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### Prognostic factors in breast cancer: one fits all?

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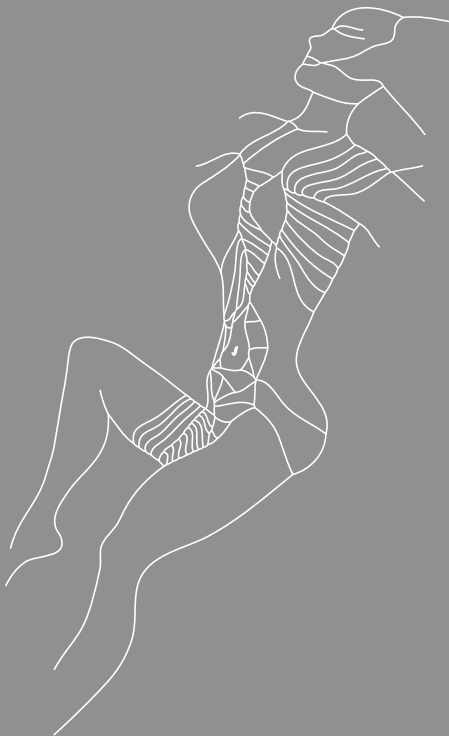
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# Chapter 10

General discussion  
and future prospects





Over the last decades, breast cancer management has changed dramatically. Primary local treatment has evolved from the extensive Halsted mastectomy to less invasive breast conservative surgery followed by radiotherapy, which is currently the standard treatment for approximately 2/3 of the breast cancer patients.<sup>1</sup> In addition, staging of the axilla by the sentinel node procedure is now widely practiced and the standard of care for patients with clinically negative nodal status.<sup>2,3</sup> Patients with a negative sentinel node can be spared complete axillary lymph node dissection and its associated side-effects.<sup>4,5</sup> The final analysis of the AMAROS trial show will show whether in case of sentinel lymph node involvement axillary lymph node dissection can be abandoned and radiotherapy of the axilla provides a safe and equivalent alternative with less morbidity. In the meantime, even more provocative results of the ASCO Z0011 trial by Giuliano and colleagues were published.<sup>6</sup> Patients with invasive breast carcinomas  $\leq 5$  cm treated with breast conserving therapy and adjuvant systemic therapy (in 96% of the patients) who had 1-3 positive sentinel nodes were randomized between axillary lymph node dissection and no further axillary treatment. Remarkably, no difference in local and regional recurrence was observed, suggesting that for a selected group of patients with macrometastases in the sentinel lymph node further axillary treatment can be safely omitted.

All the above mentioned changes in breast cancer management touch upon the ultimate goal to optimize and tailor treatment by reducing side-effects without jeopardizing survival and were guided by a conceptual change in the theory on breast cancer etiology and progression.<sup>7</sup> Traditionally, breast cancer management was based on the Halsted theory. Halsted stated that breast cancer is a localized disease, spreading in an orderly and consecutive manner from local tissue, to regional lymph nodes and then to distant sites.<sup>8</sup> This theory justified the use of extensive loco-regional surgery (*i.e.* radical mastectomy) to remove all local and regional disease, thereby improving survival. In 1968, Fisher introduced an alternative hypothesis, which has led to a number of changes in breast cancer management.<sup>9,10</sup> Fisher postulated that breast cancer is primarily a systemic disease, with the presence of circulating cancer cells already at an early stage, thus requires treatment of the entire patient (systemic treatment). As a consequence, according to the Fisher theory, local recurrence should be considered an indicator of metastatic disease, and the development of distant metastases is a result of both tumor and patient characteristics and the interaction between them. This hypothesis and the knowledge of the incurable nature of metastatic breast cancer with the emanating fear of undertreatment have caused a substantial increase in the use of adjuvant systemic therapy.<sup>11,12,13</sup>

