Tailored to fit: balancing over- and undertreatment in early-stage triple-negative breast cancer patients

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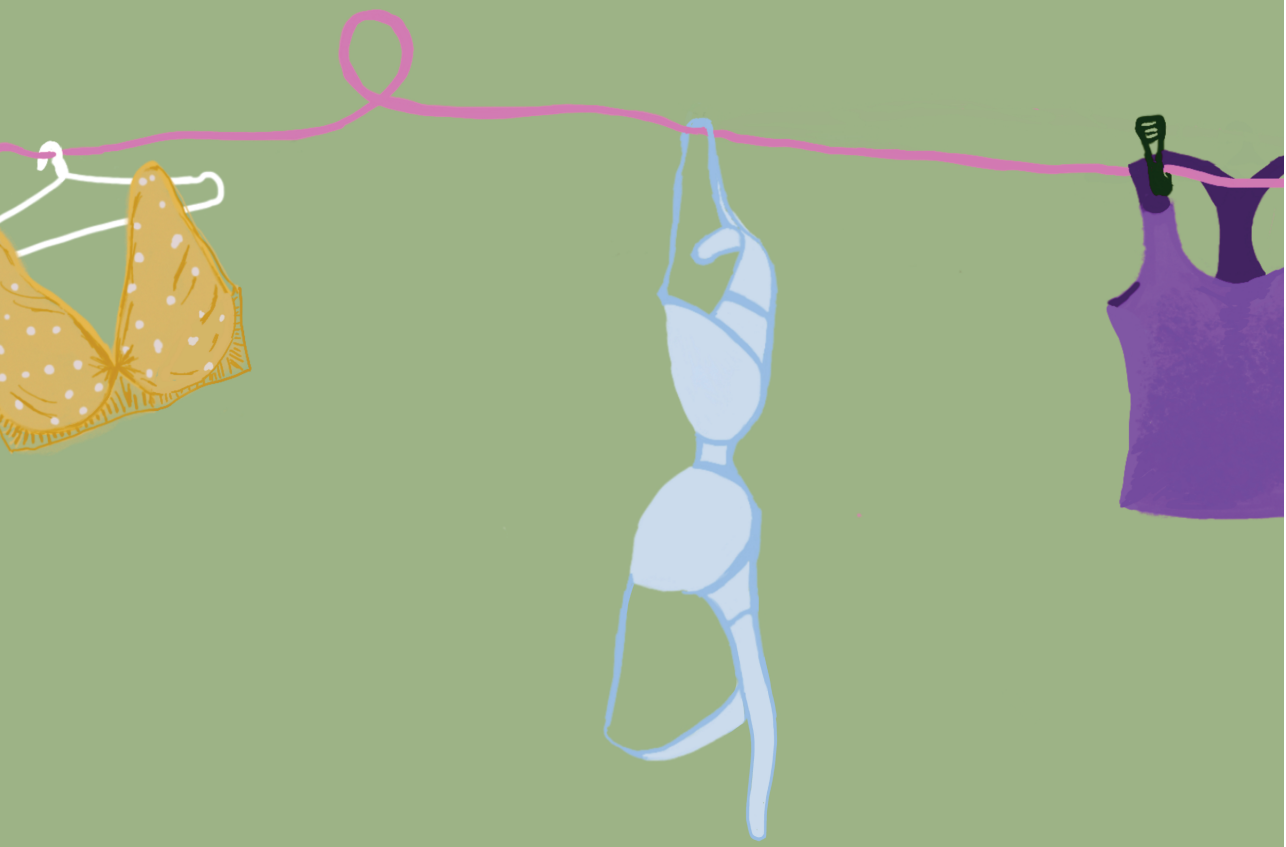

CHAPTER 1

General introduction

TRIPLE-NEGATIVE BREAST CANCER

Breast cancer is, besides skin cancer, the most common cancer and causes the most cancer-related deaths among women worldwide [1, 2]. Now recognized as a heterogeneous disease, breast cancer contains multiple subtypes with distinct biological characteristics, prognoses, and treatment responses. One of the most widely accepted breast cancer classification systems is based on immunohistochemical expression, including the expression of estrogen receptor (ER) and progestogen receptor (PR), collectively referred to as hormone receptors (HR), and human epidermal growth factor receptor two (HER2). This system classifies breast cancer into four subtypes, including HR-positive/ HER2-negative, HR-positive/ HER2-positive, HR-negative/ HER2-positive, and HR-negative/ HER2-negative (triple-negative) [3].

