**Universidade de Lisboa**

**Faculdade de Medicina**

# Adjuvant endocrine therapy for the treatment of early breast cancer: real world effectiveness, tolerability and adherence

Arlindo Júlio Rebelo da Silva Ferreira

Tese Orientada por:
Professor Doutor Luís António Marques da Costa

Tese especialmente elaborada para obtenção do grau de Doutor em Medicina, especialidade de Oncologia

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# 1. Summary

## 1.1 English language summary

Adjuvant endocrine therapy leads to substantial gains in breast cancer survival outcomes. The real-world use, effectiveness, tolerability and adherence to recent innovations in the field of adjuvant endocrine therapy for breast cancer is not well characterized. To tackle these concerns and hence help patient-physician decision making and future clinical research we developed a series of projects aiming to: 1) describe the implementation in real-world practice of recent innovations in the field of adjuvant endocrine therapy for breast cancer and summarize its effectiveness, 2) quantify adjuvant endocrine therapy impact on patients' quality of life and 3) quantify patients adherence and persistence to adjuvant endocrine therapy. To do this, we used different cohort studies and applied standard and novel statistical methods.

Using two retrospective cohorts from Southern Portugal Cancer Registry, one of ~1300 postmenopausal women and the other of ~1700 premenopausal women, we identified that both aromatase inhibitors (AI) and ovarian function suppression (OFS) were successfully introduced in clinical practice after landmark publication in 2005 and 2014, respectively. In the postmenopausal cohort, 41% of patients received an AI (16% as monotherapy, 25% as sequential therapy) and 59% tamoxifen with differences by center. After a median follow-up of 6.3 years, AI use was associated with a better overall survival (OS) when compared with tamoxifen (adjusted-HR 0.55, 95% CI 0.37-0.81). Using a complementary retrospective US cohort of ~800 postmenopausal women with lobular tumors, similar findings were registered and no heterogeneity in efficacy was recorded by histology, in specific comparing pure lobular carcinomas to mixed ductal and lobular carcinomas. In the premenopausal cohort, 17% of patients were treated with OFS with a substantial increase of its use from 2014 onward (16% vs. 25% after 2014), particularly for the combination with AI (0.4% vs. 8% after 2014). After a median follow-up of ~3 years, patients treated with OFS had a better OS than those not treated with OFS (adjusted-HR 0.44, 95% CI 0.19-0.96). Using a sub-cohort of ~4300 breast cancer patients with available patient reported outcomes from CANTO, a nationwide French prospective cohort, we described a substantial impact of treatment on QoL 2 years after diagnosis. Using the EORTC C30 summary score, a composite score of several functions and symptoms, endocrine therapy but not chemotherapy had a persistent impact on QoL 2 years after diagnosis with differences by specific domains. In addition, we uncovered a differential effect of treatment by menopausal status: in premenopausal patients CT seems to be the predominant driver of QoL domains deterioration, whereas in postmenopausal patients it was ET the predominant driver of QoL deterioration. Finally, using a second sub-cohort of ~1200 patients

from CANTO that were taking adjuvant tamoxifen and with serum assessment of tamoxifen, we identified that 1 in every 6 women (16%) were non-adherent, i.e. had serum tamoxifen levels were below the adherence threshold. This proportion was higher than the self-reported rate of non-adherence (12.3%). After a median follow-up time of 2 years from tamoxifen serum assessment, biochemically defined non-adherent patients had a shorter DDFS (adjusted-HR of 2.31, 95% CI 1.05-5.06).

This work detailed the kinetics of introduction in clinical practice of recent adjuvant endocrine treatment innovations. In addition, it provides real-world evidence of the effectiveness of adjuvant AIs and OFS. Nevertheless, it suggests that for a substantial number of patients endocrine therapy leads to a persistent negative impact on QoL, especially in postmenopausal women, and to an alarming proportion of non-adherence to treatment, to a certain extent related to tolerability issues.

**Keywords:** early breast cancer; adjuvant treatments; endocrine therapy; treatment effectiveness; quality of life; treatment adherence.