## **Selection of patients for adjuvant systemic therapy; current practice**

Currently, guidelines recommend adjuvant systemic therapy (AST) for all patients with lymph node positive disease and for over 80% of breast cancer patients with lymph node negative disease.<sup>13,14</sup> These guidelines base their recommendations for the use of AST on clinicopathological prognostic characteristics, such as age, tumor size, tumor grade, lymph node status and estrogen receptor status.<sup>14-17</sup> These clinicopathological factors are used to identify subgroups of patients with a poor prognosis who are expected to benefit more from adjuvant systemic therapy. However, many patients in these subgroups are overtreated since they do not have micrometastases at diagnosis and thus are likely to remain free from distant metastases.<sup>18</sup> This overtreatment is particularly poignant in lymph node negative breast cancer where over 80% is treated with AST, whereas approximately 70% of the patients are free of distant metastases at 10 years and likely to be cured with locoregional treatment alone.<sup>18</sup> Conversely, patient selection based on clinicopathological criteria can also cause undertreatment. According to some current guidelines, AST is often not recommended for patients with small tumors of less than 1 cm; however, a proportion of these small tumors may have spread before detection and should consequently be treated with AST. It is clear that patients who suffer from an apparently similar tumor with regard to pathological characteristics can have remarkably different disease outcomes. Therefore, patient selection for adjuvant systemic therapy by traditional prognostic factors has its limitations and will lead to both over- and undertreatment. Breast cancer treatment with as little as possible side effects and optimal survival requires a patient-tailored approach based on appropriate patient selection. This shift from a 'one size fits all' approach to a more personalized approach uncovers the need for better prognostic markers or tools in breast cancer and is the rationale for this thesis.

## **Multigene prognosis signatures**

The introduction of high-throughput microarray technology facilitated the development of gene-expression profiles or signatures that can measure the expression of multiple genes in a single test.<sup>19-21</sup> The 70-gene prognosis signature (MammaPrint™) is one of the new prognostic markers that can accurately discriminate between breast cancer patients at low and high risk of developing distant metastases, based on the expression level of 70 selected genes.<sup>22</sup> Validation studies confirmed that the signature can accurately predict disease outcome in premenopausal, lymph node negative breast cancer patients.<sup>23,24</sup> In addition, the prognostic value of the signature was independent of known clinicopathological prognostic factors.<sup>23-27</sup> These studies, as well as studies described in this thesis have led to the inclusion of the 70-gene signature in current guidelines.<sup>14,15</sup> Soon after the development of the 70-gene signature, the Recurrence Score (OncotypeDX™) was developed and

validated.<sup>28-31</sup> This test classifies tumors based on the expression of 16 genes into a low Recurrence Score (RS), an intermediate RS or a high RS. The RS has been validated in several patient series and has been incorporated into the St. Gallen recommendations and NCCN guidelines.<sup>15,32</sup> The recently conducted Trial Assigning Individualized Options for Treatment (Rx) (TAILORx), will address whether patients who are assigned to the intermediate RS have benefit from adjuvant chemotherapy in addition to endocrine therapy. Patient inclusion for the TAILORx was finished end 2010.<sup>33</sup>

## Prognostic value of the 70-gene signature in breast cancer subgroups

For the studies presented in this thesis independent retrospective patient series were selected to assess the prognostic value of the 70-gene signature in postmenopausal breast cancer patients and in patients with 1-3 positive lymph nodes. For the validation of the signature in small tumors patients were selected from a pooled database of previously published studies and studies described in **chapter 4 and 5** of this thesis.<sup>23-25,34,35</sup>

The majority of breast cancer patients are postmenopausal women.<sup>36</sup> Although former treatment guidelines recommended adjuvant endocrine therapy only, there is a strong increase in the use of chemotherapy for postmenopausal patients.<sup>37-39</sup> This more extensive use of adjuvant treatment is intuitively in contrast to the more favorable tumor characteristics and good disease outcome observed in many postmenopausal patients.<sup>37-39</sup> Besides, the overview data show that the benefit of chemotherapy diminishes as age increases.<sup>18</sup> In addition, the benefit of chemotherapy in postmenopausal patients seems to occur mainly in the first five years.<sup>18</sup> In **chapter 4** we show that even though the 70-gene signature was developed in premenopausal patients, it has independent prognostic value and utility in postmenopausal women. The signature identified poor prognosis patients who are at high risk of developing distant metastases early in the disease course and are therefore likely to benefit more from chemotherapy. Postmenopausal patients who were classified as low risk were likely to remain free of early disease recurrence; however, a proportion of those low risk patients did develop late metastases. This low risk subgroup consisted of estrogen receptor positive tumors and only 1 patient received endocrine therapy. Since the beneficial effect of chemotherapy in postmenopausal patients is limited to the first five years, patients classified as low risk who are at risk of late breast cancer related events are more likely to benefit from endocrine therapy.