Triple-negative breast cancer (TNBC) represents approximately 10-15% of all breast cancer subtypes [4-7]. Known for its aggressive nature, TNBC typically presents with a larger tumor size and a higher histologic grade at diagnosis compared to other breast cancer subtypes [5]. The aggressive nature of TNBC also leads to a worse prognosis in the first 5 years after diagnosis compared to other breast cancer subtypes. Population-based data suggested that TNBC patients have only a 77% of 5-year breast cancer survival rate, compared to the highest survival rate of 92% in patients with HR-positive/HER2-negative cancers [8]. A similar trend was also observed in Dutch patients [7]. Furthermore, TNBC patients tend to have early relapse within 5 years after diagnosis [9], and the metastases often spread to the lung, liver, and brain [10].

In addition to the aggressive nature, TNBC is also characterized by its remarkable heterogeneity [11]. The vast majority of TNBCs are invasive ductal carcinoma (or carcinoma of no specific type), followed by a small proportion of metaplastic carcinoma, invasive lobular carcinoma, medullary carcinoma, apocrine carcinoma, and adenoid cystic carcinoma [12]. In terms of molecular diversity, TNBC can be classified into several molecular subtypes, including basal-like 1 and 2, mesenchymal, immunomodulatory, mesenchymal stem-like, and luminal androgen receptor group [13]. This classification was later refined into four subtypes, showing distinct responses to neoadjuvant chemotherapy [14].

TRIPLE-NEGATIVE BREAST CANCER TREATMENT

Historically, due to the absence of ER, PR, and HER2 expression, chemotherapy in the neoadjuvant setting and/or adjuvant setting was the only systemic treatment option for early -stage TNBC patients [15-17]. In very recent years, targeted therapies such as PARP inhibitors (Olaparib) for germline *BRCA1/2* mutation carriers and PD-1/PD-L1 inhibitors (pembrolizumab) have become available for high-risk, early-stage TNBC patients. However, these therapies are

typically, sequentially or concurrent, combined with chemotherapy [18]. For germline *BRCA1/2* wild-type TNBC patients with stage I tumors, chemotherapy remains the sole option if omission is not viable. Population- and hospital-based cohort studies indicate that over 70% TNBC patients received (neo)adjuvant chemotherapy [5, 7], with even higher proportions among younger patients [7, 19].

However, not all early-stage TNBC patients who undergo chemotherapy will derive a survival benefit from this treatment. According to the Dutch breast cancer guideline, chemotherapy is recommended if the predicted 10-year mortality risk is about 10-15% [16]. This recommendation indicates that in 100 patients who are recommended chemotherapy, only 10 to 15 may derive benefit from treatment, and even then, some may not survive despite the treatment. Evidence of clear overtreatment can be observed from the PARADIGM cohort, a Dutch population-based cohort with systemic treatment-naïve, node-negative breast cancer patients who were diagnosed under 40 years old and treated per guidelines at their diagnosis time [20]. In this group of 377 patients with grade-3, T1c-T3 TNBCs, over 70% survived at least 10 years without chemotherapy [21], suggesting significant overtreatment under current Dutch guidelines, where all these patients would have been advised to receive chemotherapy [16]. Chemotherapy overtreatment provides no survival benefit but exposes patients to an unnecessary risk of side effects, including leukemia, cardiotoxicity, fatigue, premature menopause, infertility, and impaired sexual functioning [22, 23]. These side effects significantly impact quality of life of survivors, especially of younger women.