## 1.2 Portuguese language summary

O tratamento hormonal adjuvante de doentes com carcinoma da mama melhora a sobrevivência global. O uso em contexto de mundo real, efetividade, tolerabilidade e adesão a inovações recentes da terapia hormonal adjuvante de cancro da mama não está bem caracterizado. Para abordar estes pontos e assim apoiar a tomada de decisão de doentes-médicos e a investigação clínica futura desenvolvemos uma série de projetos com o propósito de: 1) descrever a implementação na prática clínica de mundo real de inovações recentes no campo da terapia hormonal adjuvante de cancro da mama e sumarizar a sua efetividade, 2) quantificar o impacto da terapia hormonal adjuvante na qualidade de vida das doentes e 3) quantificar a adesão e persistência das doentes à terapia hormonal adjuvante. Para concluir estas tarefas utilizámos diferentes estudos de coorte e aplicámos métodos padrão e inovadores de análise de dados.

Fazendo recurso de duas coortes retrospetivas derivadas do Registo Oncológico Regional do Sul, a primeira com ~1300 mulheres pós-menopáusicas e a segunda com ~1700 mulheres pré-menopáusicas, identificámos que quer os inibidores da aromatase (IA) quer a supressão da função ovárica (SFO) foram introduzidos com sucesso na prática clínica após publicações científicas de referência em 2005 e 2014, respetivamente. Na coorte pós-menopáusica, 41% das doentes receberam um IA (16% em monoterapia e 25% em sequência) e 59% tamoxifeno com diferenças por centro. Após um acompanhamento mediano de 6.3 anos, os IA associaram-se a melhor sobrevivência global (SG) quando comparados com tamoxifeno (HR-ajustado 0.5, IC 95% 0.37-0.81). Fazendo recurso complementar de uma coorte retrospetiva estado-unidense de ~800 mulheres pós-menopáusicas com tumores lobulares, registámos resultados semelhantes e não foi identificada heterogeneidade de eficácia por tipo histológico, em específico quando comparando carcinomas lobulares puros a carcinomas ductais e lobulares mistos. Na coorte pré-menopáusica, 17% das doentes foram tratadas com SFO com um crescimento substancial do uso de 2014 em diante (16% vs. 25% após 2014), particularmente para a combinação com IA (0.4% vs. 8% após 2014). Após um acompanhamento mediano de ~3 anos, doentes tratadas com SFO tiveram melhor SG que doentes não tratadas com SFO (HR-ajustado 0.44, IC 95% 0.19-0.96). Fazendo recurso de uma sub-coorte de ~4300 doentes com cancro da mama com resultados reportados por doente (*patient reported outcomes*) disponíveis do estudo CANTO, uma coorte prospetiva francesa, descrevemos um impacto substancial do tratamento na qualidade de vida (QdV) 2 anos após o diagnóstico. Usando o C30 *summary score* da EORTC, um resultado compósito de várias funções e sintomas, a terapia hormonal, mas não a quimioterapia, teve um impacto persistente na QdV 2 anos após o diagnóstico com diferenças nos domínios impactados. Adicionalmente, expusemos um efeito diferencial do tratamento por estado menopausico: em doentes pré-menopáusicas a

quimioterapia parece ser o promotor principal da deterioração de domínios de QdV, enquanto que em doentes pós-menopáusicas foi a terapia hormonal o promotor principal da deterioração da QdV. Finalmente, fazendo recurso de uma segunda sub-coorte de ~1200 derivada do estudo CANTO e de doentes que estavam a tomar tamoxifeno e com avaliação sérica do tamoxifeno, identificámos que 1 em cada 6 mulheres (16%) eram não-aderentes ao tratamento, i.e. tinham os valores séricos de tamoxifeno abaixo da linha de corte de adesão. Esta proporção foi maior que aquela auto-reportada (12.3%). Após um acompanhamento mediano de 2 anos após a avaliação do tamoxifeno sérico, doentes não aderentes quando avaliadas por via bioquímica tiveram uma sobrevivência livre de recidiva à distância mais curta (HR-ajustado de 2.31, IC 95% 1.05-5.06).

Este trabalho detalhou a cinética de introdução na prática clínica de inovações recentes na terapia hormonal adjuvante. Adicionalmente, revelou evidência de mundo real da efetividade de IA e SFO adjuvantes. Estes dados sugeriram porém que para uma proporção substancial de doentes a terapia hormonal leva a um impacto persistente e negativo na QdV, especialmente em mulheres pós-menopáusicas, tal como a uma proporção alarmante de não-adesão ao tratamento, até certo ponto relacionada com questões de tolerabilidade.

