In order to minimise the risk of recurrence, nearly all patients with hormone receptor positive breast cancer are offered adjuvant endocrine treatment after surgical treatment. Traditionally, this adjuvant endocrine treatment is administered for five years. However, recent studies are providing more and more evidence on extending adjuvant therapy beyond 5 years. These studies indicate that extending adjuvant endocrine therapy by two to three years increases disease-free and overall survival. Following these results, both the ASCO and ESMO guidelines now recommend discussing extended adjuvant endocrine therapy for all patients with hormone receptor positive breast cancer, except for those with very low risk of relapse. Extending adjuvant endocrine therapy has been shown to be a cost effective measure.

Scientific impact
The DATA study showed that the absolute benefit of extending adjuvant endocrine therapy (with two to three additional years of anastrozole) increases with the a-priori risk of recurrence. Currently, we assess the risk of distant recurrence on baseline classical pathologic tumour characteristics. With additional molecular markers that predict for distant disease recurrence, it may be possible to more accurately identify patients that would benefit from extended endocrine therapy. This thesis contributes to the search for additional DNA methylation based prognostic markers for hormone receptor positive breast cancer.

Considerable amounts of evidence have been provided by previous studies into the topic of prognostic DNA methylation markers for breast cancer. However, there was no comprehensive overview of this evidence. In the first chapter of this thesis we do provide this overview of evidence, and though it proved impossible to perform a meta-analysis on the extracted data, we identified DNA methylation markers that merit further investigation based on the existing literature.

The main study presented in this thesis endeavours to identify new markers prognostic for disease recurrence in hormone receptor positive breast cancer by genome wide DNA methylation analysis in a well-defined cohort of patients derived from the DATA study, a large Dutch randomised controlled trial, allocating patients to five or eight years of sequential endocrine therapy. The new markers discovered and validated in this analysis do, however, not outperform the currently available prognostic factors, and thus do not directly improve the identification of patients at increased risk of distant recurrence. However these markers do provide new insights in the role of DNA methylation in breast cancer recurrence. Future research into these discovered DNA methylation markers may identify a functional role of this methylation, which can provide new opportunities for targeted therapy. Moreover, as more follow-up of the included patients becomes available and more events may occur, the prognostic value of these identified methylation markers may still be proven.

In addition to assessing the role of DNA methylation in breast cancer prognosis, this thesis aims to improve the quality of DNA methylation research in general. The number of papers in the field of molecular biomarker research in cancer is far larger than the number of clinically applicable assays. In this thesis we provide insight into the reasons why so few DNA methylation studies in breast cancer lead to clinically applicable results. Moreover, we point out what improvements are required in current DNA methylation research in order to facilitate translation of this research into clinically applicable assays.

With the trans-DATA study we provide an example of a genome wide DNA methylation study performed according to the improvements we have suggested in this thesis. We have accompanied the results of the trans-DATA study with a protocol paper that discusses in further detail our methods, along with the reasoning behind these methods. This thesis can inform future research into prognostic DNA methylation markers, and help improve the quality of future research into this topic.

This thesis has further contributed to DNA methylation research as it has shown in chapter 3 that formalin fixed paraffin embedded tissue can reliably and reproducibly be used for genome wide DNA methylation studies using bead chip assays. Prior to publication of this study, the general consensus was that DNA-methylation analysis using bead chip assays required fresh frozen tissue. The publication of this study, along with two studies by different authors has enabled the use of archival tissue, greatly expanding the number of patients included in prospective studies for whom tumour tissue can be made available.
**Societal impact.**
Better prediction of the risk of recurrence in breast cancer may allow clinicians and patients to make better informed treatment decisions concerning adjuvant treatment of hormone receptor positive disease. Current ASCO and ESMO guidelines advice all hormone receptor positive breast cancer patients to be treated with extended adjuvant endocrine therapy, except for patients that have a very low risk of recurrence. The search for prognostic and predictive molecular markers may in the end help to better estimate the risk of recurrence and may also improve the selection of targeted therapy with the highest reduction of risk of recurrence. This may improve quality of life as highly selected patients may be offered more effective targeted therapies.

This thesis provides a fair and realistic representation of its results, with consideration for clinical utility and clear endpoints. Moreover, the recommendations given in multiple chapters of this thesis can help improve the design and reporting of future translational research into prognostic DNA methylation markers. In addition, lack of consideration for clinical utility may lead to overly optimistic presentation of study results. Unmindful presentation of optimistic results can give rise to unrealistic expectations and thereby does a disservice to the patients for whom the research is intended.

The preceding aspects highlight that this thesis has provided a step forward in unravelling the role of DNA methylation markers in breast cancer, and has helped improve the quality of DNA methylation biomarker research as a whole.

**REFERENCES**

1. Dutch Cancer Registration, NKR (in Dutch).
12. Janssens C. Hopen op een wonder. NED TIJDSSCHR GENEESKD. 2021;165(B1876).