Gene signature for risk stratification and treatment of breast cancer: Incorporating tumor biology in clinical decision-making
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Chapter 9
General discussion and future prospects
Several years ago the ultimate goal to personalize medicine was set by the medical society worldwide. Breast cancer treatment should be tailored to fit the individual patient and the unique characteristics of the tumors they are diagnosed with. Understanding the biology of breast cancer and translating this knowledge into new treatment options that will change clinical practice are the steps to be taken in the journey towards reaching this ultimate goal. Personalizing medicine starts by further stratifying patients in subgroups based on clinicopathological factors and molecular subtypes and adjusting screening, risk assessment and treatment decisions accordingly.

Prognostic factors and clinical guidelines

Until recently, solely clinicopathological factors such as age, tumor size, grade, hormone receptor status, HER2 status and involvement of the axillary lymph nodes were used to estimate a patient’s risk of recurrence and the benefit that could be derived from adjuvant treatment.1-3 Most of these clinicopathological factors are included in clinical guidelines and risk estimation tools such as Adjuvant! Online (AOL), the Nottingham Prognostic Index (NPI) and the St. Gallen expert panel recommendations.1-3 The definition of high and low risk and the importance of the individual clinicopathological factors can be debated, as described in chapter 2, 3 and 4. Currently, only 15% to 20% of the node-negative breast cancer patients are identified as low risk based on risk assessments by clinical guidelines, while the other 80% to 85% is classified as high risk and therefore considered eligible to receive adjuvant chemotherapy.4 An overview by the Early Breast Cancer Trialists’ Collaborative Group shows that approximately 70% of the node-negative breast cancer patients remains free of distant metastases at 10 years in the absence of adjuvant systemic therapy.4 This indicates that a substantial number of patients is currently treated with adjuvant chemotherapy without deriving significant benefit from it.5

The potential benefit of adjuvant systemic therapy is most debatable in patients who balance on the edges of the definitions of high and low risk. Often these patients are between 45 and 55 years old at the time of diagnosis and have a grade II, ER-positive, HER2-negative breast cancer of 1-2 cm. To improve accuracy in the selection of patients who will benefit from adjuvant chemotherapy, further stratification of patients into specific subgroups is necessary. Stratifying solely based on clinicopathological factors has proven to be insufficient. A new approach on breast cancer from a more biological point of view may be helpful.

Insight in the biology of breast cancer: using gene signatures

An important step towards personalized or stratified medicine was the introduction of microarray analyses. At first, breast tumors were stratified into molecular subtypes, as first identified by Perou et al. Four types of breast cancer related to different features of molecular tumor biology were identified from 65 specimens from 42 different patients using unsupervised analysis: Luminal A, Luminal B, Basal-like and ERBB2 type breast cancers.6 Soon after, supervised analysis was used to compare gene-expression data from patients with known clinical outcomes to identify
genes associated with prognosis. Based on the knowledge derived from these analyses, gene signatures were developed. The 70-gene signature was the first gene signature described and the results were published in 2002.\textsuperscript{7} Other gene signatures, such as the Oncotype Dx recurrence score (2004), PAM50 (2009) and EndoPredict (2011), followed a few years later (\textit{chapter 1}).\textsuperscript{8-10} A cause of confusion among clinicians is that the group of patients for whom the different gene signatures that are commercially available can be used is inconsistent. For example, the Oncotype Dx test is only validated in patients with ER-positive, node-negative disease who are treated with Tamoxifen, while the Pam50 test can be used in patients with ER-positive, node-negative disease treated with any type of adjuvant endocrine therapy.\textsuperscript{8,9} The 70-gene signature is validated for patients with ER-positive or ER-negative, node-negative disease.\textsuperscript{11,12} Because the 70-gene signature is the most commonly used gene signature in the Netherlands and extensive experience is gained with this prognostic tool in our country, this gene signature was used in the studies described in this thesis.