Historically, lymph node status is considered to be the most powerful prognostic factor in breast cancer, with the presence and number of involved nodes being associated with poor disease outcome.<sup>40-42</sup> As a consequence, patients with lymph node positive disease are currently offered chemotherapy, regardless of other clinicopathological characteristics. However, a subset of patients (approximately 25-30%) will remain free of distant metastases for at least 10 years without AST and are presumably cured by locoregional treatment

alone.<sup>18,43</sup> So far, no prognostic factor that can identify this subset of good prognosis lymph node positive patients has been identified. New prognostic markers or signatures will only have medical utility when they can identify a clinically relevant subset of patients with potential treatment consequences. For example, the identification of a subset of patients who have a low risk of recurrence will only be relevant when the risk is sufficiently small to justify withholding chemotherapy. In **chapter 5** we have demonstrated that the 70-gene signature was able to identify a subset of patients with 1-3 positive lymph nodes who are at sufficiently low risk to consider withholding chemotherapy. The appreciation of the existence of low risk lymph node positive patients who might not benefit from chemotherapy was also confirmed for the Recurrence Score by Albain and colleagues.<sup>28</sup> Based on these results the currently conducted randomized MINDACT trial has extended its eligibility criteria to patients with up to 3 positive nodes. Results of this prospective trial might end the long existing and persistent idea that all patients with lymph node involvement will be confronted with metastatic disease and should receive adjuvant chemotherapy.

In addition to lymph node status, tumor size is a traditional prognostic factor that is taken into account when selecting patients for adjuvant systemic therapy.<sup>40-42</sup> Current guidelines are inconsistent in the systemic treatment recommendations for patients with small breast tumors.<sup>14,15,17,32</sup> In the study described in **chapter 6** we show that the 70-gene signature can select patients with pT1c tumors (pT1c: 11-20cm) who are at low risk of distant recurrence and therefore could be safely spared chemotherapy. Moreover, the 70-gene signature could identify patients with small breast tumors (pT1ab  $\leq$ 10mm) who do have a substantial risk of developing distant metastases despite the small tumor size. The above mentioned results support the Fisher hypothesis that metastatic capacity is an early inheritance, and that lymph node involvement is an indicator and not instigator of distant disease.<sup>9,44</sup> In addition, our studies suggest that the metastatic capacity depends (at least partially) on the genetic makeup of a tumor and thus can be identified by the measurement of tumor gene expression.

## Retrospective validation

Retrospective studies are of indispensable value to identify potential biomarkers that deserve further evaluation; however, there are some drawbacks that one needs to be aware of.

In retrospective series that were not part of a randomized trial, patients have received treatment according to the guidelines present at that time. Therefore, clinicopathological markers will have influenced treatment decision, which will complicate extrapolation of the results. However, recommendations for the use of adjuvant systemic therapy in the Netherlands have been conservative for a long time, as described in the introduction of

this thesis.<sup>14,45,46</sup> As a result, retrospective series of Dutch breast cancer patients who were diagnosed before 2000 will consist of a relatively large proportion of untreated patients. For instance, the first validation study of the 70-gene signature included a true consecutive series of lymph node negative patients of whom only 10% received AST.<sup>24</sup> In addition, the postmenopausal patient series described in **chapter 4** was a true consecutive series and no patients were excluded because of chemotherapy treatment. One might argue that the prognostic value of a marker cannot be assessed in a (partially) treated population. However, selecting patients who did not receive adjuvant systemic therapy will introduce selection bias. Markers that can define the residual risk of recurrence when a patient will be treated with endocrine therapy alone are of utmost importance in determining the potential value and necessity of additional chemotherapy. Including patients who have only received adjuvant endocrine therapy seems to be a reasonable compromise. The evaluation of the 70-gene signature in postmenopausal patients (**chapter 4**) was performed in a consecutive series of patients who did not receive AST or were only treated with endocrine therapy. The Recurrence Score (OncotypeDX™) provides an estimate of the additive effect of adjuvant chemotherapy in combination with 5 years of endocrine treatment with Tamoxifen.<sup>28-31</sup> However, for patients who are assigned to the intermediate RS, the additional benefit of chemotherapy remains uncertain while having a considerable risk of recurrence and result of the TAILORx needs to be awaited.<sup>33</sup>