**Palavras-chave**: cancro de mama precoce; tratamentos adjuvantes; hormonoterapia; efetividade terapêutica; qualidade de vida; adesão ao tratamento.

## 1.3 Publication list

### 1.3.1 Original publications generated

1. **Ferreira AR**, Palha A, Correia L, et al. Treatment adoption and relative effectiveness of aromatase inhibitors compared to tamoxifen in early breast cancer: A multi-institutional observational study. *Breast* 2018. doi:10.1016/j.breast.2017.11.003

2. **Ferreira AR**, Ribeiro J, Miranda A, et al. Effectiveness of Adjuvant Ovarian Function Suppression in Premenopausal Women With Early Breast Cancer: A Multicenter Cohort Study. *Clin Breast Cancer* 2019. doi:10.1016/j.clbc.2019.06.003

3. *(co-first author)* Metzger-Filho O, **Ferreira AR**, Jeselsohn R, et al. Mixed Invasive Ductal and Lobular Carcinoma of the Breast: Prognosis and the Importance of Histologic Grade. *Oncologist* 2019. doi:10.1634/theoncologist.2018-0363

4. **Ferreira AR**, Di-Meglio A, Pistilli B, et al. Differential impact of endocrine therapy and chemotherapy on quality of life of breast cancer survivors: a prospective patient reported outcomes analysis. *Annals of Oncology* 2019. doi:10.1093/annonc/mdz298

5. Pistilli B, Paci A, **Ferreira AR**, et al. Detection of non-adherence to adjuvant tamoxifen using drug serum assessment and breast cancer recurrence risk. Journal of Clinical Oncology 2020. doi: 10.1200/JCO.19.01758

## 1.4 Abbreviation list

**AE** – adverse events
**AI** – aromatase inhibitor
**AJCC** – American joint committee on cancer
**ASR** – age-standardized rates
**ATLAS** – Adjuvant Tamoxifen: Longer Against Shorter
**ATAC** – Arimidex, Tamoxifen, Alone or in Combination
**ATTOM** – Adjuvant Tamoxifen–To Offer More?
**BCSS** – breast cancer specific survival
**BCS** – breast conserving surgery
**BCFI** – Breast cancer free interval
**BIG** – breast international group
**BMI** – body mass index
**BSA** – body surface area
**CANTO cohort** – CANcer Toxicities cohort
**CI** – confidence interval
**CPH** – Cox proportional hazards
**CRF** – clinical report form
**CRN** – clinical research nurse
**CT** – chemotherapy
**CTCAE** – common terminology criteria for adverse events
**DDFS** – Distant disease-free survival
**DFS** – Disease-free survival
**DFCI** – Dana Farber Cancer Institute
**EBCTCG** – early breast cancer trialists' collaborative group
**ECOG** – eastern cooperative oncology group
**EORTC QLQ C30** – European Organisation for the Research and Treatment of Cancer quality of life questionnaire C30
**EORTC QLQ BR23** – European Organisation for the Research and Treatment of Cancer quality of life questionnaire breast module 23
**ER** – estrogen receptor
**EU** – European Union
**ET** – endocrine therapy
**FACT-B TOI** – Functional Assessment of Cancer Therapy – Breast Trial Outcome Index
**FACT-ES** – Functional Assessment of Cancer Therapy – Endocrine Subscale
**HER2** – human epidermal growth factor 2
**HR** – hazard ratio
**HRe** – hormone receptor
**HRPROs** – health related patient reported outcomes
**iDFS** – invasive disease-free survival
**IHC** – immunohistochemistry
**IPTW** – inverse probability treatment weighting
**MeSH** – medical subject heading
**n** – number
**NA** – not applicable
**NCCN** – national comprehensive cancer network
**OFS** – ovarian function suppression
**OR** – odds ratio
**OS** – overall survival
**PRO** – patient reported outcome
**PS** – propensity score
**QoL** – quality of life
**RFS** – relapse-free survival
**ROR-Sul** – cancer registry of southern Portugal
**RR** – rate ratio
**SE** – standard error
**SERM** – selective estrogen receptor modulator
**SERD** – selective estrogen receptor degrader
**SOFT** – suppression of ovarian function trial
**TEXT** – tamoxifen and exemestane trial
**UNICANCER** – National French Cancer Centers Cooperative Group