Therefore, there is a pressing need to better balance potential over- and undertreatment in early-stage TNBC patients, emphasizing the importance of precise risk classification. Prognostic biomarkers can refine the risk classification by distinguishing patients who are likely to achieve excellent survival without chemotherapy or other targeted therapy from those who will likely face extremely poor survival without such treatments.

PROGNOSTIC BIOMARKERS IN TRIPLE-NEGATIVE BREAST CANCER

Definition of prognostic biomarkers and their assessment

In oncology studies, biomarkers are classified into prognostic and predictive biomarkers. Prognostic biomarkers play an important role in predicting future clinical outcomes in patients, such as risk of disease recurrence or mortality, regardless of intervention or treatment, while predictive biomarkers specifically predict the response to a certain treatment [24]. Both biomarker types are essential when making systemic treatment decisions, with prognostic biomarkers

aiding in the identification of high-risk and low-risk patients, and predictive biomarkers identifying patients who would respond to a particular treatment [24].

Ideally, to accurately assess the association between the prognostic biomarkers and the disease outcomes, studies should focus on patients who have not undergone any adjuvant systemic treatment [25]. However, this is particularly challenging in the context of TNBC, where (neo)adjuvant chemotherapy is commonly administered. Studies that predominantly involve chemotherapy-treated TNBC patients often struggle to differentiate between the biomarkers' prognostic and predictive values. Moreover, selecting patients based on their treatment status introduces the risk of indication bias.

The abovementioned PARADIGM study effectively avoids these challenges. Specifically initiated to study prognostic biomarkers, the PARADIGM study includes 2286 young, systemic treatment-naïve, lymph node-negative breast cancer patients diagnosed and prospectively registered in the Netherlands in the nationwide Netherlands Cancer Registry between 1989 and 2000, including 485 patients with TNBC [20]. Before 2000, chemotherapy and other systemic treatments were only recommended to breast cancer patients with positive nodal status, minimizing the risk of indication bias within the PARADIGM cohort. The study retrospectively collected the formalin fixed paraffin embedded tumor blocks and match normal tissue blocks. Fresh tumor slides were then prepared to facilitate the evaluation of histological characteristics and biomarkers, providing a unique resource for understanding the prognostic values of biomarkers in breast cancer patients without potential mediating or confounding effects of systemic treatment.

BRCA1 related genetic biomarkers

One of the most widely studied biomarkers in breast cancer is *BRCA1* mutation. In 1991, geneticist Mary Claire King made a groundbreaking discovery by identifying the association between breast cancer and a gene located on chromosome 17 [26], later named the *BRCA1* gene. This was soon followed by the discovery of the *BRCA2* gene [27]. The *BRCA1* and *BRCA2* genes play a key role in repairing DNA double-strand breaks through homologous recombination, thereby maintaining genome stability [28]. Dysfunction in *BRCA1/2* leads to the increased use of error-prone DNA repair pathways in the cells, resulting in a higher incidence of deletions, translocations and chromosomal instability, ultimately promoting tumorigenesis [28, 29].

Germline mutations in the *BRCA1/2* genes significantly increase cancer risks. By the age of 80 years, women with a germline *BRCA1* mutation face a 72% cumulative risk of developing breast cancer, and those with a germline *BRCA2* mutation have a 69% risk [30]. Despite the similar cumulative breast cancer risk associated with these two mutations, they present

distinct risk patterns. Women with a germline *BRCA1* mutation typically develop breast cancer at a younger age than those with germline *BRCA2* mutations [30]. Furthermore, germline *BRCA1* mutation carriers are significantly more likely to develop TNBCs compared to other breast cancer subtypes, with a 40% cumulative risk by the age of 80 years [31]. This risk is much higher than that for germline *BRCA2* mutation carriers and carriers of germline mutations in other breast cancer susceptibility genes [31].