To avoid selection bias as mentioned above, we included patients with lymph node positive disease regardless of adjuvant systemic therapy for the validation study described in **chapter 5**. As a consequence, 56% of the patients received adjuvant chemotherapy. These patients had more often estrogen receptor negative and poorly differentiated tumors, which in general are believed to have more benefit from chemotherapy.<sup>18</sup> In addition, patients treated with chemotherapy were more often classified as poor prognosis by the 70-gene signature. In the study presented in **chapter 7**, we analyzed 541 patients who had received adjuvant systemic therapy and who were classified by the 70-gene signature. Patients with a 70-gene poor prognosis signature treated with chemotherapy followed by endocrine therapy had a significantly better distant disease-free survival compared with poor prognosis patients who were treated with endocrine therapy alone. Conversely, patients with a low risk 70-gene signature who were treated with chemotherapy and endocrine therapy had similar disease outcomes as low risk patients treated with endocrine therapy alone. This study provides evidence that patients with a high risk 70-gene signature are more likely to benefit from adjuvant chemotherapy, whereas a low risk 70-gene signature indicates limited benefit from adjuvant chemotherapy, in addition to a low recurrence risk to begin with. Additionally, in a recently published study the predictive value of the 70-gene signature was assessed in patients treated with neoadjuvant chemotherapy.<sup>47</sup> Although patients included in this study were considered as clinically high risk patients (95% of the tumors were > 2 cm and 72% had lymph node positive disease), the 70-gene signature identified 14% good prognosis tumors. Among patients with a good prognosis

tumor none achieved a pathological complete response, which confirmed the predictive value of the 70-gene signature with regard to chemotherapy. As a consequence of the predictive value of the signature, including patients who have received chemotherapy in validation series might even diluted the real prognostic value of the signature. Patients with a poor prognosis signature more often received chemotherapy and were more likely to benefit from this treatment, therefore, disease outcome of poor prognosis patients will be more substantially improved by chemotherapy and difference in disease outcome between good and poor signature patients could have been weakened.

The above mentioned studies assessing the predictiveness of the 70 gene signature showed that patients with a low risk 70-gene signature had no substantial benefit from chemotherapy. This supports the results and conclusions of the studies presented in **chapter 4, 5 and 6** that withholding chemotherapy in 70-gene signature low risk patients seems justified, first because of the good prognosis and second because the expected benefit of chemotherapy seems to be very limited. To what extent the omission of chemotherapy in low risk patients will negatively impact survival, particularly in relation to serious long term side effects of chemotherapy, is currently studied in the MINDACT trial.<sup>48,49</sup>

## **Prospective validation and feasibility of the 70-gene signature**

The MINDACT study is a large, multicentric, randomized controlled trial that started accrual in February 2007 and is anticipated to finish recruiting the 6,000 needed patients mid 2011. Patients enrolled in the trial will have their risk of recurrence assessed by both the 70-gene signature and the clinicopathological criteria using Adjuvant!. If these risk assessments disagree, patients will be randomized to allocate adjuvant chemotherapy according to the 70-gene risk or the predicted risk by Adjuvant!. If both methods classify the risk of recurrence as low, adjuvant chemotherapy is withheld; in case of high risk according to both methods patients will receive chemotherapy (**chapter 1**). The trial will evaluate whether patients with a high clinical risk and a low risk according to the 70-gene signature can be spared chemotherapy without jeopardizing survival.<sup>48,49</sup>