# 2. Introduction

## 2.1 Epidemiology of breast cancer

Female breast cancer is the most frequently diagnosed cancer in women in the world with approximately 2.1 million diagnosis in 2018.[1] Globally approximately every 1 in 8 women from those reaching the age of 85 are affected by breast cancer.[1] In the European Union (EU), breast cancer is also the main cause of female cancer and cancer related death with an estimated absolute number of diagnosis, incidence and mortality of 364 450 cases, 108.8 cases per 100 000 women/year (age-standardized rate, ASR) and 22.4 cases per 100 000 women/year (ASR), respectively.[2] In Portugal, slightly lower rates are recorded with an estimated absolute number of diagnosis, incidence and mortality of 6 900 cases, 85.6 cases per 100 000 women/year (ASR) and 18.4 cases per 100 000 women/year (ASR), respectively.[2] In addition, of the estimated 90 580 deaths due to breast cancer recorded annually in the EU, 1 570 occur in Portugal.[2]

Of all cases of breast cancer, between 90 and 95% are diagnosed in stage I-III and are thus eligible for curative treatments.[3] The wide implementation of screening contributed to the identification of tumors in earlier stages.[4] In addition, the adoption of cumulatively better adjuvant treatments contributed to the improvement of cancer outcomes in patients with early breast cancer.[4,5] Currently, the estimated 5-years overall survival for female BC in Europe is of approximately 81%, while it is 83.4% (95% CI 82.5 - 84.3) in Portugal.[6,7]

## 2.2 Subtypes of breast cancer and implications to treatment

Breast cancer is a heterogeneous disease for which a group of biomarkers are routinely assessed as tools to inform about disease prognosis and sensitivity to treatment.[8] As a matter of fact, breast cancer was the first type of cancer in which routine biological markers started to be used in routine clinical practice to define therapy. Cancer biomarkers are molecules that can either be produced by the tumor cells or other cells of the body as a response to the tumor and can be genes, gene products, molecules, enzymes, hormones or specific cells. These markers can be detected in blood, secretions (urine, sputum, sweat or others) or tissues and can be used for cancer screening, as prognostic factors for outcomes but also as predictive factors of response to therapies.[9] Two of such biomarkers are the estrogen and progesterone receptors (collectively referred to as the hormone receptors [HRe]). More recently, the human epidermal growth factor receptor 2 (HER2) was also described as a useful molecular marker in breast cancer. The prognostic and predictive power of these biomarkers to identify patients responding to endocrine therapy (those with HRe-positive tumors) and HER2-directed therapy (those with HER2-positive tumors) led to the systematic characterization of the estrogen, progesterone and the HER2 receptors in routine

clinical practice.[8,10,11] The combination of such receptors defines four immunohistochemistry (IHC)-defined subtypes of breast cancer with clinical implications: HRe+/HER2-, HR+/HER2+, HRe-/HER2+ and HRe-/HER2- (triple negative). Subsequent studies dissecting the underpinnings of the breast cancer biology identified genomically-defined intrinsic subtypes of breast cancer that include the luminal types A and B, the HER2 amplified/overexpressing and the basal-like subtypes.[12] These genomically-defined subgroups are derived from the evaluation of the gene expression profile of tumors and partially overlap with the IHC-defined subgroups of breast cancer, especially when also assessing the cell proliferation marker ki-67 and histologic grade: luminal A tumors tend to overlap with HRe+ (high ER and PR)/HER2- with low ki-67 (and low grade) tumors, luminal B tumors tend to overlap with HRe+ (lower ER and/or PR)/HER2- with high ki-67 (and high grade) tumors or HRe+/HER2+ tumors, HER2 amplified/overexpressing with HRe-/HER2+ tumors and the basal-like with HRe-/HER2- tumors.[13,14] While the intrinsic subtypes of breast cancer add to our biological understanding of breast cancer, such classification defined by gene expression profile does not encapsulate all the complexity of the biology of breast cancer. To that end, other classifications were also developed, remarkably the integrative clusters looking to the genome and transcriptome in the overall landscape of breast tumors, and the Lehman and Burstein classifications in the subgroup of HRe-/HER2- (triple negative) breast tumors.[15–17] Both the pan-breast cancer classifications of the intrinsic subtypes of breast cancer and the integrative clusters of breast cancer have clear prognostic implications[15,18], but the predictive value to define the most appropriate therapy in the clinics is still mostly defined by the tissue evaluation of the HRe and the HER2 receptor.[19]

