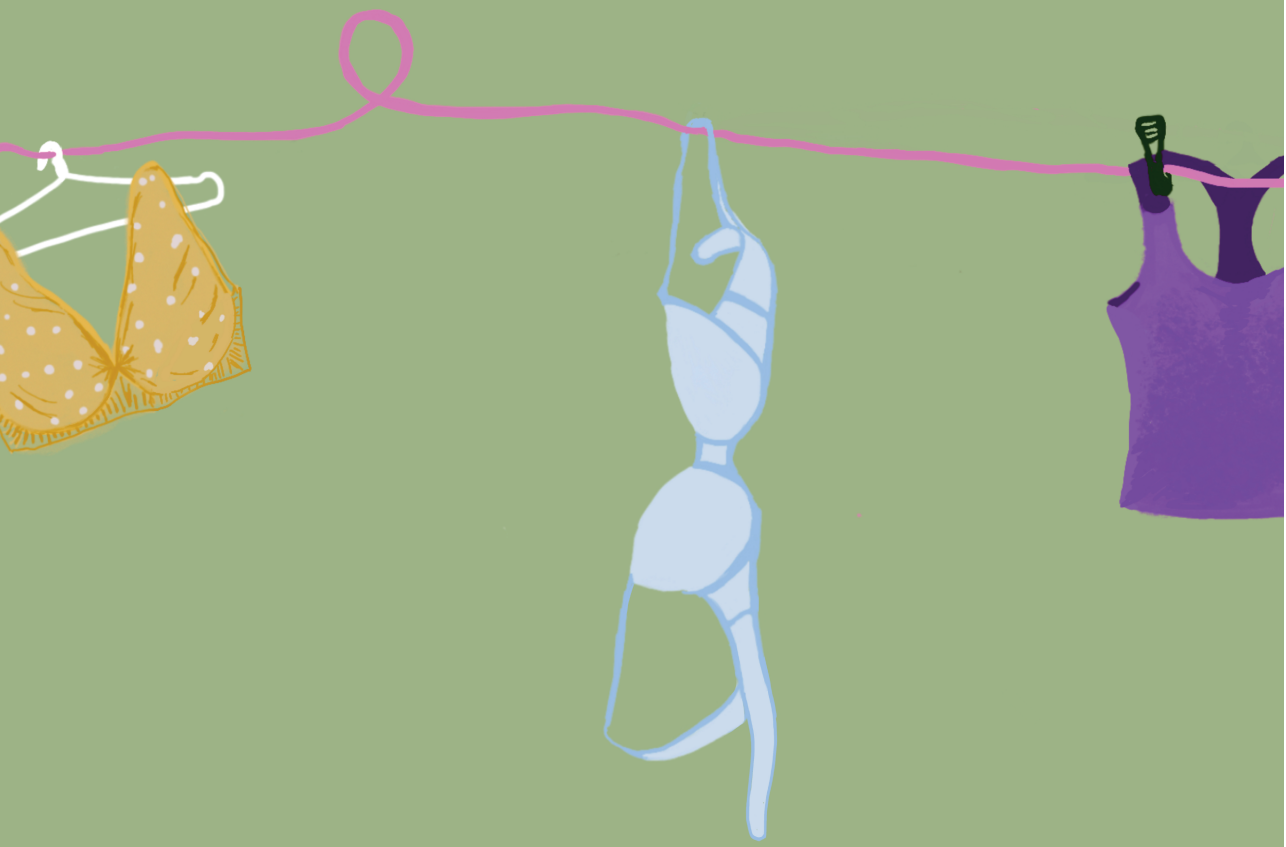
Tailored to fit: balancing over- and undertreatment in early-stage triple-negative breast cancer patients

Wang, Y.



CHAPTER 6

General discussion

Triple-negative breast cancer has been recognized as an aggressive subtype that lacks effective targeted therapies for early-stage patients. Consequently, most women with early-stage TNBC, regardless of their nodal status, are treated with (neo)adjuvant chemotherapy in accordance with international guidelines [1]. This reliance on chemotherapy has led to a scarcity of unbiased data on chemotherapy-naïve TNBC patients, making it challenging to study the true prognostic values of biomarkers in this population. Moreover, the absence of such data raises concerns about the potential overtreatment of low-risk patients who might not benefit from (neo)adjuvant chemotherapy.

TNBC is widely recognized as a heterogeneous disease, with considerable variation in genetic and immune profiles [2]. In very recent years, targeted therapies addressing these profiles have been incorporated into treatment guidelines for early-stage TNBC patients. For instance, olaparib has been recommended for patients with germline *BRCA1/2* mutations and high-risk disease, and pembrolizumab has been included for TNBC patients with stage II to III tumors [3, 4]. Despite these advances, chemotherapy, including taxane-based with or without carboplatin, is still recommended for most TNBC patients to prevent (distant) recurrences. Furthermore, the most recent Dutch guideline has not yet included all these novel therapies, leaving chemotherapy ± pembrolizumab as the primary treatment option for most TNBC patients [5].

However, chemotherapy de-escalation for TNBC remains largely unexplored. Unnecessary chemotherapy fails to prevent distant recurrences that may never occur and instead introduces adverse side effects that can significantly diminish patients' quality of life or even pose life-threatening risks such as cardiotoxicity and secondary malignancies [6, 7]. This concern is particularly relevant for young cancer survivors who have a long-life expectancy after treatment.

Due to its potential severe side effects, chemotherapy cannot be administered "just in case" a recurrence might occur [7]. Instead, the survival benefits of chemotherapy must be carefully weighed against its side effects, aiming to strike a balance between the risks of over- and undertreatment in TNBC patients. For instance, the Dutch breast cancer guideline recommends adjuvant chemotherapy only when an absolute 10-year survival benefit of at least 3-5% is anticipated [5]. This corresponds to an estimated 10-15% 10-year breast cancer-specific mortality risk, assuming chemotherapy provides a 40-60% relative risk reduction [5]. Clinicians, therefore, use these guidelines, which compile a wealth of knowledge from numerous clinical trials and the collective experience of expert clinicians, to help them evaluate and strike the right balance. In addition to the guidelines, many breast cancer prognostication models have been developed using big data to assist clinicians in making informed treatment decisions. Once the model is widely validated, it can be used alongside treatment guidelines to facilitate shared decision-making between clinicians and patients.

