0.4)	0 (0.0)	1 (0.2)	0 (0.0)	3 (0.5)
Infections and infestations	28 (2.5)	21 (1.9)	13 (2.3)	11 (1.9)	15 (2.7)	10 (1.8)
Injury and procedural complications	9 (0.8)	11 (1.0)	4 (0.7)	6 (1.1)	5 (0.9)	5 (0.9)
Investigations	9 (0.8)	2 (0.2)	6 (1.1)	1 (0.2)	3 (0.5)	1 (0.2)
Metabolism and nutrition disorders	2 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.4)	0 (0.0)
Musculoskeletal and connective tissue	304 (27.2)	16 (1.4)	143 (25.3)	10 (1.8)	161 (29.2)	6 (1.1)
Neoplasms[1]	9 (0.8)	5 (0.4)	6 (1.1)	(0.4)	3 (0.5)	3 (0.5)
Nervous system disorders	39 (3.5)	15 (1.3)	24 (4.2)	9 (1.6)	15 (2.7)	6 (1.1)
Psychiatric disorders	18 (1.6)	7 (0.6)	11 (1.9)	4 (0.7)	7 (1.3)	3 (0.5)
Renal and urinary disorders	7 (0.6)	4 (0.4)	4 (0.7)	3 (0.5)	3 (0.5)	1 (0.2)
Reproductive system and breast disorders	16 (1.4)	7 (0.6)	7 (1.2)	6 (1.1)	9 (1.6)	1 (0.2)
Respiratory and thoracic disorders	7 (0.6)	2 (0.2)	2 (0.4)	1 (0.2)	5 (0.9)	1 (0.2)
Skin and subcutaneous tissue disorders	35 (3.1)	4 (0.4)	17 (3.0)	2 (0.4)	18 (3.3)	2 (0.4)
Vascular disorders	115 (10.3)	28 (2.5)	50 (8.8)	13 (2.3)	65 (11.8)	15 (2.7)
All	778 (69.7)	155 (13.9)	391 (69.2)	82 (14.5)	387 (70.2)	72 (13.2)

Table 6: All adverse events by system organ class and Common Terminology Criteria for Adverse Event (CTCAE) grade (version 4.03).

All values are N (%).

[1] Excluding basal cell carcinoma.

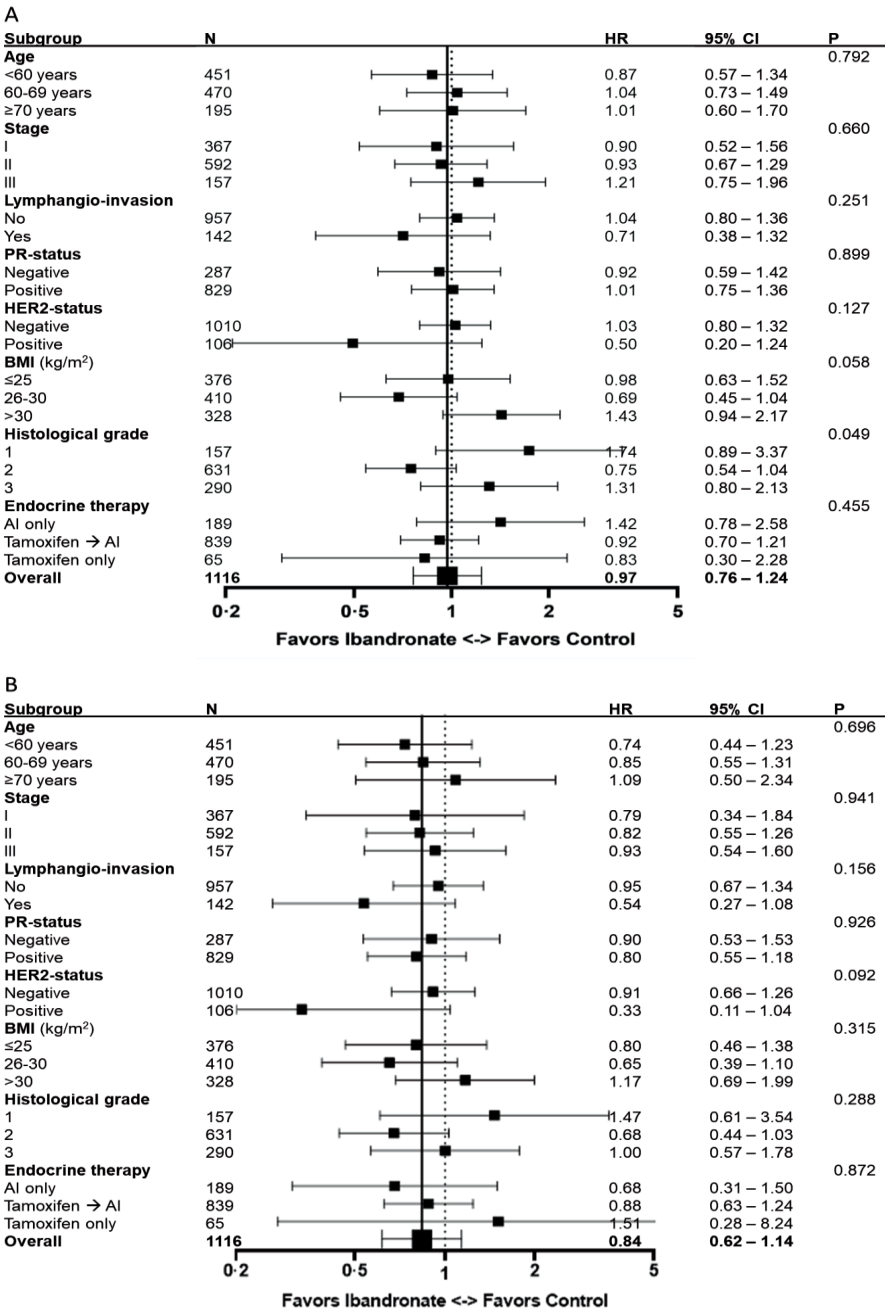

Figure 5: Univariable subgroup analyses of disease-free survival (A) and recurrence-free interval (B) at 8 years after randomization in the intention-to-treat population.

P-values are derived from Cox-regression interaction tests.

HR = hazard ratio. CI = confidence interval.

DISCUSSION

TEAM-IIB, the largest randomized controlled trial in specifically postmenopausal women with ER+ breast cancer, evaluates the benefit of adding an oral nitrogen-containing bisphosphonate, ibandronate, to adjuvant ET, and found no difference in overall DFS between the ibandronate arm and the control arm. A significant difference was observed in the first 3 years after diagnosis, which disappeared with longer follow-up. An interaction between time and treatment effect was observed, although a landmark analysis of DFS starting at 3 years after random assignment until end of follow-up showed no significant difference in DFS between the treatment arms.

Evaluation of secondary outcomes also showed only a short-term benefit of ibandronate. During the first 5 years after random assignment, patients in the ibandronate arm had few recurrences overall, and also less recurrences in bone, specifically. This is in line with results from preclinical research and the EBCTCG meta-analysis.[10] Despite the favorable short-term effects of ibandronate on disease-free survival and (bone) recurrence rate, ibandronate was not beneficial with longer follow-up. After 8 years of follow-up, the (bone) recurrence rate was similar between the ibandronate arm and the control arm. These results were also consistent with the EBCTCG meta-analysis, and it was especially notable that the point estimates for bone recurrence presented here were almost identical to those of the EBCTCG meta-analysis, namely 7.8% and 8.8% for the ibandronate arm and control arm, respectively, versus 7.8% and 9.0% in the meta-analysis. All landmark analyses starting at 3 years after random assignment showed no statistically significant differences between treatment arms.

The short-term results were not statistically significant in the multivariable analyses either. This suggests that the observed differences could be due to chance. Second, a potential explanation could be the delaying effect bisphosphonates have on recurrences instead of a preventive effect. Nitrogen-containing bisphosphonates greatly reduce osteoclast activity and can inhibit bone resorption by up to 2 years after discontinuing treatment.[19] Metastatic breast cancer cells that are present in osseous tissue are less likely to grow into detectable metastases while bone turnover is still suppressed, and stay dormant. However, when osteoclasts regain regular activity, the osseous micro-environment changes in favor of the metastatic cells, and opportunity arises for metastases to grow.[20]

Finally, since most patients in TEAM-IIB switched from tamoxifen to an AI after 2-3 years, the type of ET in combination with bisphosphonates might matter. However, the relation between the type of ET including switch and recurrences should be interpreted with caution, as these analyses may be influenced by immortal time bias.

Another limitation of this study is the use of HR as a measure of treatment effect. Considering that the proportional hazards assumption is not met for the primary end point, the HR might be a potentially inaccurate measure of treatment effect. The landmark analyses starting at 3 years after random assignment were performed to adjust for this

potential imprecision, which showed no significant differences between treatment arms either.

Other trials investigating nitrogen-containing bisphosphonates did not observe a discordance between short-term and long-term effects of bisphosphonates.[7,21] The GAIN trial, which also studied adjuvant ibandronate 50mg once daily for 2 years, did not observe any benefit of ibandronate for DFS or OS.[22] Studies such as the GAIN, AZURE, and NSABP-B34 trials showed that the benefit of bisphosphonates seems largely restricted to women with low estrogen levels at the time of treatment, although the mechanism behind this remains unclear.[7,9,22] Estrogens may interfere with the antitumor effect of bisphosphonates, or the altered bone structure in the absence of estrogens may be relevant. The results from the TEAM-IIB trial demonstrate that in the long-term, ibandronate is not beneficial for postmenopausal patients.

Moreover, ibandronate treatment carries considerable side effects. Bisphosphonates are associated with flu-like symptoms, musculoskeletal pain, and hypocalcemia. Incidence of serious AEs, such as ONJ and nephrotoxicity, is low. Most trials report an incidence of < 1% for both toxicities. Notably, in TEAM-IIB, the incidence of ONJ was 1.9%, mostly in women using an AI, which raises the question whether the combination with AIs in postmenopausal women may increase this risk. Tamoxifen increases bone mineral density in postmenopausal women by acting as an estrogen agonist in osseous tissue, whereas AIs cause osteoporosis through disrupting the bone remodeling cycle by increasing osteoclast-mediated bone resorption.[23,24] Bisphosphonates decrease bone remodeling, but also decrease angiogenesis and cause poor wound healing. Therefore, the concurrent administration of AIs and high-dose ibandronate may increase the risk of developing osteonecrosis compared with the combination of ibandronate and tamoxifen.[25,26]

Although patient satisfaction with oral formulations is generally high and oral bisphosphonates are usually well accepted, 18% of TEAM-IIB patients stopped their ibandronate treatment early because of AEs, and approximately a third of those had GI complaints.

It is still an unresolved question which is the optimal class, dose, schedule, and duration of bisphosphonates, and which postmenopausal patients should be selected for bisphosphonate treatment. The results presented here suggest that daily ibandronate for 3 years should not be the recommended strategy. The planned update of the EBCTCG meta-analysis might also provide more insights to answer these questions.