In TNBC patients, germline *BRCA1* mutations are present in up to 16% of cases [32-35], and this prevalence is even higher in younger TNBC patients [19, 31, 32, 36]. However, somatic *BRCA1* mutations are relatively rare, accounting for only 2% to 4% of the cases [37-39]. Additionally, methylation in the *BRCA1* promoter regions is also frequently observed in TNBC patients, contributing to about one-third of the cases [40]. This methylation, along with mutations in the *BRCA1* gene, can lead to a complete loss of *BRCA1* function during tumorigenesis. Tumor cells with such loss often present a specific genomic profile known as "*BRCAness*" [29], and show an increased sensitivity to DNA damaging agents. This sensitivity has led to targeted therapies including platinum-based chemotherapy [41] and PARP inhibitors [42]. Carboplatin, often in combination with anti-PD-(L)1 is currently recommended for patients with high-risk early, or metastatic TNBC in Europe and the USA [42]. PARP inhibitors are indicated for germline *BRCA1/2* carriers with high-risk early, or metastatic HER2-negative breast cancer [18]. For stage I TNBC patients, standard chemotherapy remains the primary treatment.

The association between the *BRCA1*-related biomarkers and TNBC prognosis has been extensively studied [19, 34, 43, 44]. Yet, it is challenging to determine whether these associations are prognostic or predictive, or both, as most TNBC patients are treated with chemotherapy or targeted therapies. Disentangling the prognostic value of these *BRCA1*-related biomarkers from their predictive value could provide deeper insights into the disease course and find more suitable treatment strategies for these patients.

TUMOR-INFILTRATING LYMPHOCYTES

In addition to genetic biomarkers, immune biomarkers are also extensively studied in TNBC due to their prevalence in this subtype of breast cancer, with tumor-infiltrating lymphocytes (TILs) emerging as the most important concept. TILs are immune cells that infiltrate tumor tissue, which reflect the patient's adaptive immune response to the tumor and pre-existing immunity [45, 46]. These cells, comprising mainly cytotoxic T cells along with helper T cells, B cells, macrophages, and NK cells [45], infiltrate both the tumor and surrounding microenvironment [46]. The presence and abundance of TILs differs across breast cancer subtypes, with the highest levels observed in TNBCs and HR-negative/ HER2-positive breast cancers [47]. Notably, about

30% TNBC cases show at least 30% stromal TILs [48], suggesting a link between the abundance of TILs, genomic instability [46, 49] and a high tumor mutational burden [50, 51]. It is therefore hypothesized that tumors with *BRCA1/2* mutations have higher TILs levels. However, the relationship between TILs and *BRCA1/2* mutations remains uncertain, with mixed findings [52-55] potentially influenced by the mediating effect from the age at diagnosis [30, 48, 56].

TILs offer significant value as a biomarker in oncology, particularly as their assessment can be performed on standard hematoxylin and eosin-stained tumor slides. This aligns with routine clinical pathology practices at low cost, making TILs assessment accessible even in low-income countries. In practice, TILs are divided into stromal TILs and intratumoral TILs, where the former are the immune cells infiltrating the fibrous stroma adjacent to tumor cells, and the latter refer to the immune cells that has direct contact with tumor cells. The two scores are highly correlated, while the stromal TILs show less interobserver variances and are thus more reliable to be used [57]. Recent research has been trying to use artificial-intelligence algorithms for TILs scoring [58, 59], which may further lower the cost and reduce interobserver variation among pathologists.

The very first study describing the positive association between increased TILs and favorable breast cancer prognosis was conducted over a century ago by two clinicians at Mayo Clinic [60]. This association was reaffirmed in later years, establishing a link between lymphocyte infiltrates and breast cancer prognosis [61, 62]. In recent years, a growing body of research has delineated the prognostic value of TILs for TNBC and HER2-enriched subtypes [63-65]. Notably, in early-stage TNBC, numerous studies have consistently demonstrated that higher TILs level is associated with improved clinical outcomes [48, 63-67], the results of which are summarized in the table below. These studies, mostly adhering to the international standard for stromal TILs assessment [57], have revealed remarkably consistent adjusted hazard ratios for TILs on overall survival, distant recurrence-free survival (or distant recurrence-free interval), and invasive disease-free survival (or disease-free survival) among TNBC patients. However, the majority of these studies focused on TNBC patients who received adjuvant chemotherapy, with one exception being the study from Park et al, which included a cohort of untreated TNBC patients, predominantly diagnosed after 50 years of age [67], thereby underrepresenting younger patients.