The primary aim of this thesis was to improve risk classification for early-stage TNBC patients, with a particular focus on young patients. We investigated the prognostic values of genetic and immune biomarkers in young, early-stage TNBC patients. By integrating the knowledge generated in this thesis with existing evidence from other literature, we aimed to refine risk classification for early-stage TNBC patients by updating an existing breast cancer prognostication model. In this Chapter, we discuss the main findings of this thesis and interpret them with a broader context. We also address the practical and methodological challenges encountered in these studies, as well as the limitations and strengths of this thesis. Finally, we concluded with potential clinical implications and suggestions for future research.

MAIN FINDINGS IN CONTEXT OF OTHER LITERATURE

Validity and potential clinical utility of the PREDICT model in young, early-stage breast cancer patients

As of 2017, at least 58 different breast cancer prognostication tools have been published, most of which include standard clinicopathological characteristics as predictors, such as nodal status, tumor size, tumor grade, and age at diagnosis [8]. Among these models, PREDICT remains one of the most widely used models for early breast cancer prognostication, including breast cancer-specific survival and overall survival. The predictors of the model (version 2.2 and 2.3) includes age at diagnosis, estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), tumor size, grade, number of positive lymph nodes, screening, and Ki67 (the last two predictors only for ER-positive tumors) [9]. All these predictors are routinely collected in a standard manner in clinical practice, making the PREDICT model easy to use without additional cost. Multiple external validation studies have shown that PREDICT has a good discrimination and calibration-in-the-large in the overall breast cancer population [9-13], indicating that the model has a good potential to perform risk classification using classic predictors. However, it remains uncertain whether PREDICT performs well in more specific and homogenous groups. For example, when focusing on young, node-negative breast cancer patients—a group where chemotherapy remains the dominant treatment despite an urgent need for de-escalation—can PREDICT still distinguish between low-risk and high-risk patients?

In **Chapter 2**, we evaluated the PREDICT model in a population-based cohort with women diagnosed with node-negative breast cancer under 40 years of age. These patients did not receive any systemic treatment, neither chemotherapy, hormone therapy, nor anti-HER2 therapy, following standard practice at the time of their diagnosis. We found that PREDICT underestimated all-cause mortality in these patients. The discriminative ability of PREDICT in those with ER-positive tumors was acceptable, while poor in those with ER-negative breast

cancer. Since PREDICT has been used in clinical practice to aid adjuvant chemotherapy decision-making, we also evaluated its clinical utility in this context using decision curve analysis. We found that the model showed a slightly higher clinical utility compared to the strategy of giving every patient chemotherapy in patients with ER-positive tumors, but no extra benefit in patients with ER-negative tumors was observed compared to the strategy to give all of them chemotherapy.

The poor discriminative ability of PREDICT in young, early-stage ER-negative breast cancer patients and TNBC patients was expected, as the distribution of the predictors for these patients was homogeneous. This result showed the necessity of updating the PREDICT model with new predictors to improve its discrimination in this patient group. In the next two chapters, we investigated a few prognostic biomarkers in TNBC, which can be candidates to be incorporated into PREDICT.

Prognostic biomarkers in young early-stage TNBC patients

In **Chapter 3** and **Chapter 4**, we investigated the prognostic values of sTILs, germline *BRCA1* mutations, and other *BRCA1*-related biomarkers in young, node-negative TNBC patients who did not receive chemotherapy. By using this chemotherapy-naïve cohort with minimized indication bias, we were able to examine the prognostic values of the aforementioned biomarkers without the potential mediating effect from systemic treatment.

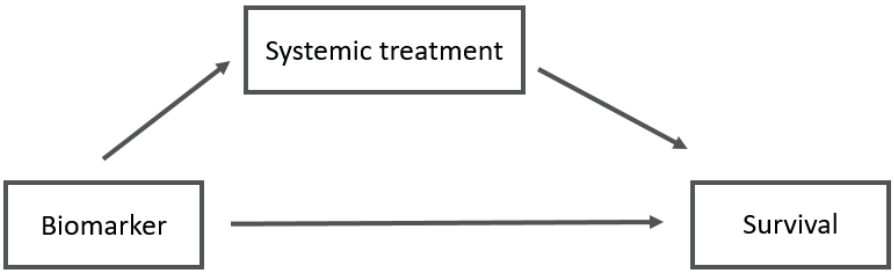


Illustration of the relationships between biomarkers, systemic treatment, and survival. The arrows depict the direct influence of biomarkers (e.g., sTILs, germline *BRCA1* mutations) on both systemic treatment decisions and survival outcomes, as well as the effect of systemic treatment on survival. In the context of a chemotherapy-naïve cohort, the absence of systemic treatment allows for the assessment of biomarker prognostic value without mediation by treatment effects

Prognostic value of sTILs

Previous studies have confirmed a positive association between sTILs and survival in TNBC patients treated with (neo)adjuvant chemotherapy [14-18]. However, it remains unclear whether

this association reflects the prognostic value of sTILs alone or their predictive value for response to chemotherapy. To exclude the possibility that sTILs' predictive value is driving the association, analyses should be restricted to systemic treatment-naïve TNBC patients. Such analyses have been performed in systemic treatment-naïve TNBC patients, primarily in those diagnosed at age 40 or older [19], but evidence for younger patients has been lacking. In **Chapter 3**, we addressed this gap by demonstrating the high prognostic value of sTILs in early-stage chemotherapy-naïve TNBC patients diagnosed before 40 years of age. Two recent studies further corroborated the prognostic value of sTILs in early-stage TNBC patients across all age groups in chemotherapy-naïve TNBC patients, with findings that strongly aligned with ours [20, 21].

In this chapter, we also showed how sTILs may improve risk classification in young, early-stage TNBC patients. Most current treatment guidelines primarily rely on tumor stage, ER, and HER2 status to guide systemic treatment decisions in breast cancer. For example, the 2024 ESMO guideline suggests that patients with T1a tumors may forgo chemotherapy, while those with T1b or larger tumors are still recommended to receive 6-8 cycles chemotherapy [3]. However, in **Chapter 3**, we showed that among T1b/c patients, those with high sTILs had excellent long-term overall survival. Additionally, patients with stage II tumors and high sTILs exhibited better survival outcomes than those with stage IB and low sTILs, suggesting that the impact of sTILs on survival may be greater than tumor size in young early-stage TNBC patients. Although the 2024 ESMO guideline acknowledges that sTILs may provide additional prognostic information, detailed recommendations on how sTILs could influence chemotherapy decisions remains absent [3].