In conclusion, the data presented here are an important contribution to the field and the results from TEAM-IIB do not support using daily ibandronate as adjuvant treatment in unselected postmenopausal women with ER1 stage I-III breast cancer.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, et al: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: Cancer J Clin 68:394-424, 2018
2. Brockton NT, Gill SJ, Laborge SL, et al: The Breast Cancer to Bone (B2B) Metastases Research Program: a multi-disciplinary investigation of bone metastases from breast cancer. BMC Cancer 15:512, 2015
3. Coleman RE, Rubens RD: The clinical course of bone metastases from breast cancer. Br J Cancer 55:61-6, 1987
4. van Acker HH, Anguille S, Willemen Y, et al: Bisphosphonates for cancer treatment: Mechanisms of action and lessons from clinical trials. Pharmacol Ther 158:24-40, 2016
5. Rogers MJ, Crockett JC, Coxon FP, et al: Biochemical and molecular mechanisms of action of bisphosphonates. Bone 49:34-41, 2011
6. Tamura T, Shomori K, Nakabayashi M, et al: Zoledronic acid, a third-generation bisphosphonate, inhibits cellular growth and induces apoptosis in oral carcinoma cell lines. Oncol Rep 25:1139-43, 2011
7. Coleman RE, Collinson M, Gregory W, et al: Benefits and risks of adjuvant treatment with zoledronic acid in stage II/III breast cancer. 10 years follow-up of the AZURE randomized clinical trial (BIG 01/04). J Bone Oncol 13:123-135, 2018
8. Gnant M, Mlineritsch B, Schippinger W, et al: Endocrine therapy plus zoledronic acid in premenopausal breast cancer. N Engl J Med 360:679-91, 2009
9. Paterson AH, Anderson SJ, Lembersky BC, et al: Oral clodronate for adjuvant treatment of operable breast cancer (National Surgical Adjuvant Breast and Bowel Project protocol B-34): a multicentre, placebo-controlled, randomised trial. Lancet Oncol 13:734-42, 2012
10. EBCTCG: Adjuvant bisphosphonate treatment in early breast cancer: meta-analyses of individual patient data from randomised trials. Lancet 386:1353-1361, 2015
11. Pocock SJ, Simon R: Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. Biometrics 31:103-15, 1975
12. Nationaal Borstkanker Overleg Nederland; https://www.oncoline.nl/borstkanker; accessed on April 8, 2021.
13. Fontein DB, Charehbili A, Nortier JW, et al: Efficacy of six month neoadjuvant endocrine therapy in postmenopausal, hormone receptor-positive breast cancer patients--a phase II trial. Eur J Cancer 50:2190-200, 2014
14. Gourgou-Bourgade S, Cameron D, Poortmans P, et al: Guidelines for time-to-event end point definitions in breast cancer trials: results of the DATECAN initiative (Definition for the Assessment of Time-to-event Endpoints in CANcer trials) 2020. Ann Oncol 26:873-879, 2015
15. van de Velde CJH, Rea D, Seynaeve C, et al: Adjuvant tamoxifen and exemestane in early breast cancer (TEAM): a randomised phase 3 trial. Lancet 377:321-31, 2011
16. Fine JP, Gray RJ: A Proportional Hazards Model for the Subdistribution of a Competing Risk. J Am Stat Assoc 94:496-509, 1999
17. Grambsch PM, Therneau TM: Proportional hazards tests and diagnostics based on weighted residuals. Biometrika 81:515-526, 1994
18. Therneau TM, Grambsch PM: Testing Proportional Hazards. Modeling Survival Data: Extending the Cox Model. . 6, 2000
19. Grey A, Bolland MJ, Wattie D, et al: The Antiresorptive Effects of a Single Dose of Zoledronate Persist for Two Years: A Randomized, Placebo-Controlled Trial in Osteopenic Postmenopausal Women. J Clin Endocrinol Metab 94:538-544, 2009
20. Cremers SC, Pillai G, Papapoulos SE: Pharmacokinetics/pharmacodynamics of bisphosphonates: use for optimisation of intermittent therapy for osteoporosis. Clin Pharmacokinet 44:551-70, 2005
21. Gnant M, Mlineritsch B, Stoeger H, et al: Zoledronic acid combined with adjuvant endocrine therapy of tamoxifen versus anastrozol plus ovarian function suppression in premenopausal early breast cancer: final analysis of the Austrian Breast and Colorectal Cancer Study Group Trial 12. Ann Oncol 26:313-20, 2015
22. von Minckwitz G, Möbus V, Schneeweiss A, et al: German adjuvant intergroup node-positive study: a phase III trial to compare oral ibandronate versus observation in patients with high-risk early breast cancer. J Clin Oncol 31:3531-9, 2013
23. Love RR, Mazess RB, Barden HS, et al: Effects of tamoxifen on bone mineral density in postmenopausal women with breast cancer. N Engl J Med 326:852-6, 1992
24. Perez EA, Weilbaecher K: Aromatase inhibitors and bone loss. Oncology (Williston Park) 20:1029-39; discussion 1039-40, 1042, 1048, 2006
25. Payne KF, Goodson AM, Tahim AS, et al: Why worry about bisphosphonate-related osteonecrosis of the jaw? A guide to diagnosis, initial management, and referral of patients. Br J Gen Pract 67:330-331, 2017
26. Ruggiero SL: Guidelines for the diagnosis of bisphosphonate-related osteonecrosis of the jaw (BRONJ). Clin Cases Miner Bone Metab 4:37-42, 2007

Chapter 7

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

Iris Noordhoek

RISK ESTIMATION AND PREDICTION FOR (EXTENDED) ENDOCRINE THERAPY

Breast cancer is the most common cancer in women.[1,2] In the past decades, much progress has been made in terms of treatment options, and breast cancer survival has improved significantly. Nevertheless, concerns in the treatment of hormone receptor positive (HR+) breast cancer remain. Even after optimal locoregional management and adjuvant systemic therapy, patients with HR+ breast cancer still have a lifetime risk of 20 to 41% of developing a recurrence.[3] Several prognostic and predictive models have been developed for early breast cancer. To improve tailoring of adjuvant treatment to the individual patient, much effort has been made to identify biomarkers or biomarker profiles capable of predicting the individual risk of recurrence and sensitivity to endocrine treatment. One objective of this thesis was to examine several prognostic and predictive models, to determine which ones are most suitable to use in daily clinical practice.

In **chapter 2** of this thesis, with a review of the literature, we investigated whether the quantitative assessment of hormone receptors is a better method to select patients for endocrine therapy (ET) than the single cut-off value of 10%.[4] We concluded that in patients with an estrogen receptor (ER)-positive tumor (defined as ER ≥10%), a higher ER load as assessed by immunohistochemistry (IHC) is not correlated to better outcome, and no evidence could be found for using quantitative ER load as a prognostic marker. In other words, patients with a higher ER load (e.g. 100%) do not inherently have a better prognosis than patients with a lower ER load (e.g. 20%). Furthermore, no evidence was found for using quantitative ER load as a predictive marker, i.e. patients with a higher ER load do not have more benefit of ET than patients with a lower ER load.

This chapter also concluded that in patients with an HR+ tumor, higher progesterone receptor (PR) load does not seem to be correlated to better outcome. Based on the included studies, quantitative PR load is not a suitable prognostic marker; patients with a higher PR load do not inherently have a better prognosis than patients with a lower PR load, nor is it suitable as a predictive marker. Furthermore, PR load seems to interact with ER load and is therefore not recognized as an independent predictor.

The studies included in the systematic review differed from each other in their way of quantitatively measuring HR load; some studies used a continuous percentage or histoscore, some studies used groups of HR-negative, low HR expression and high HR expression and some divided patients in four or more groups based on Allred score or percentage. This influenced outcomes. Studies were more likely to find a positive association between HR load and outcome if a continuous score was used. However, using a continuous quantitative measure to assess HR expression was questioned by several authors. Inter-observer variability is high, and samples get assigned different HR percentages depending on the pathologist and the lab it was reviewed in. Most importantly, staining breast cancer tissue using IHC does not allow for precise enough measurement of HR load to generate a continuous score and can only quantify into negative, weak positive and strong positive. The problem with this approach is defining “weak” and “strong”. A lack

of generally accepted definition resulted in pathologists and papers choosing their own definition, making it difficult to compare multiple studies.

Based on the results of the review, we propose using both ER and PR expression only as a qualitative measure; defining tumors with less than 10% of cells expressing this receptor as negative, and tumors with ≥10% of cells expressing the receptor as positive. Using a continuous quantitative measure does not seem feasible without centralized, unambiguous and clear pathological measurement. The implications for the daily clinical practice of pathologists are that more detailed information on the HR status beyond "positive" or "negative" should no longer be provided, to prevent oncologists subconsciously or instinctively making different treatment decisions based on this information. Since there is no evidence for different treatment strategies, providing extra information is both unnecessary and undesirable.

In **chapter 3** of this thesis, we studied the utility and accuracy of the clinical treatment score post-5 years (CTS5) to determine the risk of late distant recurrences (DR) and whether it can be used to predict benefit from extended ET. A substantial risk of late DR exists for patients with ER+ breast cancer, even after 5 years of ET, and extended ET may be beneficial.[5] The CTS5 was trained with data from postmenopausal patients who had HR+ breast cancer and were randomly assigned to receive anastrozole alone or tamoxifen alone, and were distant recurrence free after 5 years of follow-up. It was internally validated with data from postmenopausal women with HR+ early-stage breast cancer who were randomized to receive 5 years of letrozole or tamoxifen or sequential therapy (2 years of letrozole followed by 3 years of tamoxifen or opposite sequence), and were distant recurrence free after 5 years of follow-up. The CTS5 aims to estimate the DR rate between 5 and 10 years from diagnosis for postmenopausal patients with ER-positive breast cancer who remain disease free after 5 years of standard ET.

We applied the CTS5 to the TEAM and IDEAL cohorts.[6] The TEAM cohort comprised postmenopausal patients with ER-positive breast cancer randomly assigned to either 5 years of exemestane or 2-3 years of tamoxifen followed by 2-3 years of exemestane, and were disease free at 5 years.[7] The IDEAL cohort consisted of postmenopausal patients with HR+ early breast cancer, who were disease free after 5 years of standard ET and were randomized to either 2.5 or 5 years of extended treatment with letrozole.[8] The CTS5 was developed to discriminate postmenopausal patients with ER+ breast cancer into three risk categories with respect to late DR.[5] In these two large cohorts of trial patients, the risk of late DR as predicted by the CTS5 corresponded to the observed DR rates in low-risk patients, but the CTS5 overestimated the observed risk of DR in patients with a higher predicted risk. Furthermore, the CTS5 could not predict benefit of 5 years over 2.5 years of extended ET using data from the IDEAL cohort.

In conclusion, the CTS5 as applied to patients treated in the TEAM and IDEAL cohorts categorizes patients into low-, intermediate-, and high-risk groups. In low-risk patients, the predicted late DR risks correspond with the observed DR rates, but the CTS5 overestimated the risk of late DRs in high-risk patients from the TEAM and IDEAL cohorts. Using patient

data from the IDEAL cohort, the CTS5 cannot be validated as a predictive tool for extended ET either. Especially in high-risk patients, an unrealistic assessment of the risk to develop a late DR could potentially lead to overtreatment. Therefore, the numerical risk assessment from the CTS5 calculator in its current form should be interpreted with caution when used in daily clinical practice, particularly when used in high-risk patients.

Both the hormone receptor analysis studied in **chapter 2**, and the CTS5 studied in **chapter 3** are models based on clinical factors. They are able to discriminate between low risk and high risk patients, but not capable of further discrimination. Moreover, quantification does not lead to more accurate risk assessment, and both of these models are unable to select patients who are suitable for extended endocrine therapy.

In general, prognostic tools aim to distinguish patients with an inherently worse prognosis from those with a better prognosis, while predictive tools aim to distinguish those patients who will respond well to treatment from those who will not. Often, prognostic tools are not predictive and should not be used as such because patients with a worse prognosis are not necessarily the same patients who benefit from more extensive therapy. This is illustrated with the results from **chapter 2** and **chapter 3**.