Gene signatures, like the 70-gene signature, are able to identify patients at a high risk of recurrence based on the biological background of the tumor, and are therefore creating a new perspective on risk assessment. As one can anticipate, for a subgroup of patients discordance is seen between risk assessment by clinical guidelines and by a gene signature. A substantial proportion of breast cancer patients is considered high risk based on clinicopathological factors, but low risk according to a gene signature and the other way around. This discordancy is especially prevalent in the subgroup of patients described earlier who are balancing on the thresholds for clinically high and low risk (\textit{chapter 3}). This suggests that gene signatures can be especially helpful in case of uncertainty about the benefit that an individual patient will derive from adjuvant systemic treatment. Initially, the 70-gene signature was developed for patients < 55 years old with stage 1 or 2, ER-positive or -negative, node-negative breast cancer. In the many additional validation studies performed in the last decade, the test was also retrospectively validated for breast cancer patients who are 55-70 years of age, patients with HER2-positive disease, and patients with 1-3 positive nodes in the axilla (\textit{chapter 2}).\textsuperscript{13-16} Recently, the 5-year follow-up results of the RASTER study were became available (\textit{chapter 3}). This is the first prospective data on patients for whom the 70-gene signature was used in the decision whether or not to treat a patient with adjuvant chemotherapy. An excellent survival is seen in patients who did not receive adjuvant chemotherapy based on their low risk 70-gene signature result. Even patients who had unfavorable clinicopathological factors and therefore a high clinical risk assessment according to AOL, had a distant-recurrence-free-interval of 100%. Even though no randomization was included in the design of the RASTER study, this study provides an accurate reflection of daily clinical practice where the 70-gene signature will be used in addition to clinical guidelines. The RASTER study provides the first prospective data suggesting that chemotherapy can safely be omitted in case of a low risk 70-gene signature. These results might justify use of the 70-gene signature in daily
clinical practice when in doubt whether a patient will benefit from adjuvant systemic therapy, even though this assumption is based on data with a relatively short follow-up time. While the prognostic value of the 70-gene signature was thoroughly investigated, knowledge about long-term outcome was limited. An update of the consecutive validation series of van de Vijver et al (chapter 6) showed even after 25 years a significantly better survival in patients with a low risk 70-gene signature compared to patients with a high risk 70-gene signature (p<0.0001). The 25-year Hazard Ratios (HR) for distant-metastasis-free-survival (DMFS) and Overall Survival (OS) were 3.1 (95%CI: 2.02-4.86) and 2.9 (95%CI: 1.90-4.28), respectively. Both HR’s were largest in the first five years after diagnosis (9.6 (95%CI: 4.2-22.1) and 11.3 (95%CI: 3.5-36.4) respectively), indicating that the 70-gene signature is a strong prognostic factor for DMFS in the first five years after diagnosis. After the first 5 years this effect slowly diminished.

Personalized or stratified medicine not only consists of adjuvant therapy for systemic control, but also locoregional treatment with radiotherapy to achieve optimal locoregional control. The estimations of the risk of locoregional recurrence (LRR) are currently also based on clinicopathological factors, especially age, lymphovascular invasion and axillary lymph node involvement. LRR rates of 6% after 10 years can be achieved using these conventional factors, which is reasonable but still suboptimal.17 Further stratification based on the previously mentioned molecular subtypes defined by Perou et al. does not improve LRR risk estimations as was shown in a recent study by Metzger-Filho et al. In this study 1951 node-negative early stage breast cancer patients were stratified into molecular subtypes and their risk of LRR was estimated.18 The results show no significant difference in LRR risk after 13 years for all four subtypes, indicating the need for better estimation methods for the risk of LRR. Even though the 70-gene signature was developed to predict the risk of distant metastases,7 given the strong association between locoregional and distant recurrence19, we hypothesized that the 70-gene signature will also be able to predict the risk of LRR. Incorporating tumor biology in the risk assessment of LRR enables us to identify the outliers with a very low risk of recurrence after breast conserving surgery who might not benefit from whole breast irradiation. On the other hand, it might also identify a subgroup of patients at such a high risk of recurrence after mastectomy that these patients are eligible to receive chest wall irradiation after surgery (chapter 8). A randomized controlled trial to validate the findings reported in this thesis and to evaluate whether for patients with a low risk 70-gene signature result whole breast irradiation can safely be avoided is currently considered. A trial like this could be the first step towards more restrictive adjuvant locoregional treatment with radiotherapy. Whether the 70-gene signature in its current form is sufficient or a gene signature especially developed to predict LRR is required to optimize the prediction of LRR is unknown. This optimization of LRR risk prediction will not only lower LRR rates, but may eventually lead to more restrictive and minimally invasive surgical treatment for specific subgroups of patients.
Low or high: who knows?
As described earlier, one of the main issues in current clinical practice is the large amount of
discordance between clinical guidelines. Adding another prognostic factor, like the 70-gene
signature, showed a slight improvement in agreement among oncologists regarding their risk
estimations. Nevertheless, the most important conclusion of chapter 5 is that the agreement
among oncologists regarding risk estimations and adjuvant systemic treatment recommendations
is lower than expected and desired. This again underlines the need for more standardization
in breast cancer treatment. The use different and sometimes multiple guidelines by a single
oncologist might (partially) explain why the agreement is not as high as we would like it to be. To
evaluate whether this lack of agreement among these oncologists in these cases is representative
for clinical practice, a similar survey will be performed among a larger number of oncologists.
The performance of the most commonly used guidelines and the additional value of the 70-gene
signature was investigated to further evaluate the magnitude of the variance in risk estimations
and whether the 70-gene signature can contribute in resolving this lack of agreement among
oncologists (chapter 4). Clinical guidelines, such as Adjuvant! Online (AOL), Nottingham Prognstic
Index (NPI), the St. Gallen expert panel recommendations, the Dutch National guidelines of 2004
and the updated version of 2012, and the relatively new online risk estimation tool PREDICT plus,
have similar but slightly different definitions of high and low risk. The use of clinicopathological
factors among these clinical guidelines differs. For example, NPI uses tumor size, number of
involved axillary lymph nodes and grade, while AOL also incorporates age, co-morbidities and ER-
status. PREDICT plus is the only risk estimation tool which takes both HER2-status and method
of detection into account. Among all tools and guidelines included in the analyses, NPI, the
Dutch CBO 2004 guidelines and the PREDICT plus tool are the most restrictive, meaning they
classify a relatively large proportion of patients as low risk. The results of our analyses show that
adding the 70-gene signature to clinical guidelines improves risk predictions. The most accurate
risk predictions were seen when using the PREDICT plus tool in combination with the 70-gene
signature, suggesting that a more restrictive guideline combined with a gene signature may be
the best way to select patients for whom adjuvant chemotherapy can safely be omitted. One
should consider that incorporating HER2-status and method of detection might attribute to the
improvement of clinical risk estimations made by PREDICT plus. The importance of especially the
method of detection is also addressed in chapter 7.