Prognostic value of germline BRCA1 mutation and other BRCA1-related biomarkers

While immune biomarkers like TILs provide valuable insights in TNBC prognosis, genetic biomarkers, such as germline *BRCA1* mutations, also play a critical role due to their relatively high prevalence in TNBC patients. Like TILs, the prognostic value of germline *BRCA1* mutations remains understudied in systemic treatment-naïve breast cancer patients, especially in young TNBC patients, due to predominant reliance on chemotherapy in this patient population. In **Chapter 4**, we expanded our focus to *BRCA1*-related biomarkers. This chapter includes two publications: the first is a letter to the editor discussing unresolved questions regarding the association between germline *BRCA1/2* mutations and breast cancer prognosis, while the second is a study examining the prognostic value of *BRCA1*-related biomarkers in young women under 40 years old with node-negative TNBC and their relationship with TILs scores.

In the letter to the editor, we summarized six systematic reviews and meta-analyses published since 2010 that examined the association between germline *BRCA1/2* mutations and survival in breast cancer patients. Most of these meta-analyses did not stratify their analyses based

on chemotherapy usage and reported pooled results suggesting worse overall survival in germline *BRCA1* mutation carriers, although not all analyses reached a statistically significant conclusion [22-28]. More recently, a meta-analysis which combined results from 18 studies that included patients treated with chemotherapy or non-PARP targeted therapies, found no differences in overall survival between germline *BRCA1/2* mutation carriers and non-carriers [29]. Based on these pooled findings, we raised the question: what is the mediating role of chemotherapy in the prognosis of *BRCA1/2* mutation carriers? In other words, what is the true prognostic value of germline *BRCA1/2* mutations in systemic treatment-naïve breast cancer patients, and what is their predictive value for response to chemotherapy and other targeted therapies in the treated patients?

In the second part of **Chapter 4**, we investigated the true prognostic value of germline *BRCA1* mutation, *BRCA1* promoter methylation, and somatic *BRCA1* mutation in young, node-negative, chemotherapy-naïve TNBC patients. Our goal was to exclude the mediating effects of chemotherapy and examine the natural disease course of TNBC patients with different *BRCA1* status. We found that young, node-negative, TNBC patients with a germline *BRCA1* mutation who did not receive chemotherapy had worse long-term overall survival compared to those without *BRCA1* alteration or those with *BRCA1* promoter methylation in their tumors. This poorer overall survival was partly mediated by a higher incidence of second primary tumors, mostly contralateral breast and ovarian tumors, a finding that aligns with a previous study [30]. Furthermore, we showed that the prognostic value of sTILs remained significant in women with or without germline *BRCA1* mutation. No statistically significant difference of sTILs scores was observed in women with germline *BRCA1* mutation, *BRCA1* promoter methylation, somatic *BRCA1* mutation, and *BRCA1* non-alteration. So far, only a few studies have compared TIL abundance in TNBC based on germline *BRCA1/2* mutation status. These studies have reported inconsistent results, potentially due to their small sample sizes and lack of consideration for patient age during comparison [31-34]. Interestingly, we observed that the positive association between sTILs and improved overall survival was stronger in women with *BRCA1* promoter methylation than in those without (including both germline *BRCA1* mutation carriers and women without any *BRCA1* alterations). This suggested that the composition of sTILs may vary across TNBCs with different *BRCA1* status. However, supporting evidence for this hypothesis is limited.

The validity of the existing breast cancer prognostic model, and update the model with TILs

Personalized treatment based on widely validated prognostic biomarkers in early-stage TNBC patients is highly anticipated by both clinicians and patients. In **Chapter 3 and 4,** we showed the independent prognostic values of sTILs and germline *BRCA1* mutation in young, early-stage TNBC patients. These new biomarkers have not yet been incorporated into PREDICT, the

widely-used prognostic model. In **Chapter 5**, we aimed to translate the knowledge into clinical applications. Using data from two large, pooled cohorts with individual patient-level data from early-stage TNBC patients [17, 20], we updated the PREDICT model by incorporating sTILs as an additional predictor. The updated PREDICT model, called PREDICT_sTILs, performed well in leave-one-region-out cross-validation and showed promising clinical utility, especially in identifying low-risk women who might safely forgo chemotherapy.

Currently, clinical guidelines recommend chemotherapy for all TNBC patients with or without targeted therapies, except for those with T1a tumors [3, 4]. Compared to the guidelines, PREDICT not only considers tumor stage but also age at diagnosis and tumor grade. However, since many TNBC patients have high-grade tumors, the model still classifies most as high-risk and recommends chemotherapy, in line with existing guidelines. By integrating sTILs into the PREDICT model, we improved risk classification for early-stage TNBC patients, identifying those at low risk who might survive with less intense regimens or even without chemotherapy.

In **Chapter 5**, we presented the first prognostic model to incorporate sTILs for improving risk classification and assisting clinicians in chemotherapy decision making for early-stage TNBC patients. However, it is not the only model that considers immune profiles in TNBC patients. Several other prognostic models have been developed using immune biomarkers, such as immune signatures and immune multigene predictors [35-37]. One of the main limitations of these models is the high cost of biomarker measurement, which makes external validation and routine clinical use expensive. In contrast, sTILs can be easily evaluated in routine clinical practice using standard H&E slides by a trained pathologist. In addition, a recent study showed the potential of using AI-based tool to reduce the cost of scoring sTILs while maintaining scoring accuracy [38].

Emerging clinical evidence further strengthens the rationale for integrating sTILs into treatment decision frameworks. A pooled analysis of two recent trials involving stage II to stage III TNBC patients with sTILs > 30% showed excellent overall survival when using anthracycline-free chemotherapy [39]. Furthermore, recently started clinical trials are now investigating whether early-stage TNBC patients with high sTILs can safely forgo chemotherapy (NCT06078384, NCT06476119). These studies, together with our results, could support the notion that sTILs have the potential to refine personalized treatment in TNBC patients.