More promising as a predictive model may be the assessment of the activity of the ER-pathway to distinguish in which patients the estrogen receptor is not only expressed, but also active and thus a suitable target for ET. In **chapter 4** of this thesis, we examined the BCI [H/I] biomarker panel.[9] BCI [H/I] is a gene expression profile that examines the ratio between two genes, HOXB13 and IL17BR (H/I), that reflects activity of estrogen signaling pathways in breast tumors. When this H/I ratio is high, estrogen signaling is upregulated, and the proliferation of the tumor is likely influenced by the availability of estrogens.

To date, the net benefit of extended endocrine therapy based on tumor biology has incorporated individualized assessment of risk but has lacked individualized assessment of endocrine responsiveness to predict the likelihood to benefit from longer durations of endocrine treatment. Findings from this study confirm the predictive ability of BCI by H/I status to classify patients who demonstrate a high or low degree of endocrine responsiveness with categorical differences in outcome from extended endocrine therapy. Similar relative improvements in outcome based on recurrence-free survival (RFI) by BCI [H/I] category were generally observed in all clinical and pathologic factors examined.

Consistent with previous data, BCI [H/I] expression did not show a strong correlation with canonical endocrine biomarkers, ER and PR. In addition, multiple translational studies in the ATAC, BIG 1-98, and TEAM trials, and our own results from **chapter 2** have reproducibly demonstrated that quantitative ER and PR expression levels in patients with HR breast cancer do not predict benefit from endocrine therapy, suggesting that BCI [H/I] predictive effects are predicated on distinct biological mechanisms that are not directly coupled to ER/PR expression levels.[10-12]

We demonstrated significant prediction of extended endocrine benefit based on BCI [H/I] classification in patients treated with contemporary standards of care for primary adjuvant

endocrine therapy. In conjunction with previous data from MA.17 and Trans-aTTom, BCI predictive performance is established across a comprehensive range of treatment scenarios involving tamoxifen and AIs.[13] Clarifying the magnitude and level of efficacy of extended endocrine therapy with approaches that provide additive and distinct information is important to ensure that overtreatment and undertreatment do not occur. BCI may provide the rationale as a standardized molecular tool that measures preferential response and magnitude of benefit to help individualize patient selection for extending endocrine therapy to 10 years.

In a head-to-head comparison of BCI [H/I] to the CTS5, one analysis revealed that no CTS5-group derived significant benefit from extended ET.[14] The treatment by biomarker interaction was significant for BCI [H/I], but not for CTS5. When re-stratifying CTS5 categories by BCI [H/I] or vice versa, only BCI [H/I]-high patients showed consistent absolute benefit regardless of CTS5 category. In contrast, CTS5-high patients did not show any benefit in the BCI [H/I]-low group. These results demonstrate that CTS5 does not provide predictive information to support extended endocrine therapy decision-making. Only BCI [H/I] was a predictive biomarker of benefit from extended endocrine therapy. These results are consistent with our findings from **chapter 3** and **chapter 4**.

RISK ESTIMATION IN OLDER PATIENTS

A third of all patients with breast cancer are aged 70 years or older at diagnosis, and in this growing population two specific age-related issues arise.[1] As it takes time for a recurrence to develop, and older patients have an increased probability to die of causes unrelated to their breast cancer, the risk of developing a recurrence is inversely correlated to age and the competing risk of other-cause mortality.[15] Also, older patients usually have more comorbidities and frailty than younger patients. Therefore, they may experience more side effects and complications of cancer treatment and are at higher risk of hospitalization and long-term loss of quality of life.[16,17]

Risk estimation in older patients is generally based on the same factors as in younger patients. Therefore, these age-related factors regarding other cause mortality and (adverse) effects of treatment, are often not taken into account when determining the treatment strategy, which can lead to significant overtreatment of this population. Thus, instruments are needed that are validated specifically for the older population. The 70-gene signature test, or MammaPrint, is a genomic risk profile that is already established as an accurate prognostic model in younger breast cancer patients.[18] Previous studies showed that MammaPrint can be used to de-escalate the use of chemotherapy and ET in genomic low and ultralow risk patients, respectively.[19] However, these trials did not include patients aged 70 years or older. In **chapter 5** of this thesis, the validity and accuracy of MammaPrint in older patients was examined.

Our data showed that the 70-gene signature test can be used to accurately estimate DR risk in patients with breast cancer aged ≥70 years.[20] MammaPrint-ultralow risk patients had excellent clinical outcome up to ten years after diagnosis, despite 48% of them not

receiving any systemic therapy. Significantly more MammaPrint-high risk patients developed DR, even though 72% of MP-high risk patients did receive adjuvant ET. Multivariate analyses adjusted for ET usage still showed significantly lower 10-year DR rates for MP-ultralow risk patients.

This is the first study examining a gene-expression profile in the older population. Our data show that genomic ultralow risk patients had excellent long-term outcomes even if clinically high risk. This may be explained by the discontinuation of routine screening at the age of 75. With increasing age, and reduced rates of participation in breast cancer screening, breast cancer is more often diagnosed at higher clinical stages.[21] Larger tumors and more elaborate regional spread may therefore in older women not be signs of aggressive tumor biology, but rather of late diagnoses. Thus, using only clinical parameters to determine recurrence risk for older patients may result in inaccurate risk estimation. Our data show that the 70-gene signature test provides more accurate risk assessment, and it seems reasonable to suggest that all older patients with ultralow risk breast cancer could forgo adjuvant endocrine therapy. This would apply to both patients with a high risk of competing events, and also to those who are relatively fit, since the risk of developing a distant recurrence seems extremely low. Prospective randomized trials should examine whether MammaPrint can indeed be used to decide if these older patients can safely forgo adjuvant ET.

This analysis adds to the growing body of data demonstrating the validity of MammaPrint's ultralow risk threshold. Women with ultralow risk, regardless of clinical stage or grade, had an extremely low risk of recurrence. These data are especially relevant for clinicians working with older patients, who may be frailer and more susceptible to adverse effects of treatment.

ADJUVANT THERAPY WITH BISPHOSPHONATES

Aside from determining which patients have most to gain from (extended) ET, other therapeutic agents might also assist in decreasing the risk of recurrences. Since hormone receptor positive breast cancer cells prefer osseous microenvironments, about 70% of breast cancer metastases are bone recurrences.[22,23] When metastatic cells infiltrate bone tissue, the equilibrium between osteoclasts and osteoblasts is disturbed. The tumor cells stimulate the activity of osteoclasts, which increases bone resorption and the release of growth factors and cytokines. These instigate the proliferation and survival of tumor cells, creating a vicious cycle.

Nitrogen-containing bisphosphonates also affect bone metabolism by inhibiting key enzymes of the intracellular mevalonate pathway.[24] This decreases osteoclast-mediated bone resorption and osteoclast survival, causing an increase in bone density and a decreased release of cytokines and growth factors. It is hypothesized that this makes bone a less attractive environment for metastatic breast cancer cells.

Several trials have investigated the effect of (neo)adjuvant bisphosphonates on (breast) cancer recurrence.[25-27] In 2015, a meta-analysis comparing patients treated with and

without adjuvant bisphosphonates showed a reduction in breast cancer recurrence and mortality in the subgroup of women who were postmenopausal at the onset of treatment or chemically castrated using therapy, but not in the premenopausal subgroup.[28] Thus far, the use of high-dose nitrogen-containing bisphosphonates has not been studied in exclusively postmenopausal patients. The TEAM-IIB trial investigated the effect of daily oral ibandronate on the development of (bone) recurrences in postmenopausal patients with breast cancer, and its results were described in **chapter 6**.[29]

TEAM-IIB, the largest randomized controlled trial in specifically postmenopausal women with HR+ breast cancer, evaluates the benefit of adding an oral nitrogen-containing bisphosphonate, ibandronate, to adjuvant ET, and found no difference in overall disease-free survival (DFS) between the ibandronate arm and the control arm. A significant difference was observed in the first 3 years after diagnosis, which disappeared with longer follow-up. An interaction between time and treatment effect was observed, although a landmark analysis of DFS starting at 3 years after random assignment until end of follow-up showed no significant difference in DFS between the treatment arms. Evaluation of secondary outcomes also showed only a short-term benefit of ibandronate. During the first 5 years after random assignment, patients in the ibandronate arm had few recurrences overall, and also less recurrences in bone, specifically. This is in line with results from preclinical research and the EBCTCG meta-analysis.[28] Despite the favorable short-term effects of ibandronate on disease-free survival and (bone) recurrence rate, ibandronate was not beneficial with longer follow-up. After 8 years of follow-up, the (bone) recurrence rate was similar between the ibandronate arm and the control arm. These results were also consistent with the EBCTCG meta-analysis, and it was especially notable that the point estimates for bone recurrence presented here were almost identical to those of the EBCTCG meta-analysis, namely 7.8% and 8.8% for the ibandronate arm and control arm, respectively, versus 7.8% and 9.0% in the meta-analysis. All landmark analyses starting at 3 years after random assignment showed no statistically significant differences between treatment arms.

Moreover, ibandronate treatment carries considerable side effects. Bisphosphonates are associated with flu-like symptoms, musculoskeletal pain, and hypocalcemia. Incidence of serious AEs, such as osteonecrosis of the jaw (ONJ) and nephrotoxicity, is low. Most trials report an incidence of about 1% for both toxicities. Notably, in TEAM-IIB, the incidence of ONJ was 1.9%, mostly in women using an AI, which raises the question whether the combination with AIs in postmenopausal women may increase this risk. Tamoxifen increases bone mineral density in postmenopausal women by acting as an estrogen agonist in osseous tissue, whereas AIs cause osteoporosis through disrupting the bone remodeling cycle by increasing osteoclast-mediated bone resorption. Bisphosphonates decrease bone remodeling, but also decrease angiogenesis and cause poor wound healing. Therefore, the concurrent administration of AIs and high-dose ibandronate may increase the risk of developing osteonecrosis compared with the combination of ibandronate and tamoxifen. Although patient satisfaction with oral formulations is generally high and oral bisphosphonates are usually well accepted, 18% of TEAM-IIB patients stopped their

ibandronate treatment early because of adverse events, and approximately a third of those had gastro-intestinal complaints.

In conclusion, the data presented here are an important contribution to the field and the results from TEAM-IIB do not support using daily ibandronate as adjuvant treatment in unselected postmenopausal women with HR+ stage I-III breast cancer.

FUTURE PERSPECTIVES

Overall, identifying the breast cancer patients that could benefit most from ET remains important. Roughly, patients can be categorized into four groups.

First of all, there is a group of patients with HR+ breast cancer that have such favorable tumor characteristics, that they will never develop a recurrence, even if they forgo adjuvant treatment altogether. Secondly, some patients will definitely develop a recurrence, even if they are treated with ET. Thirdly, some patients would develop a recurrence, but this can be prevented with the use of ET. And lastly, some patients are elderly or frail, and although they might develop a recurrence, this will not influence their survival nor their quality of life, as they are likely to succumb to "other cause mortality" related to comorbidities before the breast cancer recurs.

Ideally, only the patients from the third group are treated with ET. If oncologists are able to predict to which group of patients people belong, they could better advise them on the use of ET. Considering the frequent and sometimes severe side effects of ET, an improved selection of those who need treatment will lower the treatment burden.

To identify patients belonging to the first group, the 70-gene signature test as described in **chapter 5** can be used. Our data show that genomic ultralow risk patients had excellent long-term outcomes even if clinically high risk. It seems reasonable to suggest that all patients with ultralow risk breast cancer could forgo adjuvant endocrine therapy, since the risk of developing a distant recurrence seems extremely low.