Besides the prognostic value, the predictive value of TILs for chemotherapy has been widely studied in early-stage TNBC patients, and such value is likely to be regimen-specific. A previous study using data from two randomized trials with patients treated with anthracycline-based chemotherapy and chemotherapy-naïve patients suggested that no interaction effect was

observed between sTILs and chemotherapy, indicating no predictive effect of TILs towards anthracycline-based chemotherapy [65]. However, studies involving taxane-based chemotherapy with or without carboplatin suggested that higher TILs are associated with a better pathological complete response [47, 68]. Preclinical research has shown that taxanes can trigger T cells to release cytotoxic extracellular vesicles that directly kill cancer cells, giving such predictive value a biological mechanism backup [69]. This novel finding points to a specific predictive value of T cells to taxane-based therapies and has recently been validated using data from the MATADOR trial. There was a significant interaction observed between sTILs (≥20%) and regimens with or without taxane [70].

A summary of adjusted hazard ratios of stromal tumor-infiltrating lymphocytes per 10% increment on different clinical outcomes

Author (year)	Study type	HR of 10% increment of sTILs on OS	HR of 10% increment of sTILs on iDFS or DFS	HR of 10% increment of sTILs on DDFS, DDFI or DRFS	Adjustment set
Loi et al., (2013)	Clinical trial -based	0.83 (0.71-0.98)	0.85 (0.74-0.98)	-	Age, tumor size, histologic grade, positive lymph nodes, type of surgery, radiotherapy, mode of drug administration, menopausal status
Adams et al., (2014)	Clinical trial -based	0.79 (0.67-0.92)	0.84 (0.74-0.95)	0.81 0.68-0.97)	Age, tumor size, nodal status
Loi et al., (2014)	Clinical trial -based	0.81 (0.61-1.10)	-	0.77 (0.61-0.98)	Age, tumor size, histologic grade, nodal status
Dieci et al., (2015)	Hospital-based	0.85 (0.74-0.99)	-	-	Age, tumor size, histologic grade, nodal status, chemotherapy
Krishnamurti et al., (2017)	Hospital-based	0.95 (0.91-1.00)	0.95 (0.91-1.00)	-	Tumor stage, Nottingham histologic grade, lymphovascular invasion, nodal status
Loi et al., (2019)	Clinical trial-based	0.84 (0.79-0.89)	0.87 (0.83-0.91)	0.83 (0.79-0.88)	Age, tumor size, histologic grade, positive lymph nodes, treatment
Park et al., (2019)	Clinical trial and hospital-based	0.88 (0.79-0.98)	0.90 (0.83-0.98)	0.86 (0.77-0.95)	Age, tumor size, histologic grade, positive lymph nodes, radiotherapy
Leo-Ferre et al. (2024)	Mostly hospital-based	0.88 (0.85-0.91)	0.92 (0.89-0.94) [iDFS]	0.87 (0.84-0.90) [DRFS]	Age, tumor size, histologic grade, lymph node metastases, and radiotherapy

Abbreviation: HR = hazard ratio; sTILs = stromal tumor infiltrating lymphocytes; OS = overall survival; iDFS = invasive disease-free survival; DFS = disease-free survival; DDFS = distant disease-free survival; DDFI = distant disease-free interval

OTHER BIOMARKERS IN TNBC

Several reviews have introduced a range of biomarkers in TNBC [71-74]. These biomarkers, reflecting the distinct characteristics of TNBC, can be classified based on their functions and potential therapeutic values. For example, genetic biomarkers that regulate homologous recombination, including *BRCA1*-related biomarkers and *BRCA2* mutation, are sensitive to DNA damaging agents and PARP inhibitors [71-73]. Another important group of biomarkers includes the immune checkpoints, such as PD-1 and PD-L1, which have been widely recognized for their prognostic and predictive value [71, 74]. Pembrolizumab, a PD-1 inhibitor, has been approved for TNBC in the US and Europe [75]. Other biomarkers in TNBC, including epidermal growth factor receptor and vascular endothelial growth factor, which regulate cells proliferation and angiogenesis, and TP53, which regulates apoptosis, have been extensively reviewed [71-73] and will not be discussed in this thesis.