STRENGTHS, LIMITATIONS AND METHODOLOGICAL CHALLENGES

This thesis aimed to improve the risk classification of TNBC patients through two association studies exploring potential prognostic biomarkers and two prediction modeling studies to validate and update the PREDICT model, translating our knowledge into potential clinical applications. When interpreting the findings of this thesis, it is crucial to consider both the strengths and limitations of the data and methodologies used in our studies.

In **Chapter 2, 3, and 4**, we used data from the TNBC patients in the PARADIGM cohort. This cohort is uniquely suited for evaluating the prognostic value of biomarkers independently of their potential predictive value, as all patients were systemic treatment naïve. Additionally, since these patients were treated according to guidelines at the time of their diagnosis, we effectively minimized indication bias. Such dataset is particularly valuable because it is increasingly difficult to find young breast cancer patients not treated with systemic therapy today, regardless of nodal status [40, 41].

However, the PARADIGM cohort also has its limitations. This cohort combined data from multiple resources, including vital status from CBS (Centraal Bureau voor de Statistiek, the national registry of vital status of Dutch Inhabitants, treatment, recurrence, and second primary tumors from IKNL (Integraal Kankercentrum Nederland/ Netherlands Comprehensive Cancer Organization), and pathological data from PALGA (Pathological-Anatomical National Automated Archive), which is the nationwide database in the Netherlands that collects and stores pathology and anatomy data. Pooling these real-world data is challenging, and decisions based on plausible assumptions within the disease's context in case of imperfect or missing data are necessary. In the PARADIGM cohort, recurrence status was missing for some patients while the death status was complete. Since a recurrence tend to occur within the first 5 years after diagnosis for TNBC patients [42], and patients typically die within about one year of distant recurrence [43], we assumed that patients who remained alive 5-15 years after diagnosis according to the CBS database and did not have a registered distant recurrence were free of this event, although chance of missing a few events may still exist.

In **Chapter 5**, we collected data of TNBC patients from two large, pooled cohorts to update the PREDICT model for TNBC patients. These large cohorts provided sufficient sample size and allowed us to perform leave-one-region-out cross-validation. However, we lacked information on the cause of death in these cohorts, while breast cancer-specific survival was the primary outcome in **Chapter 5**, consistent with the model we aimed to update. We assumed deaths occurring after distant recurrences or within 5 years of diagnosis breast cancer-related, which might have led to some misclassification. In addition, 10 to 15% of

TNBC patients carry *BRCA1/2* mutations, which increase the risk of second primary tumors, such as contralateral breast or ovarian cancers [30, 44]. If a second primary tumors occurred within 5 years after the TNBC diagnosis, we still attribute the subsequent death to the primary breast cancer due to missing data on second primary tumors and cause of death. However, it is difficult to determine the extent of misclassification's impact, especially it has been shown that overall survival and breast cancer-specific survival were not significantly different between *BRCA1/2* mutation carriers and non-carriers when treated with chemotherapy [44].

Another challenge is identifying TNBC cohorts with sufficient sample sizes and comprehensive data on both TILs and *BRCA1*-related biomarkers, along with long-term follow-up and treatment information. The TILs-scored TNBC cohorts used in **Chapter 5** lack data on *BRCA1*-related biomarkers, which limited our ability to incorporate these biomarkers into the PREDICT model, despite demonstrating their prognostic values in **Chapter 4**. Additionally, external validation of the update model across different settings, time periods, and patient ethnicities is highly challenging due to the lack of suitable datasets. However, these validations are crucial because variations in settings, time periods, and patient ethnicities are associated with patients' survival [45, 46]. To address this, we have initiated collaborations with the POSH cohort in the UK [40] and the FUDAN cohort in China [47] to explore potential external validation of PREDICT_sTILs in patients from diverse ethnic backgrounds and more recent diagnostic periods. However, progress has been hindered by the ongoing collection of sTILs data in the POSH cohort and the insufficient number of events in the FUDAN cohort for robust validation.

One of the challenges shared across all studies in this thesis is the presence of missing data in key tumor characteristics, such as tumor size, tumor grade, and *BRCA1*-related biomarkers in the TNBC subtype of the PARADIGM cohort. Completely excluding these missing values could lead to insufficient sample size and potentially biased results [48]. Several methods can help address a large amount of missing data, including multiple imputation and complete case analysis. Multiple imputation is widely recommended to reduce bias and improve precision when the missing pattern is missing at random or missing completely at random, especially when the amount of missing values is high [49]. However, when the missing pattern of the exposure and confounders is missing not at random, complete case analysis might give more valid results [48].

In chapter 4, *BRCA1* status was missing in approximately 18% of the patients, and a missing-at-random mechanism is assumed. *BRCA1*-related biomarkers were evaluated using DNA from archived tissues; however, in cases where tumor size was small, the DNA extracted from the tumor may have been insufficient for conclusive results. This indicates that *BRCA1* status was not missing completely at random, as smaller tumor size contributed to the higher chance of missing data. Furthermore, there is no evidence suggesting that young patients

with *BRCA1* mutations exhibit different tumor sizes compared to those without *BRCA1* mutations, especially during a time when genetic testing was not widely accessible [30, 40], which eliminates the possibility of missing not at random. Therefore, we can reasonably assume that the mechanism for missing *BRCA1* status in chapter 4 is missing at random. Nonetheless, it is impossible to completely rule out the possibility of missing not at random in the data. In **Chapter 2** and **4**, in addition to multiple imputation, we performed complete case analysis as a sensitivity analysis, and the aligned results between the two approaches suggest that no significant bias was introduced by missing values. In **Chapter 3**, missing data was not an issue since the missing percentage was negligible. In **Chapter 5**, where two pooled cohorts were used for the main analysis, some variables were completely unavailable in a few datasets. This type of systemic missing data can also be considered missing at random, as it can be fully explained by the fact that some studies did not collect such data [50]. In **Chapter 5**, we addressed this issue using multiple imputation with the study indicator as a covariate. Additionally, for some continuous variables that were missing, categorical variables provided information on the range of the continuous variables. For example, a patient with an unknown tumor size (continuous) but classified as stage T1c would have a tumor size between 10 and 20 mm. To ensure consistency after imputation, we used post parameter in the mice function (mice package) [51], maximizing the available data and avoiding discrepancies between imputed continuous and categorical variables.