To differentiate between the second and the third group, a potential method to identify those patients is to measure the activity of the ER pathway to distinguish in which patients the estrogen receptor is not only expressed but also active and thus a suitable target for ET. BCI [H/I] as described in **chapter 4** could be a suitable method for this. Other pathway sensitivity analyses could also be advantageous for this goal.[30]

The fourth group could be identified using the PORTRET tool. The PORTRET tool aims to predict recurrence, overall mortality, and other-cause mortality in older patients with breast cancer, including individualized estimations of adjuvant treatment benefits.[31] The tool showed good internal and external validation performance, with improved accuracy in older patients compared with existing breast cancer prediction models, by incorporating comorbidity and geriatric predictors. The accurate prediction of the competing risk of death is a substantial aspect of the PORTRET tool. Although there are existing tools that estimate remaining life expectancy in older adults available such as Lee-index/ePrognosis,

the major advantage of the PORTRET tool is that it combines this outcome with breast cancer-specific outcomes.[32]

This thesis aimed to provide guidance in navigating different risk assessment tools. Once we are able to accurately assign patients to one of the four groups by using risk assessment tools, we can optimize and personalize adjuvant treatment strategies for patients with HR+ breast cancer.

REFERENCES

1. Dutch Cancer Registry (NKR), IKNL. Obtained through https://www.iknl.nl/nkr-cijfers, accessed on April 4 2021.
2. Allemani C, Matsuda T, Di Carlo V, et al: Global surveillance of trends in cancer survival 2000-14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. Lancet 391:1023-1075, 2018
3. Pan H, Gray R, Braybrooke J, et al: 20-Year Risks of Breast-Cancer Recurrence after Stopping Endocrine Therapy at 5 Years. N Engl J Med 377:1836-1846, 2017
4. Noordhoek I, de Groot AF, Cohen D, et al: Higher ER load is not associated with better outcome in stage 1-3 breast cancer: a descriptive overview of quantitative HR analysis in operable breast cancer. Breast Cancer Res Treat 176:27-36, 2019
5. Dowsett M, Sestak I, Regan MM, et al: Integration of Clinical Variables for the Prediction of Late Distant Recurrence in Patients With Estrogen Receptor-Positive Breast Cancer Treated With 5 Years of Endocrine Therapy: CTS5. J Clin Oncol 36:1941-1948, 2018
6. Noordhoek I, Blok EJ, Meershoek-Klein Kranenbarg E, et al: Overestimation of Late Distant Recurrences in High-Risk Patients With ER-Positive Breast Cancer: Validity and Accuracy of the CTS5 Risk Score in the TEAM and IDEAL Trials. J Clin Oncol 38:3273-3281, 2020
7. Derks MGM, Blok EJ, Seynaeve C, et al: Adjuvant tamoxifen and exemestane in women with postmenopausal early breast cancer (TEAM): 10-year follow-up of a multicentre, open-label, randomised, phase 3 trial. Lancet Oncol 18:1211-1220, 2017
8. Blok EJ, Kroep JR, Meershoek-Klein Kranenbarg E, et al: Optimal Duration of Extended Adjuvant Endocrine Therapy for Early Breast Cancer; Results of the IDEAL Trial (BOOG 2006-05). J Natl Cancer Inst 110, 2018
9. Noordhoek I, Treuner K, Putter H, et al: Breast Cancer Index Predicts Extended Endocrine Benefit to Individualize Selection of Patients with HR+ Early-stage Breast Cancer for 10 Years of Endocrine Therapy. Clin Cancer Res 27:311-319, 2021
10. Sgroi DC, Sestak I, Cuzick J, et al: Prediction of late distant recurrence in patients with oestrogen-receptor-positive breast cancer: a prospective comparison of the breast-cancer index (BCI) assay, 21-gene recurrence score, and IHC4 in the TransATAC study population. Lancet Oncol 14:1067-1076, 2013
11. van de Water W, Fontein DB, van Nes JG, et al: Influence of semi-quantitative oestrogen receptor expression on adjuvant endocrine therapy efficacy in ductal and lobular breast cancer - a TEAM study analysis. Eur J Cancer 49:297-304, 2013
12. Viale G, Regan MM, Maiorano E, et al: Prognostic and predictive value of centrally reviewed expression of estrogen and progesterone receptors in a randomized trial comparing letrozole and tamoxifen adjuvant therapy for postmenopausal early breast cancer: BIG 1-98. J Clin Oncol 25:3846-52, 2007
13. Bartlett JMS, Sgroi DC, Treuner K, et al: Breast Cancer Index and prediction of benefit from extended endocrine therapy in breast cancer patients treated in the Adjuvant Tamoxifen-To Offer More? (aTTom) trial. Ann Oncol 30:1776-1783, 2019
14. Sgroi D, Zhang Y, Schnabel CA: Comparative performance of Breast Cancer Index (BCIN+) and CTS5 in hormone receptor-positive (HR+) lymph node-positive (N+) breast cancer patients with one to three positive nodes (N1). J Clin Oncol 37:555, 2019
15. van de Water W, Bastiaannet E, Hille ET, et al: Age-specific nonpersistence of endocrine therapy in postmenopausal patients diagnosed with hormone receptor-positive breast cancer: a TEAM study analysis. Oncologist 17:55-63, 2012
16. Klepin H, Mohile S, Hurria A: Geriatric assessment in older patients with breast cancer. J Natl Compr Canc Netw 7:226-36, 2009
17. Lee SY, Seo JH: Current Strategies of Endocrine Therapy in Elderly Patients with Breast Cancer. Biomed Res Int 2018:808-820, 2018
18. Cardoso F, van't Veer LJ, Bogaerts J, et al: 70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer. N Engl J Med 375:717-29, 2016
19. Lopes Cardozo JMN, Drukker CA, Rutgers EJT, et al: Outcome of Patients With an Ultralow-Risk 70-Gene Signature in the MINDACT Trial. J Clin Oncol 40:1335-1345, 2022
20. Noordhoek I, Bastiaannet E, de Glas NA, et al: Validation of the 70-gene signature test (MammaPrint) to identify patients with breast cancer aged ≥ 70 years with ultralow risk of distant recurrence: A population-based cohort study. J Geriatr Oncol 13:1172-1177, 2022
21. Cappellani A, Di Vita M, Zanghì A, et al: Prognostic factors in elderly patients with breast cancer. BMC Surg 13 Suppl 2:S2, 2013
22. Brockton NT, Gill SJ, Laborge SL, et al: The Breast Cancer to Bone (B2B) Metastases Research Program: a multi-disciplinary investigation of bone metastases from breast cancer. BMC Cancer 15:512, 2015
23. Coleman RE, Rubens RD: The clinical course of bone metastases from breast cancer. Br J Cancer 55:61-6, 1987
24. van Acker HH, Anguille S, Willemen Y, et al: Bisphosphonates for cancer treatment: Mechanisms of action and lessons from clinical trials. Pharmacol Ther 158:24-40, 2016

25. Coleman RE, Collinson M, Gregory W, et al: Benefits and risks of adjuvant treatment with zoledronic acid in stage II/III breast cancer. 10 years follow-up of the AZURE randomized clinical trial (BIG 01/04). J Bone Oncol 13:123-135, 2018
26. Gnant M, Mlineritsch B, Stoeger H, et al: Zoledronic acid combined with adjuvant endocrine therapy of tamoxifen versus anastrozol plus ovarian function suppression in premenopausal early breast cancer: final analysis of the Austrian Breast and Colorectal Cancer Study Group Trial 12. Ann Oncol 26:313-20, 2015
27. Paterson AH, Anderson SJ, Lembersky BC, et al: Oral clodronate for adjuvant treatment of operable breast cancer (National Surgical Adjuvant Breast and Bowel Project protocol B-34): a multicentre, placebo-controlled, randomised trial. Lancet Oncol 13:734-42, 2012
28. EBCTCG: Adjuvant bisphosphonate treatment in early breast cancer: meta-analyses of individual patient data from randomised trials. Lancet 386:1353-1361, 2015
29. Vliek SB, Noordhoek I, Meershoek-Klein Kranenbarg E, et al: Daily Oral Ibandronate With Adjuvant Endocrine Therapy in Postmenopausal Women With Estrogen Receptor-Positive Breast Cancer (BOOG 2006-04): Randomized Phase III TEAM-IIB Trial. J Clin Oncol 40:2934-2945, 2022
30. Inda MA, Blok EJ, Kuppen PJK, et al: Estrogen Receptor Pathway Activity Score to Predict Clinical Response or Resistance to Neoadjuvant Endocrine Therapy in Primary Breast Cancer. Mol Cancer Ther 19:680-689, 2020
31. van der Plas-Krijgsman WG, Giardiello D, Putter H, et al: Development and validation of the PORTRET tool to predict recurrence, overall survival, and other-cause mortality in older patients with breast cancer in the Netherlands: a population-based study. Lancet Healthy Longev 2:e704-e711, 2021
32. Lee SJ, Lindquist K, Segal MR, et al: Development and validation of a prognostic index for 4-year mortality in older adults. Jama 295:801-8, 2006

Appendices

NEDERLANDSE SAMENVATTING
LIST OF PUBLICATIONS
CURRICULUM VITAE
DANKWOORD

GESCHIEDENIS VAN DE ENDOCRINE THERAPIE

De behandeling van borstkanker bestaat al lange tijd uit een combinatie van locoregionale behandelingen en systemische cytotoxische en endocriene behandelingen. Al in 1896 werd ontdekt dat borsttumoren gevoelig konden zijn voor hormonen en dat groei van uitzaaiingen verminderd werd door de ovaria te verwijderen.[1] Echter, slechts een derde van de patiënten ondervond duidelijke voordelen van deze behandeling.[2] De ontdekking van expressie van de oestrogeen- en progesteronreceptor (ER en PR) in 1958 maakte een betere selectie van patiënten mogelijk die baat zouden hebben bij hormoondepletie.[3] Wanneer hormonen zich aan deze receptoren binden, resulteert dat in celdeling en tumorgroei.[4] Borstkankercellen die geen hormoonreceptoren tot expressie brengen, ontvangen groeisignalen via andere routes en worden niet beïnvloed door de aan- of afwezigheid van hormonen.

In 1960 werd oraal tamoxifen, een selectieve oestrogeenreceptormodulator, geïntroduceerd als alternatief voor chirurgische ovariumablatie.[5] Tamoxifen heeft een antagonistische werking in borstweefsel, waardoor oestrogeen niet aan de receptor kan binden en de signaalcascade geblokkeerd wordt.[6] Verschillende onderzoeken bij patiënten met ER-positieve borstkanker toonden aan dat het gebruik van tamoxifen het risico op terugkeer van borstkanker verminderde. Later toonde een grote meta-analyse van de *Early Breast Cancer Trialists' Collaborative Group* (EBCTCG) aan dat het gebruik van tamoxifen gedurende vijf jaar de terugkeer van borstkanker en de sterfte significant verminderde.[7]

Begin jaren 2000 kwam een ander type geneesmiddel beschikbaar om de groei van hormoonreceptor-positieve tumoren te remmen: de aromatase-inhibitor (AI).[8] Bij postmenopauzale vrouwen is de enige bron van oestrogeen de conversie van androgenen naar oestrogeen door het enzym aromatase.[9] Wanneer deze conversie wordt geremd, is er geen oestrogeen beschikbaar om aan de hormoonreceptoren te binden, en ontvangt de tumor dus geen signaal voor celdeling. Verschillende onderzoeken werden uitgevoerd om verschillende soorten endocriene therapieën (ET) voor postmenopauzale patiënten te vergelijken. Een andere meta-analyse door de EBCTCG toonde aan dat vijf jaar AI-monotherapie vergelijkbare resultaten oplevert als twee tot drie jaar tamoxifen, gevolgd door twee tot drie jaar AI. Het gebruik van vijf jaar tamoxifen-monotherapie werd bewezen een minder effectieve behandeling te zijn.[10]

EPIDEMIOLOGIE VAN BORSTKANKER

Borstkanker is nog steeds de meest voorkomende kankersoort bij vrouwen, hoewel de verdeling over verschillende leeftijdscategorieën aanzienlijk is veranderd.[11,12] Het percentage vrouwen dat postmenopauzaal is bij de diagnose is gestegen van 74% in 1989 naar 80% in 2019, en patiënten van 70 jaar of ouder vertegenwoordigen momenteel een derde van alle borstkankerpatiënten.[11] Tegenwoordig wordt de systemische behandelstrategie voor postmenopauzale patiënten met borstkanker bepaald door klinische, histopathologische en genetische parameters, en hormoonreceptoren zijn nog steeds de belangrijkste van deze biomarkers. Ongeveer 85% van alle postmenopauzale patiënten heeft borstkankertumoren met ER- of PR-expressie, of beide.[13,14] Richtlijnen bevelen 5 jaar AI-behandeling aan voor deze patiënten.[15]

UITDAGINGEN IN DE BEHANDELING VAN BORSTKANKER

Er is veel vooruitgang geboekt in de afgelopen decennia, en de overleving van patiënten met borstkanker is aanzienlijk verbeterd. Desondanks blijven er verschillende zorgen bestaan over de behandeling van hormoonreceptor-positieve borstkanker.