PROGNOSTICATION MODELS

Using a single prognostic biomarker to accurately classify patients' risk is challenging; therefore, researchers often combine different biomarkers into a single tool to aid in risk classification. For example, the TNM cancer staging system integrates the extent of the tumor, extent of spread to the lymph nodes, and presence of metastasis, providing a more comprehensive risk classification. Similarly, a statistical model can combine different prognostic biomarkers, providing a predicted probability of a clinical outcome. Such predictions about one's future health condition or the outcome of a disease over a specific period are commonly referred to as prognosis [66, 76]. The statistical model which integrates different clinical characteristics to estimate an individual's prognosis is referred to as a prognostication model [76]. Importantly, in many countries and regions, including European Union [77] and the United States [78], prognostication models are classified as medical devices. Therefore, they must undergo extensive evaluation—including model validation, and assessment of clinical utility—to demonstrate their positive impact on clinical decisions before being implemented.

Model validation encompasses both internal and external processes. Internal validation evaluates the model's performance in a population similar to that used during the model's development [79], using approaches such as cross-validation and bootstrapping. This process is crucial for preventing overfitting, especially in cases of small sample size and low event numbers [80]. In contrast, external validation tests the model's performance in a different population, such as different settings, regions, and time periods, thereby assessing the model's generalizability [81]. Both internal and external validation focus on calibration and discrimination. Calibration measures the concordance between observed outcomes and model predictions,

with recommended methods including the observed-to-predicted ratio, calibration slope, and calibration plot. Discrimination, on the other hand, reflects the model's ability to differentiate between high- and low-risk patients, typically using metrics like *c*-index [82, 83], and area under the receiver operating characteristic curve [84].

Clinical utility indicates the benefit from model-based decisions to individual patients or to the healthcare system compared to the standard of care. The gold standard of showing the clinical utility needs randomized trials [85, 86]. For example, genomic tests like TAILORx and MammaPrint have undergone randomized clinical trials to demonstrate their clinical utility [87-89]. However, such trials are rarely conducted in prognostication models, which might be due to substantial demands on time and resources, the necessity for large sample sizes, and ethical concerns regarding patient willingness to participate in randomized studies. Therefore, theoretical clinical utility based on observational studies can provide valuable insights into the actual clinical utility. In the context of a prognostication model designed to facilitate therapy de-escalation, the demonstrated theoretical clinical utility of the model may motivate a single-arm trial to evaluate the safety of therapy de-escalation. Methods to calculate theoretical clinical utilities include using risk reclassification tables to calculate net reclassification index [90], decision curve analysis to compare the net benefits [91, 92], and emulating target trials [86].

Breast cancer prognostication models

In clinical practice, prognostication models can help to identify low-risk patients who have good prognosis without systemic treatment, so that these low-risk patients can avoid the side effects of the treatment to exchange for the small potential benefit [85]. In breast cancer, prognostication modeling has been a popular research topic. A recent systematic review showed that from 1982 to 2016, 58 breast cancer prognostication models were published, predominantly using Cox regression models [93]. A more recent scoping review identified 21 models published since 2008, which aim to support decisions related to surgery, radiation therapy, and systemic therapies [94]. Both reviews highlighted that age at diagnosis, tumor size, tumor grade, and nodal status are the most used predictors in these models [93, 94].

Among all the published breast cancer prognostication models, AdjuvantOnline [95], CancerMath [96], and PREDICT [97] have been the most widely recognized and validated tools used by clinicians to assess patient outcomes and guide treatment decisions. However, AdjuvantOnline, once a popular choice, is no longer available online. Similarly, CancerMath, despite the initial promise, has not been updated since its publication in 2009, limiting its applicability in the context of current clinical practices and emerging research findings. This makes the PREDICT model the most updated and available prognostication model for breast cancer.