In **Chapter 5**, we faced a methodology challenge when estimating the baseline hazard in a Cox regression model with an offset term for chemotherapy. The offset coefficient was pre-specified based on results from meta-analysis of randomized clinical trials [52]. Initially, we used the *coxph* function (*survival package*) in R to fit the Cox regression model, and the *survfit* and *basehaz* function (*survival package)* to estimate baseline hazards [53]. However, the predicted survival was significantly different from the observed survival in the training cohorts, indicating a mistake in the model parameters. After reviewing the R source code, we discovered that *survfit* ignores the offset term when applied with a *coxph* object [53]. We then compared baseline hazards calculated using *coxph* [53] and *cph* (*rms package*) [54], and cross-checked using Python and STATA. While the coefficients were consistent across methods, the baseline hazards from *cph* aligned with other software. In addition, the predicted survival based on *cph* baseline hazards matched the observed survival in the training cohorts. Therefore, in **Chapter 5**, all baseline hazards were calculated using the *cph* in R.

SUGGESTIONS FOR FUTURE RESEARCH AND CLINICAL IMPLICATIONS

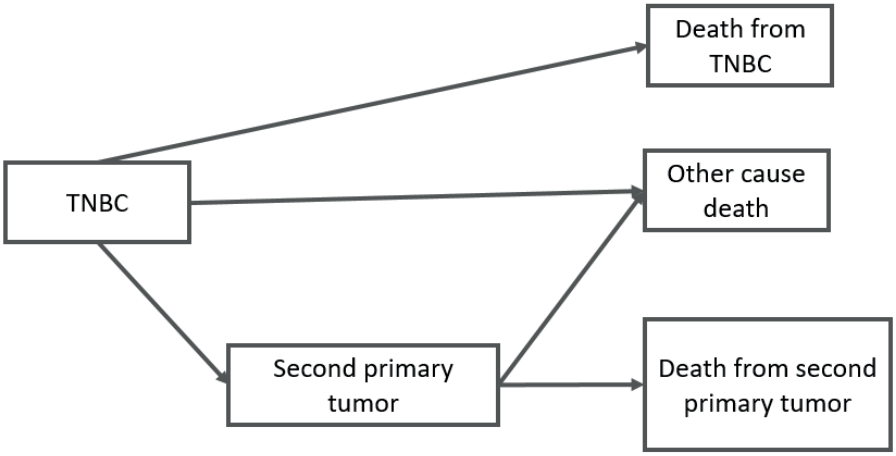
Composition and spatial perspectives of sTILs, taking *BRCA1*-related biomarkers into account

Not all early-stage TNBC patients with high TILs survived, highlighting the need to further understand TILs, tumor cells, and their interaction. TILs consist of various lymphocytes with distinct functions; for example, cytotoxic CD8+ T cells and T helper cells of type 1 support immune surveillance, whereas regulatory T cells (Tregs) counteract the antitumor responses [55]. The positive association between TILs and breast cancer prognosis is likely primarily driven by CD8+ T cells and other immune surveillance cells. Refining TILs by counting specific lymphocytes for optimal prognostic value is an area of interest, although whether these refined biomarkers show prognostic superiority over TILs alone remains unclear [56-58]. Examining the spatial relationship between immune and tumor cells may also improve prognostic accuracy [59]. TNBCs can be divided into four immune phenotypes: immune excluded, immune deserted, inflamed stroma restricted and inflamed stromal and intratumoral [60]. Small-scale studies have assessed the prognostic values of these phenotypes with mixed results [61, 62].

The genetic profile of tumors, including *BRCA1* status, likely influences immune response. Tumors with germline *BRCA1* mutations or with *BRCAness* profiles are genomically unstable and may provoke an active immune response due to the high volume of tumor-associated antigens [63]. Yet, few studies have found a positive association between the abundance of TILs and germline *BRCA1/2* mutation [34]. Notably, in **Chapter 4**, we observed a stronger prognostic value of TILs in young, node-negative TNBC patients with *BRCA1* promoter methylation compared to those with other *BRCA1* status. This finding requires validation; if confirmed, it suggests distinct immune profiles in patients with tumors harbouring *BRCA1* promoter methylation. Future research may therefore explore TILs composition and their spatial relationship with the tumor cells in TNBC patients with different *BRCA1* status and provide valuable insights into personalized treatment.

Including new predictors into the prognostic model

PREDICT_sTILs, like all prediction models, should be continuously updated as new prognostic or predictive biomarkers emerge and are validated for clinical use. As a prevalence prognostic biomarker, germline *BRCA1/2* mutation could be valuable in future updates once larger cohorts with both sTILs and germline *BRCA1/2* mutation data are available. Germline *BRCA1/2* mutation carriers also face higher risks of second primary tumors, adding complexity to disease progression. Future models may need to account for this by considering second primary tumors as a transitional state in the disease pathway, offering a deeper understanding of TNBC progression in mutation carriers [64].



Illustrative model of disease progression in TNBC patients, with potential pathways of second primary tumors, which may contribute to overall mortality alongside death from TNBC. Incorporating second primary tumors as a transitional state offers insights into the complex progression and survival outcomes for germline *BRCA1/2* mutation carriers.

In integrating biomarkers like sTILs and potentially *BRCA1/2* mutations, their potential predictive values should be considered, as these relate to treatment benefits. For example, HER2 status in PREDICT was incorporated both for its prognostic value in breast cancer-specific survival and for its predictive value in response to trastuzumab [65]. PREDICT_sTILs only includes the prognostic, but not the predictive value of of sTILs. A study based on two randomized trials showed no statistically significant interaction between TILs, whether assessed continuously or as binary (using a 50% cutoff), and anthracycline-based chemotherapy versus no chemotherapy, indicating that sTILs have no predictive value for anthracycline-based chemotherapy [66]. However, studies have shown a predictive value of sTILs for taxane-based chemotherapy, supported by both preclinical and clinical evidence [67, 68]. In Chapter 5, although some patients received taxane-based chemotherapy, we did not include an interaction term for sTILs and taxane-based chemotherapy in the model. This decision is based on a previous study that analyzed the same data from chemotherapy-treated patients in Chapter 5, which found no statistically significant interaction term [17]. One potential explanation for the inability to validate this predictive effect is that taxanes directly activate T cells [67] while sTILs comprise not only T cells but also other immune cells, which may attenuate this effect. While the predictive value of sTILs for taxane-based chemotherapy cannot be entirely dismissed, robust trial data are required before integrating this predictive value into the model. At this stage, we recommend focusing on the prognostic aspects of the model, using it solely to identify low-risk patients who may safely forgo chemotherapy.