Zelfs na optimale locoregionale behandeling en adjuvante systemische therapie, hebben borstkankerpatiënten nog steeds een levenslang risico van 20 tot 41% om een recidief te ontwikkelen, afhankelijk van de tumorgrootte en het lymfklierstadium bij diagnose.[16] Blijkbaar kan endocriene therapie recidieven niet in alle patiënten met hormoonreceptor-positieve tumoren voorkomen en kan de selectie van patiënten die op de lange termijn baat hebben bij ET nog steeds worden verbeterd.

Een andere uitdaging is dat hormoonreceptor-positieve borstkanker specifiek wordt gekarakteriseerd door late recidieven. Twee derde van de recidieven treedt op na vijf jaar, dat wil zeggen na het stoppen van de ET, en het risico op het ontwikkelen van een recidief blijft groeien tot 20 jaar na de diagnose.[16] Recente onderzoeken hebben aangetoond dat het verlengen van ET na vijf jaar het risico op late recidieven kan verminderen en de ziektevrije overleving kan verbeteren bij patiënten die in eerste instantie vijf jaar tamoxifen-monotherapie hebben gekregen.[17,18] Echter is het effect van het verlengen van ET bij patiënten die met een AI zijn behandeld, niet eenduidig, en tot nu toe is er geen klinisch relevant voordeel aangetoond voor deze patiëntengroep.[19-22] Dit feit, in combinatie met de ernstige bijwerkingen die gepaard gaan met langdurige ET, zou het onverantwoord maken om alle borstkankerpatiënten met verlengde ET te behandelen. Aan de andere kant kunnen sommige patiënten wel profiteren van verlengde ET, en zij moeten worden geïdentificeerd opdat zij hun optimale behandeling kunnen ontvangen.

Verder vormt de toenemende leeftijd waarop patiënten worden gediagnosticeerd een probleem. Zoals eerder vermeld, is een derde van alle borstkankerpatiënten 70 jaar of ouder bij de diagnose, en in deze groeiende populatie ontstaan twee specifieke leeftijdsgebonden problemen. Aangezien het tijd kost voor een recidief om zich te ontwikkelen, en oudere patiënten een verhoogde kans hebben om te overlijden aan oorzaken die niet gerelateerd zijn aan hun borstkanker, is het risico op het ontwikkelen van een recidief omgekeerd gecorreleerd aan de leeftijd en het daarbij komende concurrerende risico van sterfte door andere oorzaken.[23] Daarnaast hebben oudere patiënten meestal meer comorbiditeit en kwetsbaarheid dan jongere patiënten.[24] Daarom kunnen zij meer bijwerkingen en complicaties van de kankerbehandeling ervaren en lopen ze een hoger risico op ziekenhuisopnamen en langdurig verlies van kwaliteit van leven.[25] Desondanks worden deze leeftijdsspecifieke factoren vaak niet meegenomen bij het bepalen van de behandelstrategie voor oudere patiënten, wat kan leiden tot aanzienlijke overbehandeling van deze populatie.[26,27]

PROGNOSTISCHE EN PREDICTIEVE MODELLEN

Er bestaan verschillende soorten modellen die gebruikt kunnen worden om adjuvante behandeling af te stemmen op de individuele patiënt. Prognostische modellen hebben als doel om patiënten te identificeren die een van nature slechtere ziektestatus hebben en een hoger risico lopen op het ontwikkelen van recidieven. Deze risicobeoordeling kan

vervolgens worden gebruikt om patiënten te selecteren voor specifieke adjuvante behandelingsstrategieën. Predictieve modellen zijn gebaseerd op het idee dat de patiënten met een hoog risico op een recidief niet per se dezelfde patiënten zijn die baat hebben bij een meer uitgebreide therapie. Het doel van een predictief model is het identificeren van de patiënten die het meeste voordeel halen uit de behandeling, ongeacht de onderliggende prognose.[28] Een van de doelen van het onderzoek beschreven in dit proefschrift was om verschillende prognostische en predictieve modellen te onderzoeken en te bepalen welke het meest geschikt zijn voor gebruik in de dagelijkse klinische praktijk.

Kwantitatieve hormoonreceptorbepaling

Een duidelijk voorbeeld van een predictieve marker is de hormoonreceptor, aangezien patiënten met ER-negatieve borstkanker helemaal geen baat hebben bij ET en patiënten met ER-positieve borstkanker wel. Borsttumoren worden als ER- of PR-positief beschouwd wanneer 1-10% of meer van de kankercellen deze receptoren tot expressie brengen. Echter hebben sommige patiënten met ER-positieve borstkanker nog steeds weinig baat bij ET. Er wordt vaak beweerd dat wanneer tumoren meer cellen hebben die ER of PR tot expressie brengen, ze gevoeliger zijn voor ET, hoewel er nog geen consensus is bereikt over de definitie van een hoog versus laag expressieniveau.[29] In **hoofdstuk 2** van dit proefschrift werd door middel van een literatuuroverzicht onderzocht of de kwantitatieve beoordeling van hormoonreceptoren een betere methode is om patiënten te selecteren voor ET dan enkel de drempelwaarde van 10%.

We concludeerden dat bij patiënten met een ER-positieve tumor (gedefinieerd als ER ≥10%) een hogere ER-load, zoals beoordeeld door immunohistochemie (IHC), niet gecorreleerd is aan een beter prognose, en er kon geen bewijs worden gevonden voor het gebruik van kwantitatieve ER-load als prognostische marker. Met andere woorden, patiënten met een hogere ER-load (bijv. 100%) hebben niet per se een betere prognose dan patiënten met een lagere ER-load (bijv. 20%). Verder werd er geen bewijs gevonden voor het gebruik van kwantitatieve ER-load als een predictieve marker, dat wil zeggen dat patiënten met een hogere ER-load niet méér baat hebben bij ET dan patiënten met een lagere ER-load.

Dit hoofdstuk concludeerde ook dat bij patiënten met een hormoonreceptorpositieve tumor een hogere PR-load niet lijkt te correleren met een betere prognose. Op basis van de geïncludeerde studies is kwantitatieve PR-load geen geschikte prognostische marker; patiënten met een hogere PR-load hebben geen betere prognose dan patiënten met een lagere PR-load, noch is het geschikt als een predictieve marker. Bovendien lijkt er een wisselwerking te zijn tussen de PR-load en de ER-load en daarom wordt de PR-load niet als een onafhankelijke predictor gezien.

De studies die in deze literatuurreview waren opgenomen, verschilden van elkaar in hun manier van het kwantitatief meten van de HR-load; sommige studies gebruikten een continu percentage of zogenoemde *histoscore*, anderen verdeelden patiënten in groepen van HR-negatief, lage HR-expressie en hoge HR-expressie, en weer anderen verdeelden patiënten in vier of meer groepen op basis van de *Allred-score* of het percentage positieve cellen. Dit beïnvloedde de uitkomsten. Studies vonden vaker een positieve associatie tussen HR-load en prognose wanneer een continue score werd gebruikt. Dit gebruik van een continue score om HR-expressie te beoordelen werd echter door verschillende auteurs in

twijfel getrokken. De variabiliteit tussen beoordelaars is hoog, en tumoren krijgen verschillende HR-percentages toegewezen afhankelijk van de patholoog en het laboratorium waarin deze werden beoordeeld. Echter was het belangrijkste probleem in dit literatuuronderzoek het definiëren van "zwakke" en "sterke" expressie van receptoren. Een gebrek aan algemeen geaccepteerde definities zorgde ervoor dat pathologen en artikelen hun eigen definitie kozen, wat het moeilijk maakte om meerdere studies te vergelijken.

Op basis van de resultaten van dit review stellen we voor om zowel ER- als PR-expressie alleen als een kwalitatieve maat te gebruiken; tumoren met minder dan 10% van de cellen die deze receptor tot expressie brengen, worden als negatief gedefinieerd, en tumoren met ≥10% van de cellen die de receptor tot expressie brengen, als positief. Het gebruik van een continue kwantitatieve maat lijkt niet haalbaar zonder gecentraliseerde, ondubbelzinnige en duidelijke pathologische metingen. De consequentie voor de dagelijkse praktijk van de patholoog is dat meer gedetailleerde informatie over de HR-status dan slechts "positief" of "negatief" niet meer verstrekt moet worden, om te voorkomen dat oncologen onbewust of instinctief andere beslissingen nemen op basis van deze informatie. Aangezien er geen bewijs is voor verschillende behandelstrategieën, is het verstrekken van extra informatie zowel onnodig als ongewenst.

Een ander model op basis van klinische gegevens

Een voorbeeld van een prognostisch hulpmiddel dat in de praktijk wordt gebruikt, is de CTS5 (*clinical treatment score post-5 years*). De CTS5 heeft als doel het risico op late afstandsmetastasen (d.w.z. na vijf jaar) in te schatten, op voorwaarde dat de patiënt recidief-vrij was in de eerste vijf jaar na diagnose. De CTS5-calculator genereert een percentage dat het risico weergeeft op het ontwikkelen van een recidief tussen vijf en tien jaar na diagnose. Deze categoriseert patiënten ook als laag, gemiddeld of hoog risico. Deze risicobeoordeling is gebaseerd op makkelijk verkrijgbare klinische en histopathologische parameters.[30] In **hoofdstuk 3** van dit proefschrift werd de bruikbaarheid en nauwkeurigheid onderzocht van de CTS5 om het risico op late afstandsmetastasen te bepalen en of het kan worden gebruikt om het voordeel van verlengde ET te voorspellen.

Patiënten met ER-positieve borstkanker hebben een substantieel risico op late afstandsmetastasen, zelfs na 5 jaar ET. Verlengde ET kan voordelig zijn om dit risico te verlagen. De CTS5 werd getraind met gegevens van postmenopauzale patiënten die ER-positieve borstkanker hadden en waren gerandomiseerd voor behandeling met anastrozol of tamoxifen, en die recidiefvrij waren na vijf jaar follow-up. De CTS5 werd intern gevalideerd met gegevens van postmenopauzale vrouwen met ER-positieve borstkanker, die waren gerandomiseerd voor behandeling met vijf jaar letrozol of tamoxifen, of sequentiële therapie (twee jaar letrozol gevolgd door drie jaar tamoxifen of andersom), en die recidiefvrij waren na vijf jaar follow-up. De CTS5 heeft als doel het risico in te schatten op afstandsmetastasen die zich ontwikkelen tussen vijf en tien jaar na diagnose, bij postmenopauzale patiënten met ER-positieve borstkanker die ziektevrij bleven na vijf jaar standaard ET.