It is important to note that, while the PREDICT research team refers to the model as a prognostic or prognostication model, it offers not only breast cancer prognosis but also treatment benefits, albeit using different sources of evidence for prognostication and prediction. The model is a cause-specific Cox regression model derived from a UK population-based cohort. The model was initially published in 2010 [98], followed with multiple model updates and recalibration [97, 99, 100]. The most used model version 2.2 and 2.3 includes separate algorithms for ER-negative and ER-positive tumors. The ER-negative algorithm considers age at diagnosis, tumor size, number of positive lymph nodes, tumor grade, and HER2 status. The ER-positive algorithm includes these predictors plus detection mode and Ki67. Besides, the model extracted the effect of hormone therapy, extended tamoxifen therapy, trastuzumab, bisphosphonates, second, and third generation chemotherapy from multiple published or unpublished meta-analyses of clinical trials [101-103], and constrained the effect in the model. These two algorithms provide predictions for breast cancer-specific survival at multiple time points, while non-breast cancer survival is calculated separately using age as the sole predictor. Overall survival prediction is then calculated as the product of breast and non-breast cancer survivals.

Recent validation studies have shown that PREDICT in general performs reasonable across different patient populations [104-109]. However, there are notable gaps in its application for making treatment decisions. Firstly, most validation studies fall short of directly assessing the clinical utility of PREDICT. Theoretical methods such as decision curve analysis could offer insights into its potential clinical utility, although it is seldom applied in validation studies. Secondly, the predictions of PREDICT for treatment effect for young patients might not be entirely accurate, partly due to the scarcity of data from systemic treatment-naïve young patients. Lastly, the model's predictors do not adequately address the diversity found within the tumor microenvironment and genomic profiles of TNBC patients, limiting its ability to capture the full spectrum of heterogeneity in these patients.

AIM AND THESIS OUTLINE

The goal of this thesis is to improve risk classification regarding prognosis for TNBC patients. We focused on young, lymph node-negative TNBC patients due to the potential overtreatment in this group and the severe consequences of overtreatment in young women. Meanwhile, data from general TNBC patients were also collected for prognostication. The table below listed the data sources that were used in this thesis. In **Chapter 2**, we first externally validated the PREDICT model in young, node-negative breast cancer patients who did not receive systemic treatment, i.e. the PARADIGM cohort. In **Chapter 3**, we investigated the prognostic value of stromal TILs in the TNBC patients from the PARADIGM cohort. In **Chapter 4**, we

investigated the association between the *BRCA1*-related biomarkers, including germline *BRCA1* mutation, somatic *BRCA1* mutation and *BRCA1* promoter methylation, and long-term clinical outcomes in the TNBC patients from the PARADIGM cohort. In addition, we refined the analysis in **Chapter 3** with more complete data in the *BRCA1* status. We also commented on a systematic review and meta-analysis on the association between *BRCA1/2* mutations and breast cancer outcomes, with a summary of results from the systematic reviews and meta-analyses published between 2010 and 2021. To make the knowledge we generated into practice, in **Chapter 5** we incorporate stromal TILs into the PREDICT model to improve the model's performance and clinical utility in TNBC patients. We used data from pooled treated and untreated cohorts to update the model and performed leave-one-region-out cross-validation. We made our conclusion in **Chapter 6**, where we summarized the main findings in the previous chapters, discussed the strengths, limitations and potential biases of the included studies, along with their clinical relevance and future perspectives.

Data sources used in this thesis

	The PARADIGM cohort	**The pooled treated cohort**	**The pooled untreated cohort** [a]
Country	The Netherlands	International	International
Study type	Population-based	Clinical trial and hospital-based	Population- and hospital-based
Chapter	2, 3, 4, 5	5	5
Number of patients	2286	1806	1892
Number of TNBC	485	1806	1892
Median years of age at diagnosis (range)	36 (22- 39)	50 (25- 85)	55 (25- 85)
Year of diagnosis	1989-2000	-	1980-2020
Chemotherapy treatment			
Yes	0%	100%	0%
No	100%	0%	100%

[a] The pooled untreated cohort includes 443 patients from the PARADIGM cohort

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