Up to 80% of all breast carcinomas present ≥ 1% of cells positive for the estrogen and/or progesterone receptors thus being considered HRe-positive.[20–22] For these, especially those with HRe present ≥ 10% of cells, it is well established the clinical utility of the use of hormone-related therapies/endocrine therapies.[10,21]

### 2.3 The evolving field of adjuvant endocrine therapy

Endocrine therapy was the first targeted therapy used for the treatment of breast cancer. The landscape of hormone-related therapies evolved substantially over time, from pioneer approaches more than 1 century ago by Beatson and colleagues of surgical ovarian ablation (oophorectomy)[23] to more contemporaneous medical approaches using ovarian suppression (e.g. goserelin), selective estrogen receptor modulators (SERMs; e.g. tamoxifen), aromatase inhibitors (AI; e.g. letrozole) and selective estrogen receptor degraders (SERDs; e.g. fulvestrant). Dr. Beatson was a surgeon studying breast milk formation. During his research he observed that the removal of ovaries would halt breast milk production in rabbits. This fact pointed towards the possibility of the

ovaries having control over the secretion of a complete separate organ. Beatson ended up transporting this principle to breast cancer treatment without even knowing about the substance who caused it: estrogen.[24] From those days until now, knowledge on endocrine therapy for breast cancer multiplied and currently the clinical use and sequence of different classes of endocrine treatments differs by menopausal status, underlying intention of care (curative or palliative) and in patients with early breast cancer it even differs by the perceived risk of recurrence.[25]

Tamoxifen is a selective estrogen receptor modulator that acts mainly as an estrogen receptor antagonist at the tumor levels inducing growth arrest and apoptosis.[26] Nevertheless, in other tissues tamoxifen acts as an ER receptor agonist and in postmenopausal women it can prevent postmenopausal osteoporosis and have an impact in blood cholesterol.[27] Tamoxifen was the mainstay of adjuvant endocrine therapy in breast cancer for several decades and is nowadays the treatment of choice for premenopausal women with low or average risk tumors and for some postmenopausal women with low risk tumors, intolerant or with formal contra-indication to AI.[28–30] The AIs are a class of drugs that inhibit the aromatase, an enzyme responsible for the conversion of androgens into estrogens (mostly at peripheral sites as the fatty tissue).[31] While in premenopausal women the ovaries are the primary source of estrogens, in postmenopausal women, peripheral conversion of androgens is the main mechanism of estrogen production; hence, the use of AI effectively reduces estrogen levels in postmenopausal women or pre/perimenopausal women with concomitant treatment with ovarian function suppression. At present, AIs are a standard treatment for postmenopausal women or premenopausal women at high risk of recurrence, in this later case if provided in combination with ovarian suppression/ablation.[29,30]

In the postmenopausal setting, the establishment of AIs as a standard treatment was a long and winding road. While the two largest and most mature trials showed consistent results in terms of DFS, the overall survival results were inconsistent between trials and over time.[32,33] In the breast international group (BIG) 1-98 trial, 8010 patients were randomized in a 1:1:1:1 fashion to 5 years of letrozole or tamoxifen alone or their sequence. While at a median follow-up of 8.1 years there was an improvement both in terms of DFS (inverse probability treatment weighting [IPTW] HR 0.82, 95% CI 0.74 – 0.92) and OS (IPTW HR 0.79, 95% CI 0.69 – 0.90) favoring letrozole, in the most recent analysis after a median follow-up of 12.6 years only a non-significant trend was registered in favor of letrozole both for DFS (HR 0.91, 95% CI 0.81 – 1.01) and OS (HR 0.89, 95% CI 0.77 – 1.02).[34,35] In the arimidex, tamoxifen, alone or in combination (ATAC) trial 6241 women were randomized in a 1:1 fashion to 5 years of letrozole or tamoxifen. In both the 8.3 years follow-up analysis and the 10 years follow-up analysis, letrozole showed an improved DFS (10 years, HR 0.91, 95% CI 0.83 – 0.99) but not an improved OS (10 years, HR 0.87, 9% CI 0.74 – 1.02).[36,37] These observations led some