Further validation of PREDICT_sTILs and implementation

Before implementing the PREDICT_sTILs model in clinical management of early-stage TNBC patients, several steps are necessary. Firstly, further external validation is crucial. Although **Chapter 5** presented good results from leave-one-region-out cross-validation, an external validation by an independent research group is still needed to evaluate the reproducibility and transportability of PREDICT_sTILs in different settings [69] These settings include different ethnicity groups (e.g., Asian and African ancestry), patients diagnosed more recently, those treated with neoadjuvant chemotherapy, and very importantly, germline *BRCA1/2* mutation carriers. The predictive value of germline *BRCA1/2* mutations, alongside these carriers' elevated risks of second primary cancers, affects survival and warrants careful consideration for the generalization of breast cancer prognostic models. PREDICT has previously validated in germline *BRCA1/2* mutation carriers, showing a good model fit in those with ER-negative breast cancers but overestimation of breast cancer mortality, suggesting possible baseline hazard miscalibration and/or inaccurate chemotherapy benefit estimation [70]. Given the inclusion of a new predictor and recalibrated baseline hazard in PREDICT_sTILs, external validation in germline *BRCA1/2* mutation carriers is especially important to ensure accuracy in this high-risk group.

Several results could occur in PREDICT_sTILs external validation. Ideally, PREDICT_sTILs will show strong calibration, discrimination, and clinical utility, allowing direct application in new settings. A less ideal outcome is preserved discriminative but weaker calibration. In this case, model utility should still be evaluated, as calibration accuracy is most crucial at thresholds that influence clinical decisions. If recalibration is needed, the locally recalibrated model requires targeted validation before being applied to a new population/setting [71]. Moreover, constant validation and recalibration are essential even within the same setting, as patient characteristics can shift over time due to factors like immigration, diet changes, and implementation of screening programs [72]. Prognostic models predicting events over 5- or 10-year period will inherently lag, as they are based on past data for application in present-day patients. Methods such as discrete updating baseline hazards and Bayesian updating can help mitigate this delay [73].

After validating PREDICT_sTILs in independent cohorts, the next step is to implement the model in clinical practice. It is important to note that such a model is considered a medical device and must receive approval under In Vitro Diagnostic Medical Devices Regulation before being implemented. Successful implementation requires not only reliable prediction results but also clear, interpretable outcomes. For instance, displaying probabilities graphically rather than verbally can improve understanding [74]. To facilitate implementation, we recommend training sessions for clinicians, covering the model's development, data sources, outcome interpretation, and strategies for effectively communicating results to patients [75].

REFERENCES

1 Steenbruggen TG, van Werkhoven E, van Ramshorst MS et al. Adjuvant chemotherapy in small node-negative triple-negative breast cancer. Eur J Cancer 2020; 135: 66-74.

2 Kudelova E, Smolar M, Holubekova V et al. Genetic Heterogeneity, Tumor Microenvironment and Immunotherapy in Triple-Negative Breast Cancer. Int J Mol Sci 2022; 23 (23).

3 Loibl S, Andre F, Bachelot T et al. Early breast cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. Ann Oncol 2024; 35 (2): 159-182.

4 Burstein HJ, Curigliano G, Thurlimann B et al. Customizing local and systemic therapies for women with early breast cancer: the St. Gallen International Consensus Guidelines for treatment of early breast cancer 2021. Ann Oncol 2021; 32 (10): 1216-1235.

5 NABON/NIV. Federatie Medisch Specialisten - Richtlijnen database_Borstkanker adjuvante systemische behandeling. 2023. 2020.

6 Lustberg MB, Kuderer NM, Desai A et al. Mitigating long-term and delayed adverse events associated with cancer treatment: implications for survivorship. Nat Rev Clin Oncol 2023; 20 (8): 527-542.

7 Tao JJ, Visvanathan K, Wolff AC. Long term side effects of adjuvant chemotherapy in patients with early breast cancer. Breast 2015; 24 Suppl 2 (0 2): S149-153.

8 Phung MT, Tin Tin S, Elwood JM. Prognostic models for breast cancer: a systematic review. BMC Cancer 2019; 19 (1): 230.

9 Candido Dos Reis FJ, Wishart GC, Dicks EM et al. An updated PREDICT breast cancer prognostication and treatment benefit prediction model with independent validation. Breast Cancer Res 2017; 19 (1): 58.

10 van Maaren MC, van Steenbeek CD, Pharoah PDP et al. Validation of the online prediction tool PREDICT v. 2.0 in the Dutch breast cancer population. Eur J Cancer 2017; 86: 364-372.

11 Gray E, Marti J, Brewster DH et al. Independent validation of the PREDICT breast cancer prognosis prediction tool in 45,789 patients using Scottish Cancer Registry data. Br J Cancer 2018; 119 (7): 808-814.

12 Zaguirre K, Kai M, Kubo M et al. Validity of the prognostication tool PREDICT version 2.2 in Japanese breast cancer patients. Cancer Med 2021; 10 (5): 1605-1613.

13 Grootes I, Keeman R, Blows FM et al. Incorporating progesterone receptor expression into the PREDICT breast prognostic model. Eur J Cancer 2022; 173: 178-193.

14 Adams S, Gray RJ, Demaria S et al. Prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancers from two phase III randomized adjuvant breast cancer trials: ECOG 2197 and ECOG 1199. J Clin Oncol 2014; 32 (27): 2959-2966.

15 Denkert C, von Minckwitz G, Brase JC et al. Tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy with or without carboplatin in human epidermal growth factor receptor 2-positive and triple-negative primary breast cancers. J Clin Oncol 2015; 33 (9): 983-991.