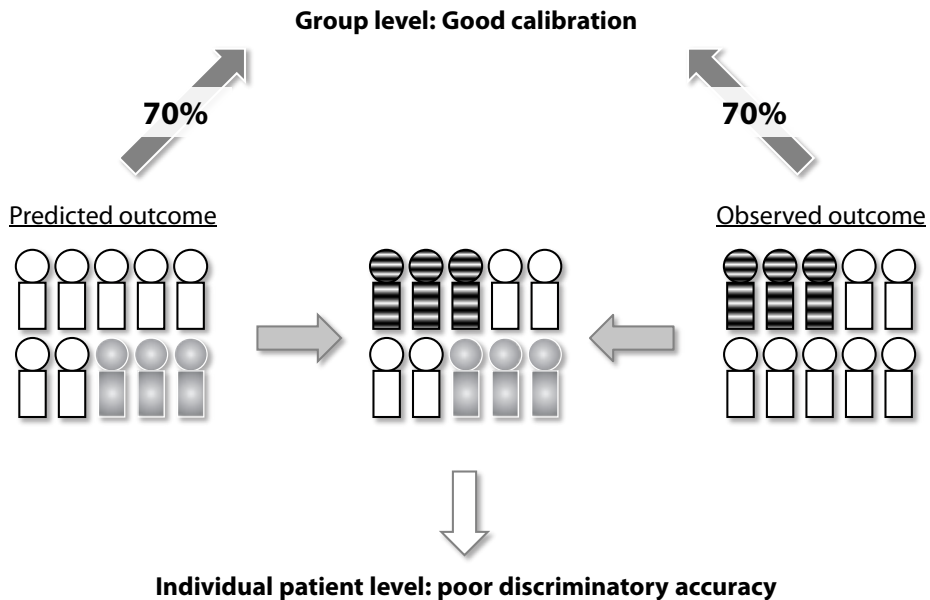
In addition, fresh frozen tumor samples, paraffin-embedded tumor tissue and blood samples from 6000 patients will be collected and stored in a biobank, representing a valuable resource for future research. Moreover, complex gene arrays of all 6,000 tumors will be available. The feasibility of performing gene expression profiles in daily clinical practice has been evaluated in the prospective RASTER (Microarray Prognostics in Breast Cancer) study.<sup>34</sup> Results of this study showed that it is feasible to implement the 70-gene signature in community hospitals in the Netherlands. In order to perform gene expression profiles, tumor samples were placed in a commercially available preservation fluid at room temperature, and were sent by conventional mail to the Netherlands Cancer Institute. Given that one of the aims of MINDACT is the establishment of a biological materials

bank for future research, including proteomics, temporarily preservation of tissue in preservation fluid might not be suitable and fresh frozen tissue is probably a more reliable source for future research. Therefore we conducted a pilot study preceding the MINDACT trial, in which we have tested and optimized the comprehensive logistics to obtain good-quality fresh frozen tumor tissue (**chapter 3**). The feasibility of performing gene expression profiling in daily practice is further reflected by the fast accrual of the MINDACT trial.<sup>50</sup>

## Clinicopathological risk assessment and individualized prognostication

### The prognostic tool Adjuvant!

In the MINDACT trial, the clinicopathological risk will be assessed by Adjuvant! to provide an internationally used and standardized method for predicting outcome. Adjuvant! predicts 10 year disease outcome with and without the use of adjuvant systemic therapy based on age, co-morbidity, tumor size, tumor grade, estrogen receptor status and lymph node status.<sup>16</sup> The model was developed in an American breast cancer population and previously validated in Canadian breast cancer patients.<sup>16,51</sup> In **chapter 8** a Dutch validation study of the model is described. The results show that in general Adjuvant! can be used for Dutch breast cancer patients; however, predictions in patients under 40 years should be carefully judged. In this validation study we assessed both the accuracy of the model to predict disease outcome in subgroups of breast cancer patients (*i.e.* the goodness of fit or calibration) and the model's ability to distinguish individuals who will experience different outcomes (discriminatory accuracy). In the era of personalized treatment, the discriminatory accuracy of a prognostic tool or marker is of paramount importance.<sup>52-54</sup> A model can predict disease outcome very accurately in the whole group or in clinically relevant subgroups, without identifying the correct patients who are at high risk of recurrence. For instance, when 30% of the patients will suffer from recurrence and the model indeed predicts a recurrence of 30% in this group, its calibration is excellent. However, the model could still identify the wrong patients as poor prognosis as is depicted in *figure 1*, hence resulting in a poor discriminatory accuracy and limited value for the individual patient. In addition to the good calibration of the Adjuvant! tool in Dutch patients (differences between predicted and observed outcomes were within 2% for most clinically relevant subgroups), the model showed moderate discriminatory accuracy. This observation was expected since we know that patients with identical clinicopathological characteristics can have different outcomes. Consequently, the prognostic information that is captured by these characteristics can only explain part of the differences in outcome. Considering the results described in this thesis, the 70-gene signature as a measurement of tumor biology will be the most obvious marker to incorporate in the Adjuvant! model. The signature will probably explain part of the residual variation and increase the discriminatory accuracy of the model and therefore could provide the opportunity to improve personalized treatment. In addition, other (new) prognostic factors could be added to the model and potentially improve outcome prediction, such as Her2 status or Ki67.<sup>55,56</sup> In the MINDACT trial, an adapted version of Adjuvant! including Her2 is used.



**Figure 1. Calibration and discriminatory accuracy of a hypothetical prognostic marker.**

The predicted distant metastases-free survival of 70% is in good agreement with the actual observed DMFS of 70%, reflecting the good calibration of the marker. However, the 30% who were identified by the prognostic marker as patients who are at risk of developing distant metastases are completely different from the patients in whom distant metastases were observed, depicting the poor discriminatory accuracy.