Breast cancer screening
Already decades ago, it was hypothesized that to optimize breast cancer treatment the disease
needs to be diagnosed as early as possible. Therefore, the population-based screening programs
were introduced. Screening entails the examination of a group of asymptomatic individuals to
detect disease at an earlier stage. The rationale for the introduction of nation-wide screening
programs is that if breast cancer is detected at an earlier stage, more treatment options would
be available, prognosis would improve and mortality would decline. The latter was achieved, alongside with a remarkable increase in incidence.\textsuperscript{22,23} This increased incidence has been suggested to be due to the detection of slow growing tumors that would never have caused symptoms or death, i.e. overdiagnosis (Figure 1).\textsuperscript{24} The possibility of overdiagnosis in breast cancer due to screening was already described in 1982 by Lundgren et al.\textsuperscript{25} Several studies have tried to quantify the proportion of patients that are overdiagnosed.\textsuperscript{26} The estimated proportion of overdiagnosis is Europe ranges from 1\% to 10\%. In the Netherlands this estimate is 2.8\% after adjustment for breast cancer risk and lead time.\textsuperscript{26} Identification of the patients that are overdiagnosed is difficult. We hypothesized that the 70-gene signature would be able to identify a subgroup of patients with screen-detected cancers with such a low risk of recurrence that these cancers are not likely to have become symptomatic without screening, even though they are invasive cancers. Since screening has proven to detect a substantial number of high risk cancers at an early stage, screening programs will continue to exist. Therefore, overdiagnosis can not be avoided, but overtreatment can. Identifying those individual patients that are currently overdiagnosed will help to avoid overtreatment in this subgroup. Previous analyses showed that screen-detected cancers are associated with favorable survival, independent of known prognostic factors.\textsuperscript{23} The question was raised whether screen-detected cancers would also have a more favorable tumor biology. Our group previously evaluated the 70-gene signature result of a group of patients diagnosed before the introduction of screening and compared this to the 70-gene signatures of screen-detected cancers and concluded that the introduction of screening leads to the detection of tumors with a more favorable tumor biology.\textsuperscript{27} In our recent analyses, described in chapter 7, we showed that screen-detected cancers are more likely to have a low or even ultralow risk tumor biology, which prospectively validates our previous analyses.\textsuperscript{27} Especially for this screen-detected patients the use of tools to differentiate breast cancers by risk of recurrence may minimize overtreatment. The ultralow risk tumors were defined as having a 70-gene signature index-score >0.6. In the original 78 patients from the van ’t Veer training set, no distant metastasis were seen in this group after 5 years.\textsuperscript{27} Whether this ultralow risk group has a significantly better survival than patients with an index-score between 0.4 and 0.6, first needs to be evaluated in a larger, independent cohort before the results of our study can be translated into changes in the treatment of these screen-detected ultralow risk tumors. Preliminary analyses evaluating outcome of a small group of patients with screen-detected cancers for whom a 70-gene signature (n=107) was available shows that patients with an ultralow risk 70-gene signature have a better breast cancer specific survival (96.7\%) compared to patients with a low (91.3\%) or high risk (78.8\%; p=0.06) 70-gene signature result (data not published). The group of patients with data on method of detection and biological background was too small to draw any firm conclusion, but it shows a trend towards excellent survival in patients with an ultralow risk tumor biology.
Another issue that needs to be addressed in the near future is the selection of women eligible for screening outside of the national screening programs. Currently, women with a BRCA1 or BRCA2 mutation and women with a strong hereditary predisposition are offered annual in-hospital screening. If a large proportion of the genetically high risk tumors has a strong hereditary component, one could hypothesize that more extensive breast cancer screening in these families might be appropriate. While at the same time more limited screening might be sufficient in families were no cases of breast cancer or a single genetically low risk case have presented. More customized risk-adapted screening should also be based on the presence of lifestyle- and environmental risk factors.