16 Denkert C, von Minckwitz G, Darb-Esfahani S et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. Lancet Oncol 2018; 19 (1): 40-50.
17 Loi S, Drubay D, Adams S et al. Tumor-Infiltrating Lymphocytes and Prognosis: A Pooled Individual Patient Analysis of Early-Stage Triple-Negative Breast Cancers. J Clin Oncol 2019; 37 (7): 559-569.
18 Loi S, Michiels S, Salgado R et al. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. Ann Oncol 2014; 25 (8): 1544-1550.
19 Park JH, Jonas SF, Bataillon G et al. Prognostic value of tumor-infiltrating lymphocytes in patients with early-stage triple-negative breast cancers (TNBC) who did not receive adjuvant chemotherapy. Ann Oncol 2019; 30 (12): 1941-1949.
20 Leon-Ferre RA, Jonas SF, Salgado R et al. Tumor-Infiltrating Lymphocytes in Triple-Negative Breast Cancer. JAMA 2024; 331 (13): 1135-1144.
21 Geurts VCM, Balduzzi S, Steenbruggen TG et al. Tumor-Infiltrating Lymphocytes in Patients With Stage I Triple-Negative Breast Cancer Untreated With Chemotherapy. JAMA Oncol 2024.
22 Lee EH, Park SK, Park B et al. Effect of *BRCA1/2* mutation on short-term and long-term breast cancer survival: a systematic review and meta-analysis. Breast Cancer Res Treat 2010; 122 (1): 11-25.
23 van den Broek AJ, Schmidt MK, van 't Veer LJ et al. Worse breast cancer prognosis of *BRCA1*/*BRCA2* mutation carriers: what's the evidence? A systematic review with meta-analysis. PLoS One 2015; 10 (3): e0120189.
24 Zhong Q, Peng HL, Zhao X et al. Effects of *BRCA1*- and *BRCA2*-related mutations on ovarian and breast cancer survival: a meta-analysis. Clin Cancer Res 2015; 21 (1): 211-220.
25 Templeton AJ, Gonzalez LD, Vera-Badillo FE et al. Interaction between Hormonal Receptor Status, Age and Survival in Patients with *BRCA1/2* Germline Mutations: A Systematic Review and Meta-Regression. PLoS One 2016; 11 (5): e0154789.
26 Zhu Y, Wu J, Zhang C et al. *BRCA* mutations and survival in breast cancer: an updated systematic review and meta-analysis. Oncotarget 2016; 7 (43): 70113-70127.
27 Baretta Z, Mocellin S, Goldin E et al. Effect of *BRCA* germline mutations on breast cancer prognosis: A systematic review and meta-analysis. Medicine (Baltimore) 2016; 95 (40): e4975.
28 Liu M, Xie F, Liu M et al. Association between *BRCA* mutational status and survival in patients with breast cancer: a systematic review and meta-analysis. Breast Cancer Res Treat 2021; 186 (3): 591-605.
29 Shao C, Chang MS, Lam FC et al. A Systematic Review and Meta-Analysis on the Prognostic Value of *BRCA* Mutations, Homologous Recombination Gene Mutations, and Homologous Recombination Deficiencies in Cancer. J Oncol 2022; 2022: 5830475.
30 Schmidt MK, van den Broek AJ, Tollenaar RA et al. Breast Cancer Survival of *BRCA1*/*BRCA2* Mutation Carriers in a Hospital-Based Cohort of Young Women. J Natl Cancer Inst 2017; 109(8):10.1093/jnci/djw329 (8).

31 Grandal B, Evrevin C, Laas E et al. Impact of *BRCA* Mutation Status on Tumor Infiltrating Lymphocytes (TILs), Response to Treatment, and Prognosis in Breast Cancer Patients Treated with Neoadjuvant Chemotherapy. Cancers (Basel) 2020; 12(12):3681.

32 Solinas C, Marcoux D, Garaud S et al. *BRCA* gene mutations do not shape the extent and organization of tumor infiltrating lymphocytes in triple negative breast cancer. Cancer Lett 2019; 450: 88-97.

33 Telli ML, Chu C, Badve SS et al. Association of Tumor-Infiltrating Lymphocytes with Homologous Recombination Deficiency and *BRCA1/2* Status in Patients with Early Triple-Negative Breast Cancer: A Pooled Analysis. Clin Cancer Res 2020; 26 (11): 2704-2710.

34 Nolan E, Savas P, Policheni AN et al. Combined immune checkpoint blockade as a therapeutic strategy for *BRCA1*-mutated breast cancer. Sci Transl Med 2017; 9(393):eaal4922.

35 Reyal F, van Vliet MH, Armstrong NJ et al. A comprehensive analysis of prognostic signatures reveals the high predictive capacity of the proliferation, immune response and RNA splicing modules in breast cancer. Breast Cancer Res 2008; 10 (6): R93.

36 Zhao J, Wang Y, Lao Z et al. Prognostic immune-related gene models for breast cancer: a pooled analysis. Onco Targets Ther 2017; 10: 4423-4433.

37 Wang S, Xiong Y, Zhang Q et al. Clinical significance and immunogenomic landscape analyses of the immune cell signature based prognostic model for patients with breast cancer. Brief Bioinform 2021; 22 (4).

38 Schirris Y, Voorthuis R, Opdam M et al. ECTIL: Label-efficient Computational Tumour Infiltrating Lymphocyte (TIL) assessment in breast cancer: Multicentre validation in 2,340 patients with breast cancer. 2025. [Epub ahead of print] doi:10.48550/arXiv.2501.14379

39 Martin M, Yoder R, Salgado R et al. Tumor-Infiltrating Lymphocytes Refine Outcomes in Triple-Negative Breast Cancer Treated with Anthracycline-Free Neoadjuvant Chemotherapy. Clin Cancer Res 2024; 30 (10): 2160-2169.

40 Copson ER, Maishman TC, Tapper WJ et al. Germline *BRCA* mutation and outcome in young-onset breast cancer (POSH): a prospective cohort study. Lancet Oncol 2018; 19 (2): 169-180.

41 van der Meer DJ, Kramer I, van Maaren MC et al. Comprehensive trends in incidence, treatment, survival and mortality of first primary invasive breast cancer stratified by age, stage and receptor subtype in the Netherlands between 1989 and 2017. Int J Cancer 2021; 148 (9): 2289-2303.

42 Dent R, Trudeau M, Pritchard KI et al. Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res 2007; 13 (15 Pt 1): 4429-4434.

43 Lin NU, Claus E, Sohl J et al. Sites of distant recurrence and clinical outcomes in patients with metastatic triple-negative breast cancer: high incidence of central nervous system metastases. Cancer 2008; 113 (10): 2638-2645.