Om de risicoschatting van de CTS5 te valideren, hebben we de CTS5 toegepast op de TEAM- en IDEAL-cohorten. Het TEAM-cohort bestond uit postmenopauzale patiënten met ER-positieve borstkanker die gerandomiseerd waren voor behandeling met vijf jaar

exemestaan of twee tot drie jaar tamoxifen gevolgd door twee tot drie jaar exemestaan, en die vijf jaar recidiefvrij waren. Het IDEAL-cohort bestond uit postmenopauzale patiënten met ER-positieve borstkanker die recidiefvrij waren na vijf jaar standaard ET en die vervolgens waren gerandomiseerd voor tweeëneenhalf of vijf jaar verlengde behandeling met letrozol. In deze twee grote cohorten van patiënten kwam het risico op late afstandsmetastasen zoals voorspeld door de CTS5 weliswaar overeen met de waargenomen mate van metastasen bij patiënten met een laag risico, maar de CTS5 overschatte het waargenomen risico op afstandsmetastasen bij patiënten met een hoger voorspeld risico. Bovendien kon de CTS5 niet voorspellen welke patiënten voordeel kunnen hebben van langer verlengde ET versus korter.

Concluderend kan de CTS5 patiënten indelen in lage-, gemiddelde- en hoge-risicogroepen. Bij laag-risicopatiënten komen de voorspelde risico's op late afstandsmetastasen overeen met de waargenomen mate van metastasen, maar de CTS5 overschatte het risico op late afstandsmetastasen bij hoog-risicopatiënten uit de TEAM- en IDEAL-cohorten. Ook kon de CTS5 niet gevalideerd worden als een predictief hulpmiddel voor verlengde ET. Vooral bij hoog-risicopatiënten kan een onrealistisch hoge inschatting van het risico leiden tot overbehandeling. Daarom moet de numerieke risicobeoordeling van de CTS5-calculator in zijn huidige vorm voorzichtig worden geïnterpreteerd bij het gebruik in de dagelijkse klinische praktijk, vooral bij hoog-risicopatiënten.

Zowel de hormoonreceptoranalyse zoals bestudeerd in **hoofdstuk 2**, als de CTS5 bestudeerd in **hoofdstuk 3** zijn modellen die gebaseerd zijn op klinische factoren. Ze kunnen onderscheid maken tussen lage- en hoog-risicopatiënten, maar zijn niet in staat om verder te discrimineren dan dat. Bovendien leidt kwantificering niet tot een nauwkeurigere risicobeoordeling, en beide modellen zijn niet in staat patiënten te selecteren die mogelijk baat zouden hebben bij verlengde ET.

Over het algemeen is het doel van prognostische hulpmiddelen om patiënten met een slechtere prognose te onderscheiden van patiënten met een betere prognose, terwijl predictieve modellen patiënten proberen te identificeren die waarschijnlijk goed zullen reageren op een behandeling. Vaak zijn prognostische hulpmiddelen niet predictief en moeten ze niet als zodanig worden gebruikt, omdat patiënten met een slechtere prognose niet altijd de patiënten zijn die profiteren van meer uitgebreide therapie. Dit wordt geïllustreerd met de resultaten uit **hoofdstuk 2** en **hoofdstuk 3**.

Predictie op basis van genetische parameters

Veelbelovender als een predictief model, is de beoordeling van de activiteit van de oestrogeenreceptor om te onderscheiden bij welke patiënten de receptor niet alleen tot expressie wordt gebracht, maar ook actief is en dus een geschikt doelwit is voor ET.

Een biomarker die primair ontwikkeld is als een predictief model, is de *Breast Cancer Index* (BCI). BCI is een genexpressieprofiel dat de verhouding onderzoekt tussen twee genen, HOXB13 en IL17BR (H/I), welke de activiteit van oestrogeensignalering in borsttumoren weergeeft.[31] Wanneer deze H/I-verhouding hoog is, wordt de oestrogeensignalering aangezet, en wordt de groei van de tumor waarschijnlijk beïnvloed door de aanwezigheid van oestrogenen.[32] Eerdere studies hebben aangetoond dat BCI patiënten kan identificeren die baat hebben bij verlengde ET na vijf jaar tamoxifen-monotherapie. Of BCI ook patiënten

kan identificeren die baat hebben bij verlengde ET na behandeling met een AI, werd beschreven in **hoofdstuk 4** van dit proefschrift.

Bevindingen uit dit hoofdstuk bevestigen het predictieve vermogen van BCI om door middel van de H/I-status patiënten te identificeren die een hoge mate van endocriene responsiviteit vertonen, waarbij deze patiënten betere uitkomsten hadden van verlengde ET dan patiënten met een lage mate van oestrogeensignalering.

In lijn met eerder gepubliceerde data toonde de BCI geen sterke correlatie met de kwantitatieve niveaus van ER en PR. Bovendien hebben meerdere translationele studies in de onder andere de ATAC-, BIG 1-98- en TEAM-trials, en onze eigen resultaten uit **hoofdstuk 2** herhaaldelijk aangetoond dat kwantitatieve ER- en PR-expressieniveaus geen voorspellende waarde hebben voor het effect van endocriene therapie, wat suggereert dat het predictievermogen van BCI gebaseerd is op andere biologische mechanismen, die niet direct gekoppeld zijn aan ER- of PR-expressieniveaus.

In **hoofdstuk 4** toonden we aan dat BCI significant kan voorspellen welke patiënten voordeel hebben van langere verlengde ET. In combinatie met eerdere data uit de MA.17- en Trans-aTTom-trials is het predictievermogen van BCI gevalideerd in een breed spectrum van patiënten die zijn behandeld met tamoxifen en AI. Het helder krijgen van de effectiviteit van verlengde ET is belangrijk om ervoor te zorgen dat zowel overbehandeling als onderbehandeling worden voorkomen. BCI kan gebruikt worden als hulpmiddel om patiënten te selecteren bij wie ET moet worden verlengd tot tien jaar.

In een vergelijkende studie tussen BCI en de CTS5, toonde de analyse aan dat geen enkele CTS5-groep significant voordeel had van verlengde ET.[33] Bij het opnieuw indelen van de CTS5-categorieën op basis van BCI, hadden alleen BCI-hoge patiënten consistent een absoluut voordeel van verlengde ET, ongeacht de CTS5-categorie. Daarentegen vertoonden CTS5-hoge patiënten geen enkel voordeel in de BCI-lage groep. Deze resultaten tonen aan dat de CTS5 geen voorspellende informatie biedt ter ondersteuning van besluitvorming over verlengde ET. Alleen BCI was een predictieve biomarker voor mate van voordeel uit verlengde ET. Deze resultaten komen overeen met onze bevindingen uit **hoofdstuk 3** en **hoofdstuk 4**.

Risicoschatting in oudere patiënten

Risicobeoordeling bij oudere patiënten is over het algemeen gebaseerd op dezelfde factoren als bij jongere patiënten. Leeftijdsgebonden factoren zoals sterfte door andere oorzaken en (negatieve) effecten van de behandeling worden vaak niet meegenomen bij het bepalen van de behandelingsstrategie, wat kan leiden tot aanzienlijke overbehandeling van deze populatie.[26] Daarom zijn er instrumenten nodig die specifiek gevalideerd zijn voor de oudere populatie. De *70-gene signature test*, ook wel *MammaPrint*, is een risicoprofiel op basis van genoomdata, dat al is gevalideerd als een nauwkeurig prognostisch model bij jongere borstkankerpatiënten.[34] Eerdere studies toonden aan dat *MammaPrint* kan worden gebruikt om het gebruik van chemotherapie en ET te de-escaleren bij respectievelijk patiënten met laag of ultralaag genomisch risico. Deze trials hebben echter geen patiënten van 70 jaar of ouder geïncludeerd. In **hoofdstuk 5** van dit proefschrift werd de validiteit en nauwkeurigheid van *MammaPrint* bij oudere patiënten onderzocht.

Onze data toonden aan dat de MammaPrint kan worden gebruikt om het risico op afstandsmetastasen nauwkeurig in te schatten bij patiënten met borstkanker van 70 jaar of ouder. Patiënten met een MammaPrint-ultralaag risico hadden een uitstekende klinische prognose tot tien jaar na de diagnose, ondanks dat 48% van hen geen systemische therapie kreeg. Significant meer patiënten met een MammaPrint-hoog risico ontwikkelden een metastase, hoewel 72% van deze hoog risico patiënten wel ET kreeg. Multivariate analyses, gecorrigeerd voor het gebruik van ET, toonden nog steeds significante lagere mate van metastasen na tien jaar voor patiënten met een MammaPrint-ultralaag risico.

Dit is de eerste studie die een genexpressieprofiel in de oudere populatie onderzoekt. Onze gegevens tonen aan dat genomische ultralaag-risico patiënten een uitstekende lange-termijn prognose hadden, zelfs als ze een klinisch hoog risico hadden. Dit kan worden verklaard door het stoppen van routinematige borstkankerscreening op de leeftijd van 75 jaar. Met toenemende leeftijd en verminderde deelname aan de borstkankerscreening wordt borstkanker vaker in een later klinische stadium gediagnosticeerd.[35] Grotere tumoren en betrokkenheid van een of meerdere lymfklieren zijn daardoor bij oudere vrouwen misschien geen tekenen van agressieve tumorbiologie, maar juist van late diagnose. Het gebruik van alleen klinische parameters om het risico op recidieven bij oudere patiënten te bepalen, kan dus leiden tot een minder nauwkeurige risicoschatting. Onze data toonden aan dat de MammaPrint een meer accurate risicoschatting biedt dan het gebruik van klinische factoren, en het lijkt daarbij een logische gedachte dat alle oudere patiënten met ultralaag-risico borstkanker op een veilige manier ET kunnen overslaan, echter moet dit worden bevestigd in gerandomiseerde trials.

De analyse uit **hoofdstuk 5** draagt bij aan de groeiende hoeveelheid data die de MammaPrint valideren. Vrouwen met een ultralaag-risico, ongeacht klinisch stadium of graad, hadden een extreem laag risico op recidief. Deze gegevens zijn vooral relevant voor clinici die werken met oudere patiënten, aangezien deze patiënten mogelijk kwetsbaarder zijn en gevoeliger voor bijwerkingen van een behandeling.

EEN ANDERE BENADERING VAN ADJUVANTE THERAPIE

Naast het bepalen welke patiënten het meeste baat hebben bij (verlengde) ET, kunnen andere therapeutische middelen ook helpen bij het verminderen van het risico op recidieven. Hormoonreceptor-positieve borstkankercellen zaaien vaak uit naar de botten, en ongeveer 70% van de borstkankermetastasen zijn botrecidieven.[36,37] Wanneer de borstkankercellen het botweefsel infiltreren, wordt het evenwicht tussen osteoclasten en osteoblasten verstoord.[36] De tumorcellen stimuleren de activiteit van osteoclasten, wat de botresorptie verhoogt en de afgifte van groeifactoren en cytokines bevordert. Deze factoren stimuleren vervolgens de groei en overleving van tumorcellen, wat een vicieuze cirkel creëert.[37]

Stikstofhoudende bisfosfonaten beïnvloeden ook het botmetabolisme, doordat zij bepaalde enzymen in de intracellulaire omgeving remmen. Dit vermindert de osteoclast-gemedieerde botresorptie en de overleving van osteoclasten, wat daardoor resulteert in een toename van de botdichtheid en een verminderde afgifte van cytokines en groeifactoren.[38] Het wordt verondersteld dat dit de botomgeving minder aantrekkelijk maakt voor de borstkankercellen.[39]

Verschillende trials hebben het effect van behandeling met (neo)adjuvante bisfosfonaten op borstkankerrecidieven onderzocht. In 2015 toonde een meta-analyse, die patiënten vergeleek die wel en niet met adjuvante bisfosfonaten werden behandeld, een vermindering van recidieven en mortaliteit aan in de subgroep van vrouwen die postmenopauzaal waren bij aanvang van de behandeling, maar toonde geen effect aan in de premenopauzale subgroep.[40] Tot nu toe is het gebruik van stikstofhoudende bisfosfonaten in hoge doseringen niet onderzocht bij uitsluitend postmenopauzale patiënten.