**Diagnostic imaging**

During implementation of the national screening programs, all screening mammographies were conducted using film-screen mammography (FSM). Over past couple of years a transition to full-field digital mammography (FFDM) has taken place. In 2010, 94% of the Dutch women participating in the screening program were screened using FFDM. Several studies have shown that FFDM is comparable or even better than FSM in the detection of clinically relevant tumors, especially in pre- or perimenopausal women with dense breasts. Whether the introduction of this new screening modality has led to the detection of different types of tumors from a biological point of view was unknown. Our results suggest that the transition from FSM to FFDM resulted in the detection of a larger proportion of biologically high risk tumors, which may indicate that FFDM is a more effective screening-modality than FSM. One should be aware that this study involves only patients were diagnosed with breast cancer. The true impact of the transition from FSM to FFDM can only be evaluated in a group of patients representative for the entire screened

![Figure 1. Overdiagnosis due to breast cancer screening.](image-url)
population, including patients who developed breast cancer and patients who did not. The report of the Dutch screening facilities, who are currently collecting data on both screening modalities, is still awaited.

Aside from conventional mammography, several other imaging techniques are used for evaluating suspicious lesions of the breast, such as magnetic resonance imaging (MRI), ultrasound and molecular imaging of the breast using positron emission tomography (PET-CT). Several studies have shown that for monitoring BRCA-mutated women the MRI is the most accurate modality. MRI has also been shown to be useful in monitoring response to neoadjuvant chemotherapy, especially in patients with triple-negative and HER2-positive tumors. On the other hand, this technique has proven to be less accurate in monitoring the response in patients with ER-positive, HER2-negative tumors. This indicates that stratification into specific subtypes is also relevant when choosing the imaging techniques that will be used for screening and monitoring of breast cancer.

**Gene signatures in current clinical practice**

Gene signatures are already increasingly used in daily clinical practice. Especially when in doubt of the benefit that the individual patient can derive from adjuvant therapy, locoregional as well as systemic, the 70-gene signature can be a useful tool. Knowledge of the heterogeneity of breast cancer and the increasing insight in the biological background of this disease, enables us to move forward towards personalized medicine by incorporating this knowledge in clinical decision-making. In the thesis of Jolien Bueno de Mesquita and Stella Mook the first steps were taken towards the implementation of gene signatures in clinical practice. They retrospectively validated the 70-gene signature for early stage, node-negative breast cancer as well as for different subgroups of patients, confirmed its feasibility in an observational study and evaluated its additional value and potential use in the clinic. The cost-effectiveness of the 70-gene signature was confirmed by Valesca Retèl and in a head-to-head comparison to the 21-gene assay of Oncotype DX, the 70-gene signature was most cost-effective in terms of quality adjusted life years. In this thesis, we showed that the 70-gene signature accurately predicts outcome and has clinical relevance in estimating the risk of distant metastasis as well as locoregional recurrence. We evaluated the impact on risk assessment and AST recommendations, and evaluated its additional value to established clinical guidelines used in breast cancer treatment. In addition, we used the 70-gene signature to evaluate the biological background of tumors detected in a population-based screening program.