clinicians to challenge the clinical superiority of adjuvant AIs when compared to tamoxifen.[38] An EBCTCG patient-level meta-analysis summarized results of BIG 1-98, ATAC and other studies showing for the comparison 5 years of letrozole vs. 5 years of tamoxifen an improved DFS favoring letrozole in years 0-1 (RR 0.64, 95% CI 0.52 – 0.78) and 2-4 (RR 0.80, 95% CI 0.68 – 0.93) but not afterwards.[39] A modest but measurable OS improvement at 10 years was also recorded (RR 0.89, 95% CI 0.8 – 0.97; death from any cause of 24.0% vs. 21.3% for tamoxifen and AIs, respectively - absolute difference of 2.7%, 95% CI 0.1 – 4.7). The same EBCTCG metanalysis also summarized the impact on clinical outcomes of switch strategies (2-3 years of tamoxifen followed by an AI for a total duration of therapy of 5 years) when compared to both tamoxifen and an AI when given for 5 years. In both cases, i.e. for the comparison of a switch strategy vs. tamoxifen or an AI, DFS is improved in favor of the arm providing an AI while treatments differ, but not thereafter. As for OS, the switch strategy also seems to improve survival when compared to 5 years of tamoxifen (RR 0.82, 95% CI 0.73 – 0.91), but does not differ when compared to 5 years of letrozol (RR 0.96, 95% CI 0.86 – 1.07). Clinical practice guidelines reconcile these observations by identifying groups of patients at higher risk of recurrence or with perceived higher sensitivity to AIs for preferred treatment with AIs, as patients with stage II/III disease, with tumors with histologic grade 3, high ki67, lobular histology and with HRe+/HER2+ receptors.[14] The real-world effectiveness of these treatments is however poorly understood.

Over the last years we have observed an escalation of adjuvant endocrine treatment options in the early disease setting, either by the extension of treatment duration or by its intensification. Seminal studies of adjuvant tamoxifen used somehow arbitrary treatment periods of 1 or 2 years. Backed by basic research findings[40] and the clinical observation that more than half of the recurrences of hormone receptor-positive tumors occur after 5 years of diagnosis[41], subsequent research steps aimed at testing the role of longer treatment durations and consistently showed that incrementally higher duration of treatment from 1 to 2, to 5 and more recently to 10 years was of additional benefit both in terms of disease-free survival and overall survival. Current clinical practice guidelines recommend the use of extended tamoxifen (or AI in postmenopausal women) for patients with baseline increased risk of disease relapse, as defined by node positive disease (stage III disease or node positive stage II disease).[14,29] It is however important to keep in mind that the added benefit of treatment beyond 7 to 8 years might be very modest. The absolute and relative merits of extending the duration of the treatment with tamoxifen were summarized by an EBCTCG meta-analysis and the recent “Adjuvant Tamoxifen: Longer Against Shorter” (ATLAS) and “Adjuvant Tamoxifen–To Offer More?” (ATTOM) trials.[10,42–44] In summary, compared to no tamoxifen, 5 years of adjuvant tamoxifen, reduced by half the risk of recurrence in the years 0-4