De TEAM-IIB trial onderzocht het effect van dagelijkse orale ibandronaat op de ontwikkeling van (bot)recidieven bij postmenopauzale patiënten met borstkanker, en de resultaten werden beschreven in **hoofdstuk 6**. Hier werd geen verschil gevonden in de algehele ziektevrije overleving tussen de ibandronaatgroep en de controlegroep. Er werd wel een significant verschil waargenomen in de eerste drie jaar na diagnose, maar dit verschil verdween naarmate de follow-up langer werd. Evaluatie van secundaire uitkomsten toonde ook alleen een voordeel van ibandronaat op de korte termijn. Gedurende de eerste vijf jaar na randomisatie hadden patiënten in de ibandronaatgroep minder recidieven in het algemeen, en ook minder recidieven in bot. Dit is in lijn met resultaten uit preklinisch onderzoek en de EBCTCG-meta-analyse uit 2015. Ondanks de gunstige effecten van ibandronaat op de ziektevrije overleving en (bot)recidiefpercentage op de korte termijn, was ibandronaat niet effectief bij langere follow-up. Na acht jaar follow-up was het (bot)recidiefpercentage vergelijkbaar tussen de ibandronaatgroep en de controlegroep.

Bovendien brengt behandeling met ibandronaat aanzienlijke bijwerkingen met zich mee. Bisfosfonaten worden geassocieerd met griepachtige symptomen, musculoskeletale pijn en hypocalciëmie. De incidentie van ernstige bijwerkingen, zoals osteonecrose van de kaak en nierschade, is laag. De meeste trials rapporteren een incidentie van ongeveer 1% voor deze bijwerkingen. Opmerkelijk is dat in TEAM-IIB de incidentie van kaaknecrose 1,9% was, vooral bij vrouwen die een AI gebruikten. Dit roept de vraag op of de combinatie van bisfosfonaten met een AI bij postmenopauzale vrouwen dit risico verhoogt. Tamoxifen verhoogt de botdichtheid bij postmenopauzale vrouwen omdat het als oestrogeenagonist werkt in botweefsel, terwijl AI osteoporose veroorzaakt door de aanmaakcyclus van bot juist te verstoren, wat leidt tot verhoogde activiteit van osteoclasten. Bisfosfonaten verminderen de activiteit van osteoclasten, maar verlagen ook angiogenese en veroorzaken slechte wondgenezing. Daarom kan de gelijktijdige toediening van een AI en hoge dosis ibandronaat het risico op het ontwikkelen van kaaknecrose verhogen in vergelijking met de combinatie van ibandronaat en tamoxifen. Hoewel de patiënttevredenheid over orale behandeling over het algemeen hoog is en orale bisfosfonaten meestal goed worden verdragen, stopte 18% van de TEAM-IIB-patiënten hun ibandronaatbehandeling eerder dan gepland vanwege bijwerkingen, en ongeveer een derde van hen had gastro-intestinale klachten.

Samenvattend kunnen de data uit **hoofdstuk 6** de keuze om ibandronaat als adjuvante behandeling bij niet-geselecteerde postmenopauzale vrouwen met ER-positieve borstkanker niet ondersteunen.

TOEKOMSTPERSPECTIEVEN

Het blijft belangrijk om juist die borstkankerpatiënten te identificeren die het meeste baat hebben bij ET. Patiënten kunnen globaal in vier groepen worden ingedeeld. Allereerst is er een groep patiënten met hormoonreceptorpositieve borstkanker die dusdanig gunstige tumorkarakteristieken heeft, dat zij nooit een recidief zullen ontwikkelen, zelfs niet als ze de adjuvante behandeling helemaal overslaan. Als tweede zijn er patiënten die zeker een recidief zullen ontwikkelen, zelfs wanneer zij optimaal worden behandeld met ET. Een derde groep patiënten zou een recidief kunnen ontwikkelen, maar dit kan worden voorkomen door middel van ET. En als laatste is er een groep oudere of kwetsbare patiënten die, hoewel ze een recidief zouden kunnen ontwikkelen, waarschijnlijk zullen overlijden door andere oorzaken, gerelateerd aan hun comorbiditeit, voordat de borstkanker opnieuw opduikt. Bij deze groep heeft de ontwikkeling van een recidief geen invloed op hun overleving of kwaliteit van leven.

Idealiter zouden alleen de patiënten uit de derde groep behandeld worden met ET. Als oncologen in staat zijn om te voorspellen tot welke groep een patiënt behoort, kunnen ze beter adviseren over het gebruik van ET. Gezien de frequente en soms ernstige bijwerkingen van ET, zou een verbeterde selectie van de patiënten die behandeling nodig hebben, de behandellast verlagen.

Identificatie van Groep 1

Om patiënten uit de eerste groep te identificeren, kan de *70-gene signature test*, zoals beschreven in **hoofdstuk 5** worden gebruikt. Onze data tonen aan dat patiënten met een genomisch ultralaag-risico uitstekende langetermijnresultaten hadden, zelfs wanneer ze klinisch als hoog risico werden geclassificeerd. Het lijkt daardoor mogelijk dat patiënten met ultralaag risico borstkanker geen ET nodig zouden hebben, aangezien het risico op het ontwikkelen van een afstandsmetastase extreem laag lijkt.

Differentiatie tussen Groep 2 en Groep 3

Om te onderscheiden tussen de tweede en derde groep patiënte kan het helpen om de activiteit van de oestrogeenreceptor te meten, zodat kan worden bepaald in welke patiënten deze receptor niet alleen tot expressie komt, maar ook actief is, en dus een geschikt doelwit is voor ET. De BCI, zoals beschreven in **hoofdstuk 4**, zou hiervoor een geschikt hulpmiddel kunnen. Andere analyses die de activiteit van de receptor meten kunnen ook nuttig zijn om dit onderscheid te maken.

Identificatie van Groep 4

De vierde groep kan worden geïdentificeerd met behulp van de PORTRET-tool. De PORTRET-tool is bedoeld om het recidief, de algehele mortaliteit, en de mortaliteit door andere oorzaken dan kanker bij oudere borstkankerpatiënten te voorspellen, en daarbij een geïndividualiseerde schatting van het effect van adjuvante behandeling te geven.[41] Deze tool toonde goede interne en externe validatie, met betere nauwkeurigheid bij oudere patiënten dan bestaande predictiemodellen, door comorbiditeit en geriatrische parameters te integreren. Het nauwkeurig voorspellen van het risico op overlijden door andere oorzaken dan de borstkanker is een belangrijk aspect van de PORTRET-tool.

Hoewel er al hulpmiddelen bestaan die de resterende levensverwachting van ouderen inschatten, zoals de Lee-index/ePrognosis, is het belangrijkste voordeel van de PORTRET-tool dat het deze uitkomst combineert met borstkanker-specifieke uitkomsten.[42]

CONCLUSIE

Dit proefschrift had als doel om overzicht te bieden bij het navigeren door het woud van verschillende prognostische en predictieve modellen. Zodra we in staat zijn om patiënten nauwkeurig toe te wijzen aan een van de vier groepen door het gebruik van dit soort modellen, kunnen we adjuvante behandelstrategieën voor patiënten met ER-positieve borstkanker optimaliseren en personaliseren. Het gebruik van deze instrumenten zal bijdragen aan een verfijnde benadering van borstkankerbehandeling, waarbij we het risico van recidief beter kunnen inschatten en de behandeling beter kunnen afstemmen op de behoeften van elke individuele patiënt.

REFERENTIES

1. Beatson GT: On the Treatment of Inoperable Cases of Carcinoma of the Mamma: Suggestions for a New Method of Treatment, with Illustrative Cases. Lancet 2:104-107, 1896
2. Boyd S: On oophorectomy in cancer of the breast. Br Med J 2:1161-1167, 1900
3. Jensen EV, Jacobson HI: Fate of steroid estrogens in target tissues. Biological Activities of Steroids in Relation to Cancer:161-174, 1960
4. Russo J, Russo IH: The role of estrogen in the initiation of breast cancer. J Steroid Biochem Mol Biol 102:89-96, 2006
5. Quirke VM: Tamoxifen from Failed Contraceptive Pill to Best-Selling Breast Cancer Medicine: A Case-Study in Pharmaceutical Innovation. Front Pharmacol 8:620, 2017
6. Lewis JS, Jordan VC: Selective estrogen receptor modulators (SERMs): mechanisms of anticarcinogenesis and drug resistance. Mutat Res 591:247-63, 2005
7. EBCTCG: Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 365:1687-717, 2005
8. Buzdar A, Howell A: Advances in aromatase inhibition: clinical efficacy and tolerability in the treatment of breast cancer. Clin Cancer Res 7:2620-35, 2001
9. Cui J, Shen Y, Li R: Estrogen synthesis and signaling pathways during aging: from periphery to brain. Trends Mol Med 19:197-209, 2013
10. EBCTCG: Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. Lancet 386:1341-1352, 2015
11. Dutch Cancer Registry (NKR), IKNL. Obtained through https://www.iknl.nl/nkr-cijfers, accessed on April 4 2021.
12. Allemani C, Matsuda T, Di Carlo V, et al: Global surveillance of trends in cancer survival 2000-14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. Lancet 391:1023-1075, 2018
13. Osborne CK, Schiff R: Estrogen-receptor biology: continuing progress and therapeutic implications. J Clin Oncol 23:1616-22, 2005
14. Rosenberg PS, Barker KA, Anderson WF: Estrogen Receptor Status and the Future Burden of Invasive and In Situ Breast Cancers in the United States. J Natl Cancer Inst 107, 2015
15. Nationaal Borstkanker Overleg Nederland; https://www.oncoline.nl/borstkanker; accessed on April 8, 2021.
16. Pan H, Gray R, Braybrooke J, et al: 20-Year Risks of Breast-Cancer Recurrence after Stopping Endocrine Therapy at 5 Years. N Engl J Med 377:1836-1846, 2017
17. Davies C, Pan H, Godwin J, et al: Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. Lancet 381:805-16, 2013
18. Goss PE, Ingle JN, Pritchard KI, et al: Extending Aromatase-Inhibitor Adjuvant Therapy to 10 Years. N Engl J Med 375:209-19, 2016
19. Blok EJ, Kroep JR, Meershoek-Klein Kranenbarg E, et al: Optimal Duration of Extended Adjuvant Endocrine Therapy for Early Breast Cancer; Results of the IDEAL Trial (BOOG 2006-05). J Natl Cancer Inst 110, 2018
20. Gnant M, Steger G, Greil R, et al: Abstract GS3-01: A prospective randomized multi-center phase-III trial of additional 2 versus additional 5 years of anastrozole after initial 5 years of adjuvant endocrine therapy – results from 3,484 postmenopausal women in the ABCSG-16 trial. Cancer Res 78:GS3-01-GS3-01, 2018
21. Mamounas EP, Bandos H, Lembersky BC, et al: Use of letrozole after aromatase inhibitor-based therapy in postmenopausal breast cancer (NRG Oncology/NSABP B-42): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 20:88-99, 2019
22. Tjan-Heijnen VCG, van Hellemond IEG, Peer PGM, et al: Extended adjuvant aromatase inhibition after sequential endocrine therapy (DATA): a randomised, phase 3 trial. Lancet Oncol 18:1502-1511, 2017
23. de Boer AZ, van der Hulst HC, de Glas NA, et al: Impact of Older Age and Comorbidity on Locoregional and Distant Breast Cancer Recurrence: A Large Population-Based Study. Oncologist 25:e24-e30, 2020
24. Klepin H, Mohile S, Hurria A: Geriatric assessment in older patients with breast cancer. J Natl Compr Canc Netw 7:226-36, 2009
25. Lee SY, Seo JH: Current Strategies of Endocrine Therapy in Elderly Patients with Breast Cancer. Biomed Res Int 2018:808-820, 2018
26. Cardoso F, Kyriakides S, Ohno S, et al: Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 30:1194-1220, 2019
27. Derks MGM, Bastiaannet E, Kiderlen M, et al: Variation in treatment and survival of older patients with non-metastatic breast cancer in five European countries: a population-based cohort study from the EURECCA Breast Cancer Group. Br J Cancer 119:121-129, 2018