44 Park WK, Chung SY, Jung YJ et al. Long-term oncologic outcomes of unselected triple-negative breast cancer patients according to *BRCA1/2* mutations. NPJ Precis Oncol 2024; 8 (1): 96.

45 Caswell-Jin JL, Sun LP, Munoz D et al. Analysis of Breast Cancer Mortality in the US-1975 to 2019. JAMA 2024; 331 (3): 233-241.

46 Maskarinec G, Sen C, Koga K et al. Ethnic differences in breast cancer survival: status and determinants. Womens Health (Lond) 2011; 7 (6): 677-687.
47 Jiang YZ, Ma D, Suo C et al. Genomic and Transcriptomic Landscape of Triple-Negative Breast Cancers: Subtypes and Treatment Strategies. Cancer Cell 2019; 35 (3): 428-440 e425.
48 Hughes RA, Heron J, Sterne JAC et al. Accounting for missing data in statistical analyses: multiple imputation is not always the answer. Int J Epidemiol 2019; 48 (4): 1294-1304.
49 Sterne JA, White IR, Carlin JB et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. BMJ 2009; 338: b2393.
50 Giardiello D. Prediction of contralateral breast cancer: statistical aspects and prediction performance. Ph.D. Leiden University; 2022.
51 van Buuren S, Groothuis-Oudshoorn K. mice: Multivariate Imputation by Chained Equations in R. Journal of Statistical Software 2011; 45 (3): 1- 67.
52 Early Breast Cancer Trialists' Collaborative G, Peto R, Davies C et al. Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. Lancet 2012; 379 (9814): 432-444.
53 Therneau TM. A Package for Survival Analysis in R. R package version 3.7-0 ed.; 2024.
54 Harrell FE. rms: Regression Modeling Strategies. R package version 6.8-1 ed.; 2024.
55 Baxevanis CN, Fortis SP, Perez SA. The balance between breast cancer and the immune system: Challenges for prognosis and clinical benefit from immunotherapies. Semin Cancer Biol 2021; 72: 76-89.
56 Ali HR, Provenzano E, Dawson SJ et al. Association between CD8+ T-cell infiltration and breast cancer survival in 12,439 patients. Ann Oncol 2014; 25 (8): 1536-1543.
57 Demir L, Yigit S, Ellidokuz H et al. Predictive and prognostic factors in locally advanced breast cancer: effect of intratumoral FOXP3+ Tregs. Clin Exp Metastasis 2013; 30 (8): 1047-1062.
58 Huertas-Caro CA, Ramirez MA, Rey-Vargas L et al. Tumor infiltrating lymphocytes (TILs) are a prognosis biomarker in Colombian patients with triple negative breast cancer. Sci Rep 2023; 13 (1): 21324.
59 Egelston CA, Avalos C, Tu TY et al. Resident memory CD8+ T cells within cancer islands mediate survival in breast cancer patients. JCI Insight 2019; 4 (19).
60 Nederlof I, Horlings HM, Curtis C et al. A High-Dimensional Window into the Micro-Environment of Triple Negative Breast Cancer. Cancers (Basel) 2021; 13 (2).
61 Gruosso T, Gigoux M, Manem VSK et al. Spatially distinct tumor immune microenvironments stratify triple-negative breast cancers. J Clin Invest 2019; 129 (4): 1785-1800.
62 Keren L, Bosse M, Marquez D et al. A Structured Tumor-Immune Microenvironment in Triple Negative Breast Cancer Revealed by Multiplexed Ion Beam Imaging. Cell 2018; 174 (6): 1373-1387 e1319.
63 Loi S. Tumor-infiltrating lymphocytes, breast cancer subtypes and therapeutic efficacy. Oncoimmunology 2013; 2 (7): e24720.

64 Eulenburg C, Schroeder J, Obi N et al. A Comprehensive Multistate Model Analyzing Associations of Various Risk Factors With the Course of Breast Cancer in a Population-Based Cohort of Breast Cancer Cases. Am J Epidemiol 2016; 183 (4): 325-334.
65 Wishart GC, Bajdik CD, Dicks E et al. PREDICT Plus: development and validation of a prognostic model for early breast cancer that includes HER2. Br J Cancer 2012; 107 (5): 800-807.
66 Dieci MV, Mathieu MC, Guarneri V et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in two phase III randomized adjuvant breast cancer trials. Ann Oncol 2015; 26 (8): 1698-1704.
67 Vennin C, Cattaneo CM, Bosch L et al. Taxanes trigger cancer cell killing in vivo by inducing non-canonical T cell cytotoxicity. Cancer Cell 2023; 41 (6): 1170-1185 e1112.
68 Opdam M, van Rossum AGJ, Hoogstraat M et al. Predictive gene expression profile for adjuvant taxane benefit in breast cancer in the MATADOR trial. iScience 2024; 27 (8): 110425.
69 Ramspek CL, Jager KJ, Dekker FW et al. External validation of prognostic models: what, why, how, when and where? Clin Kidney J 2021; 14 (1): 49-58.
70 Muranen TA, Morra A, Khan S et al. PREDICT validity for prognosis of breast cancer patients with pathogenic *BRCA1/2* variants. NPJ Breast Cancer 2023; 9 (1): 37.
71 Sperrin M, Riley RD, Collins GS et al. Targeted validation: validating clinical prediction models in their intended population and setting. Diagn Progn Res 2022; 6 (1): 24.
72 Jenkins DA, Martin GP, Sperrin M et al. Continual updating and monitoring of clinical prediction models: time for dynamic prediction systems? Diagn Progn Res 2021; 5 (1): 1.
73 Jenkins DA, Sperrin M, Martin GP et al. Dynamic models to predict health outcomes: current status and methodological challenges. Diagn Progn Res 2018; 2: 23.
74 van Weert JCM, Alblas MC, van Dijk L et al. Preference for and understanding of graphs presenting health risk information. The role of age, health literacy, numeracy and graph literacy. Patient Educ Couns 2021; 104 (1): 109-117.
75 Joseph-Williams N, Abhyankar P, Boland L et al. What Works in Implementing Patient Decision Aids in Routine Clinical Settings? A Rapid Realist Review and Update from the International Patient Decision Aid Standards Collaboration. Med Decis Making 2021; 41 (7): 907-937.