and by a third from years 5-9 with a very small effect thereafter; regarding overall survival, 5 years of tamoxifen decreased the risk of death in the first 15 years by 30% which translated into an absolute reduction in the risk of death from 33.1% to 23.9% (absolute difference of 9.2%).[10] As for continuing treatment beyond year 5, the contemporaneous read of the available evidence favors its use.[29,45] While a meta-analysis of non-individual patient data concluded that in an unselected population of hormone receptor positive tumors there is not a disease-free or overall survival benefit, the group of node-positive patients emerged as deriving a DFS benefit from extended tamoxifen (HR 0.76, 95% CI 0.63 – 0.92)[46]. In addition, a hint emerged from the observation that while in years 5 to 9 no DFS difference is seen, at longer follow-up in years 10 and beyond, a DFS difference emerges (HR 0.80, 95 CI 0.73 – 0.88). Although this meta-analysis included 5 trials and more than 21 500 patients, the results were challenged by the fact that it included trials with considerably different follow-up times (most trials had less than 10 years of follow-up), by the fact that the estrogen receptor status was unavailable in some patients and by the fact that it constituted a non-individual patient data meta-analysis. With these points in mind, the two largest trials included in the aforementioned meta-analysis (the ATLAS trial with close to 13 000 patients and the ATTOM trial with about 7000 patients) showed both an early DFS advantage and a deferred breast cancer specific and overall survival advantage (after 10 years of treatment): in the ATLAS trial, the HR for breast cancer specific survival was 0.97 (SE 0.10) in years 5 to 9, 0.70 (SE 0.10) in years 10 to 14 and 0.79 (SE 0.27) after 15 or more years after, all favoring 10 years of adjuvant tamoxifen; consistent results were found for overall survival.[43,47]

Similarly to tamoxifen, recent clinical research efforts are pushing for a similar path of validating the role of extended treatment with AI in postmenopausal women. Several combinations of treatment extension were tested, some of which including a starting period of tamoxifen ranging from 3 to 5 years. Despite the fact that no study found an overall survival improvement with extended AI in postmenopausal women, current clinical practice guidelines recommend the use of extended AI (or tamoxifen) for postmenopausal patients at higher risk of recurrence, as defined by node positive disease (stage III disease or node positive stage II disease).[14,29] As in other problematics of adjuvant endocrine therapy, the EBCTCG also performed an individual patient data meta-analysis of 12 clinical trials (including close to 25 000 patients) aiming at dissecting the role of extended adjuvant AIs.[48] In patients that received 5 years of adjuvant tamoxifen (n=7500), an extra 5 years of an AI improved any recurrence (RR 0.67, 95% CI 0.57 – 0.79) and distant recurrence (RR 0.77, 95% CI 0.63 – 0.93), but not breast cancer mortality (RR 0.77, 95% CI 0.59 – 1.00). As for patients receiving in the first 5 years of hormone treatment an adjuvant AI, an extra 5 years of an AI (n=4 800) also improved any recurrence (RR 0.76, 95% CI 0.61 – 0.95) and distant recurrence (RR

0.78, 95% CI 0.59 – 1.04), but not breast cancer mortality (RR 0.99, 95% CI 0.68 – 1.44). Similar findings were recorded for patients receiving 5 to 10 years of adjuvant tamoxifen followed by an AI (n=12 600). The absolute difference seems to be more clinically relevant as the nodal burden increases: the 5-years absolute recurrence improvements vary from 1.1% (95% CI 0.1 – 2.0) in patients with no nodal involvement, to 3.8% (95% CI 2.2 – 5.4) in patients with 1 to 3 involved nodes and to 7.7% (95% CI 3.9 – 11.6) in patients with 4 or more affected nodes. In addition, the types of recurrence were not affected similarly, with contralateral tumors being the most reduced (RR 0.61, 95% CI 0.47 – 0.78), followed by isolated local recurrences (RR 0.74, 95% CI 0.57 – 0.96) and distant recurrences (RR 0.84, 95% CI 0.72 – 0.97).