The white figures represent patients who were predicted to remain free of distant metastases by the marker and/or in whom no distant metastases were observed. The gray figures represent patients who are predicted to develop metastases according to a prognostic marker. The black and white striped figures are patients in whom distant metastases were observed.

### Method of detection

A new marker that is currently ignored when selecting patients for AST is method of detection, *i.e.* whether a tumor is detected by screening mammography or by the cause of symptoms. Adding method of detection to models such as Adjuvant! could improve the individual prediction of disease outcome. In **chapter 9** we show that Adjuvant! underestimated disease outcome in patients with screen-detected tumors. Furthermore, results of our study show that, even after adjusting for factors associated with tumor advancement and aggressiveness, patients with screen-detected tumors have a better survival compared with patients with nonscreening-related tumors. This suggests that

other yet unknown factors determine the good prognosis of screen-detected tumors. Until this factor or more likely these factors are established, method of detection can serve as a surrogate marker and should be taken into account when predicting prognosis.<sup>56</sup> Both the incorporation of method of detection and the 70-gene signature are subjected to current research. Most likely small tumors detected by screening will have a low risk 70-gene signature (Esserman, Van 't Veer, *et al.* unpublished data). Future research of the MINDACT trial will further address this issue.

### **The 70-gene signature in daily clinical practice**

The studies described in this thesis show that the 70-gene signature has prognostic value independent of current clinicopathological criteria and that it can be used to guide adjuvant treatment decision-making. In particular, for but not limited to the subgroup of patients with estrogen receptor positive, grade 2 tumors that are up to 2 cm in diameter, with or without limited lymph node involvement, the 70-gene signature provides additional information which will facilitate the selection of patients who need AST. The clinical utility of the signature has also been recognized by two currently used guidelines: the Dutch CBO guideline 2008 and the St. Gallen recommendation 2009 that is used throughout Europe.<sup>14,15</sup> Both guidelines state that validated gene expression profiles could add information to traditional prognostic factors and could assist treatment decision-making in certain patient groups. According to the concept CBO guideline 2011 the signature could have clinical utility in patients with estrogen receptor positive tumors for whom the necessity of adjuvant chemotherapy is indistinct (unpublished). The St. Gallen recommendation restricts the utility of the 70-gene signature to estrogen receptor positive patients with Her2 negative tumors.<sup>15</sup> However, recent data show that the 70-gene signature could identify a subgroup of Her2-positive patients with a favorable disease outcome, suggesting that the signature could also improve prognostication in this subgroup.<sup>15,57</sup> In patients classified as high risk according to clinicopathological characteristics, the prognostic value of the 70-gene signature seems to be of equal magnitude to that in patients with a low-moderate clinical risk, whereas the proportion of patients classified as good prognosis will be smaller (unpublished data). This will constrain the additional value of the signature to the group level; however, it is important to realize that the individual risk assessment and treatment decision could still be improved. Whether the signature will be used in these groups will be determined by both the improvement of estimation of individual prognosis (discriminatory accuracy) and the number of patients needed to test. For instance, testing 100 patients in order to identify 2 patients who are at sufficiently low risk to withhold chemotherapy might not be considered as cost-effective. In addition, with such small numbers of low risk patients it will be difficult to assess its prognostic value in this group. For patients with ER-negative tumors, and especially for patients with triple negative tumors, the additional

value seems limited since the vast majority of these patients will be classified as high risk according to the signature.

### **Future prospective**

The introduction of microarray technology will increasingly impact the management of breast cancer. It will increase our understanding about breast cancer biology and further elucidate its heterogeneity. The studies described in this thesis show that the 70-gene signature, which was developed in a well-defined subset of breast cancer patients, has prognostic value in several other breast cancer subgroups, suggesting that one prognostic test fits all patients. However, there is still room for improvement and with the currently increasing knowledge about tumor biology it is likely that new markers will be developed in more specific subgroups, such as in estrogen receptor negative or triple negative tumors. In the future we might be able to perform one assay in a certain subgroup in order to determine a patient's prognosis but also the likelihood of response to different therapies and the presence of drug targets. These developments will also influence clinical trial design, in which patients will be stratified by both prognostic and predictive markers, thereby identifying targeted therapy that will be highly effective in a (small) subgroup of patients who indeed need additional systemic treatment.

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