Even though full incorporation of gene signatures in clinical practice appears close, some areas need further investigation. In the process of further stratifying patients into different subgroups, a group of patients with triple-negative breast cancer originated. Triple-negative breast cancer is associated with a relatively poor prognosis in the first five years of follow-up. Yet, around 60% of node-negative triple-negative breast cancer patients are cured by locoregional therapy alone,
without additional adjuvant chemotherapy. Unfortunately, the 70-gene signature labels only a few percent of all triple-negative breast cancers as low risk, illustrating the inability of this gene signature to identify the majority of triple negative breast cancers with a good prognosis. Also, the majority of breast cancers evaluated in the numerous validation studies were invasive ductal carcinomas. Other histological types and especially the lobular carcinomas were underrepresented and further evaluation of these specific histological types is necessary, but challenging because of the low prevalence of these types of cancer. Currently, data from randomized studies evaluating the predictive value of the 70-gene signature is not yet available. Of course, the results of the MINDACT trial will provide a definite answer to the question whether chemotherapy can safely be omitted in case of a low risk 70-gene signature result. In many recently presented trial outcomes, such as the 5-year results of the AMAROS trial, we see that studies are underpowered due to very low numbers of events. This results in longer follow-up time necessary to meet the criteria for accurate statistical analyses, which in turn leads to a longer delay for clinicians to translate the results into clinical practice. Whether the improvement in overall, breast cancer specific and distant disease free survival among breast cancer patients in Europe will have an impact on the time needed to accurately evaluate the endpoints of the MINDACT trial is still unclear. Another consequence of the improvement of survival among breast cancer patients is the increasing number of late recurrences, especially among patients with ER-positive disease. The currently available gene signatures cannot be used to predict these late recurrences.

Future prospects
Over the past few years, clinicians are more and more incorporating gene signatures in their clinical practice. Gene signatures like the 70-gene signature are nowadays used in combination with clinicopathological factors. Incorporating the result of these signatures in clinical guidelines as an additional prognostic factor is still ongoing. Once the predictive value of the 70-gene signature has been scrutinized, this gene signature may also be used to tailor adjuvant systemic treatment. Researchers at the Netherlands Cancer Institute have the ambition to change breast cancer treatment in a way that in a few decades breast cancer will be more like a chronic disease for 90% of the patients, who would otherwise have died of their metastatic disease. Targeted therapies for early stage breast cancer as well as metastatic disease will become available, because of the increasing insight in tumor biology that will be acquired over the next couple of years. Screening methods will be optimized and fitted to the personalized character of breast cancer treatment. In patient subgroups at a high risk of developing breast cancer due to hereditary predisposition increased screening will create the possibility to detect tumors at an even earlier stage. At the same time, limited screening in women with a low risk of ever developing breast cancer will reduce overdiagnosis and overtreatment.

The 70-gene signature not only created more insight in the biology of the individual tumor, but was the first step to incorporate tumor biology in clinical decision making. This revolutionary
The introduction of next-generation (massive parallel) sequencing will enable us to personalize medicine faster, cheaper and more accurate than ever before. In a future where cancer becomes a chronic disease, the focus is likely to shift towards primary prevention. Primary prevention focuses on modifiable risk factors, such as decreasing maternal age at first childbirth, obesity, alcohol consumption and physical inactivity. To provide more individualized primary prevention strategies, there is a need to further investigate the relationship between known lifestyle- and environmental risk factors and tumor biology. The MINDACT lifestyle study was conducted by our group to evaluate lifestyle- and environmental risk factors within the Dutch MINDACT cohort. The results of this study are still awaited. Perhaps this revolution towards genome-guided medicine will evolve as far as genome-based screening, which will enable us to determine the perfect screening intervals and diagnostic imaging modalities, anticipate on the type of cancer that might lay ahead and adjust lifestyle- and environmental risk factors accordingly to minimize risk and maximize the chance of early detection.

**Concluding remarks**

Previous studies showed that the 70-gene signature is a promising tool for risk estimation in breast cancer based on the biology of the tumor. The results described in this thesis expand our knowledge on the use of the 70-gene signature in clinical practice and the impact it has on clinical decision-making. Not only did the results show that the 70-gene signature is an important prognostic factor in long-term retrospective and observational prospective data, the results also revealed new areas in which the 70-gene signature can be used. Using the 70-gene signature to estimate the risk of loco-regional recurrence and to identify screen-detected cancers at a low risk of recurrence needs further evaluation, but will likely have an important influence on clinical practice.
References