28. Sechidis K, Papangelou K, Metcalfe PD, et al: Distinguishing prognostic and predictive biomarkers: an information theoretic approach. Bioinformatics 34:3365-3376, 2018
29. Curigliano G, Burstein HJ, Winer EP, et al: De-escalating and escalating treatments for early-stage breast cancer: the St. Gallen International Expert Consensus Conference on the Primary Therapy of Early Breast Cancer 2017. Ann Oncol 28:1700-1712, 2017
30. Dowsett M, Sestak I, Regan MM, et al: Integration of Clinical Variables for the Prediction of Late Distant Recurrence in Patients With Estrogen Receptor-Positive Breast Cancer Treated With 5 Years of Endocrine Therapy: CTS5. J Clin Oncol 36:1941-1948, 2018
31. Sgroi DC, Sestak I, Cuzick J, et al: Prediction of late distant recurrence in patients with oestrogen-receptor-positive breast cancer: a prospective comparison of the breast-cancer index (BCI) assay, 21-gene recurrence score, and IHC4 in the TransATAC study population. Lancet Oncol 14:1067-1076, 2013
32. Sgroi DC, Carney E, Zarrella E, et al: Prediction of late disease recurrence and extended adjuvant letrozole benefit by the HOXB13/IL17BR biomarker. J Natl Cancer Inst 105:1036-42, 2013
33. Sgroi D, Zhang Y, Schnabel CA: Comparative performance of Breast Cancer Index (BCIN+) and CTS5 in hormone receptor-positive (HR+) lymph node-positive (N+) breast cancer patients with one to three positive nodes (N1). J Clin Oncol 37:555, 2019
34. Cardoso F, van't Veer LJ, Bogaerts J, et al: 70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer. N Engl J Med 375:717-29, 2016
35. Cappellani A, Di Vita M, Zanghì A, et al: Prognostic factors in elderly patients with breast cancer. BMC Surg 13 Suppl 2:S2, 2013
36. Brockton NT, Gill SJ, Laborge SL, et al: The Breast Cancer to Bone (B2B) Metastases Research Program: a multi-disciplinary investigation of bone metastases from breast cancer. BMC Cancer 15:512, 2015
37. Coleman RE, Rubens RD: The clinical course of bone metastases from breast cancer. Br J Cancer 55:61-6, 1987
38. Rogers MJ, Crockett JC, Coxon FP, et al: Biochemical and molecular mechanisms of action of bisphosphonates. Bone 49:34-41, 2011
39. van de Ven S, Kroep JR, Hamdy NA, et al: [Antitumour effects of bisphosphonates in breast cancer]. Ned Tijdschr Geneeskd 154:A1951, 2010
40. EBCTCG: Adjuvant bisphosphonate treatment in early breast cancer: meta-analyses of individual patient data from randomised trials. Lancet 386:1353-1361, 2015
41. van der Plas-Krijgsman WG, Giardiello D, Putter H, et al: Development and validation of the PORTRET tool to predict recurrence, overall survival, and other-cause mortality in older patients with breast cancer in the Netherlands: a population-based study. Lancet Healthy Longev 2:e704-e711, 2021
42. Lee SJ, Lindquist K, Segal MR, et al: Development and validation of a prognostic index for 4-year mortality in older adults. Jama 295:801-8, 2006

LIST OF PUBLICATIONS

Noordhoek I, Bastiaannet E, de Glas NA, Scheepens J, Esserman LJ, Wesseling J, et al. Validation of the 70-gene signature test (MammaPrint) to identify patients with breast cancer aged ≥70 years with ultralow risk of distant recurrence: a population-based cohort study. *Journal of Geriatric Oncology.* 2022; 13(8): 1172-1177.

Ünlü B, Kocatürk B, Rondon AMR, Lewis CS, Swier N, van den Akker RFP, Krijgsman D, **Noordhoek I**, et al. Integrin regulation by tissue factor promotes cancer stemness and metastatic dissemination in breast cancer. *Oncogene.* 2022; 41(48): 5176-5185.

Liefers GJ, **Noordhoek I**, Putter H, Wong J, Siuliukina N, Zhang Y, et al. Predictive performance of breast cancer index (BCI) and clinical treatment score post-5 years (CTS5) in the IDEAL study. *Journal of Clinical Oncology*. 2022; 40 (16 Suppl): 545.

Esgueva AJ, **Noordhoek I**, Meershoek-Klein Kranenbarg E, Espinosa-Bravo M, Mátrai Z, Zhygulin A, et al. Health-related quality of life after nipple-sparing mastectomy: results from the INPIRE registry. *Annals of Surgical Oncology.* 2022; 29(3): 1722-1734.

Vliek SB*, **Noordhoek I***, Meershoek-Klein Kranenbarg E, van Rossum AGJ, Dezentje VO, Jager A, et al. Daily oral ibandronate with adjuvant endocrine therapy in postmenopausal women with hormone receptor positive breast cancer (BOOG 2006-04): randomized phase 3 TEAM-IIB trial. *Journal of Clinical Oncology*. 2022; 40(25): 2934-2945.

Noordhoek I, Sohn M, Wang L, Mohamed M, Mohile S, Loh KP, et al. Validation of the cancer and aging research group (CARG) toxicity score in the community oncology setting. *Journal of Geriatric Oncology*. 2021; 12(8 Suppl 1): S38-S39.

Liefers GJ, **Noordhoek I**, Treuner K, Putter H, Wong J, Zhang Y, et al. Breast cancer index and prediction of benefit from extended endocrine therapy based on treatment compliance: an IDEAL study. *Journal of Clinical Oncology.* 2021; 39(15 Suppl): 531.

Liefers GJ, **Noordhoek I**, Zhang Y, Sgroi DC, Putter H, Treuner K, et al. An optimized breast cancer index node-positive (BCIN+) prognostic model for late distant recurrence in patients with hormone receptor-positive (HR+) node-positive breast cancer. *Annals of Oncology*. 2021; 32(Suppl 2): S21-S36.

Liefers GJ, **Noordhoek I**, Treuner K, Putter H, Zhang Y, Wong J, et al. Breast Cancer Index is a molecular signature of endocrine responsiveness that determines extended endocrine benefit independent of prognostic risk. *Cancer Research.* 2021; 81(4 Suppl): PD2-11.

Esgueva AJ, **Noordhoek I**, Meershoek-Klein Kranenbarg E, Siso Raber C, Mátrai Z, Zhygulin A, et al. Impact of endocrine therapy on quality of life in early stage breast cancer patients: prospective patient reported outcomes from the international registry INSPIRE. *Breast.* 2021; 56 (Suppl 1): S81-S82.

Noordhoek I, Putter H, Portielje JEA, Liefers GJ. Reply to M. Dowsett et al. *Journal of Clinical Oncology*. 2021; 39(4): 339-340.

Noordhoek I, Treuner K, Putter H, Zhang Y, Wong J, Meershoek-Klein Kranenbarg E, et al. Breast Cancer Index predicts extended endocrine benefit to individualize selection of patients with HR+ early-stage breast cancer for 10 years of endocrine therapy. *Clinical Cancer Research*. 2021; 27(1): 311-319.

Noordhoek I, Blok EJ, Meershoek-Klein Kranenbarg E, Putter H, Duijm-de Carpentier M, Rutgers EJT, et al. Overestimation of late distant recurrences in high-risk patients with ER-positive breast cancer: validity and accuracy of the CTS5 risk score in the TEAM and IDEAL trials. *Journal of Clinical Oncology*. 2020; 38(28): 3273-3281.

Noordhoek I, Bastiaannet E, Meershoek-Klein Kranenbarg E, Espinosa-Bravo M, Mátrai Z, Zhygulin A, et al. Preliminary results of the international nipple sparing mastectomy registry (INSPIRE): a EURECCA project. *European Journal of Surgical Oncology*. 2020; 46(2): E17-E18.

Noordhoek I, Bastiaannet E, van Eycken L, Siesling S, Walsh P, Johannesen T, et al. International comparison of treatment and survival of older patients with primary metastatic breast cancer: a population-based cohort study from the EURECCA Breast Cancer Group. *European Journal of Surgical Oncology*. 2020; 46(2): E22.

Noordhoek I, van der Hage JA. Magnetic marker localization for non-palpable breast cancer using the Magseed and Sentimag systems. *European Journal of Surgical Oncology*. 2020; 46(2): E59.

Evrard S, van de Velde CJH, **Noordhoek I**, Caballero C, Ceelen W, Polom K, et al. European Society of Surgical Oncology's strategy for clinical research: paving the way for a culture of research in cancer surgery. *European Journal of Surgical Oncology*. 2019; 45(9): 1515-1519.

Noordhoek I, de Groot AF, Cohen D, Liefers GJ, Portielje JEA, Kroep JR. Higher ER load is not associated with better outcome in stage 1-3 breast cancer. *Breast Cancer Research and Treatment*. 2019; 176(1): 27-36.

Noordhoek I, Koning MT, Vleggeert-Lankamp CLA. Evaluation of fusion after anterior cervical discectomy. *European Spine Journal*. 2019; 28(2): 386-399.

Noordhoek I, Bastiaannet E, van de Velde CJH. Improving hormonal therapy: to extend, to intermit or to readjust expectations? *Translational Cancer Research*. 2018; 7(4 Suppl): S525-S527.

Noordhoek I, Koning MT, Jacobs WCH, Vleggeert-Lankamp CLA. Incidence and clinical relevance of cage subsidence in anterior cervical discectomy and fusion. *Acta Neurochirurgica*. 2018; 160(4): 873-880.

CURRICULUM VITAE

Iris Noordhoek was born in Haarlem on May 8, 1995. In 2013, she graduated from the Stedelijk Gymnasium Haarlem. During the final two years at this school, Iris also followed the Pre-University College program from the Leiden University Honours College. This allowed her to start her Bachelor of Medicine at the Leiden University Medical Center via decentral selection.

During her study, Iris discovered her interest in scientific research. Her scientific career started by following the Honours Program with a project at the department of Neurosurgery. When she started her Master's degree in 2017 and did her internship in surgery, Iris was asked to pause her studies to start a PhD program at the Leiden University Medical Center under the supervision of prof. dr. van de Velde and later prof dr. Portielje. She researched the validation of several prediction models used for breast cancer treatment. The results are described in this thesis. In 2021, Iris resumed her Master's degree and she graduated in 2024.

Currently, Iris is training to become a General Practitioner in Zeeland.

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