In premenopausal women, the intensification of treatment with the use of ovarian function suppression/ablation has also emerged as a competing strategy to escalate adjuvant endocrine therapy. Such strategy comes from the observation that premenopausal women with hormone receptor positive tumors that have permanent chemotherapy-induce amenorrhea/premature ovarian failure after adjuvant chemotherapy seem to have a better prognosis compared to those with menstrual resumption after adjuvant chemotherapy.[49,50] Contemporaneous clinical practice guidelines recommend the use of OFS in premenopausal women with intermediate/high risk ER-positive breast cancer.[14,29] A 2007 meta-analysis by Cuzick and colleagues showed that, in patients with HRe-positive breast cancer, despite the small number of patients included (n=338) and the direction of effect favoring OFS, use of OFS in monotherapy compared to no systemic treatment did not improve relapse risk (HR 0.72, 95% CI 0.49 – 1.04) nor death after recurrence (HR 0.82, 95% CI 0.47 – 1.43). Conversely, the addition of OFS to chemotherapy (with or without tamoxifen, n=2741) improved both recurrence (HR 0.88, 95% CI 0.77 – 0.99) and death after recurrence (HR 0.85, 95% CI 0.73 – 0.99). Furthermore, a significant interaction with age (using the 40 years cut-off; p=0.046) was shown: in women aged 40 or younger, OFS reduced both recurrence and death after recurrence but not after 40. This is in line with the hypothesis that ovarian function is a relevant driver of the risk of recurrence in patients with HRe-positive tumors as the risk of chemotherapy-induced amenorrhea rises considerably after the age of 40. Recent studies further added evidence to the filed. In the suppression of ovarian function trial (SOFT), 3066 premenopausal women were randomized to 5 years of tamoxifen, tamoxifen plus OFS or exemestane plus OFS.[51,52] The randomization was stratified by receipt of chemotherapy and the primary analysis compared tamoxifen to tamoxifen plus OFS. At a median follow-up of 8 years, tamoxifen plus OFS compared to tamoxifen monotherapy showed an improved DFS (HR 0.76, 95% CI 0.62 – 0.93) and OS (HR 0.67, 95% CI 0.48 – 0.92). On the other hand, exemestane plus OFS when compared to tamoxifen monotherapy showed improved DFS (HR 0.65, 95% CI 0.53 – 0.81) but not

OS (non-significant trend; HR 0.85, 95% CI 0.62 – 1.15). A consistent effect but with larger absolute magnitude was recorded for these comparisons in the subgroup of patients receiving adjuvant chemotherapy. In the SOFT trial patients could be enrolled up to 8 months after completion of (neo)adjuvant chemotherapy to allow for proper menopausal status definition. In a similar study, the ASTRRA trial, that randomized 1282 premenopausal women to 5 years of tamoxifen or 5 years of tamoxifen plus 2 years of OFS, patients could be enrolled up to 2 years after completion of (neo)adjuvant chemotherapy.[53] Of the 1483 women screened, 1282 had menses/ovarian function resumption, and after a median follow-up of 63 months, patients receiving tamoxifen plus OFS when compared to tamoxifen monotherapy had an improved DFS (HR 0.69, 95% CI 0.48 – 0.97) and OS (HR 0.31, 95% CI 0.10 – 0.94). Taken together both SOFT and ASTRRA studies favor the use of OFS in premenopausal women that remained premenopausal after receipt of adjuvant chemotherapy. Of note, the use of OFS renders premenopausal women functionally postmenopausal thus allowing for the use of AIs. As in two of the arms of the SOFT study, the "tamoxifen and exemestane trial" (TEXT) randomized premenopausal women to OFS plus tamoxifen or OFS plus exemestane. The resemblances between the SOFT and TEXT trials allowed for a combined analysis that further refined our understanding about the most appropriate combination endocrine therapy to OFS, i.e. tamoxifen or AI.[52,54] In this analysis, 4690 patients were randomized in a 1:1 fashion to OFS plus tamoxifen or OFS plus exemestane. After a median follow-up of 9 years, OFS plus exemestane improved both DFS (HR 0.77, 95% CI 0.67 – 0.90) and distant DFS (DDFS; HR 0.80, 95% CI 0.66 – 0.96), but not OS (HR 0.98, 95% CI 0.79 – 1.22). Based on these results and at this point in time, the preferred combination therapy of OFS is still unclear.

Extending or intensifying endocrine therapy improves cancer outcomes, but it also comes at a cost of added toxicity. This toxicity might further have downstream effects as decreasing the real-world adherence to treatment and in the process compromise treatment effectiveness. The real-world use, effectiveness, tolerability and adherence to these treatments is poorly characterized.

## 2.4 Cancer survivorship in the intersection with safety, tolerability and quality of life impact of endocrine therapy

Major improvements in early diagnosis, treatment and supportive care lead to a growing community of cancer survivors.[55] While relevant during all the continuum of treatment, for this group of patients, tolerability issues and how the treatment trajectory impacts QoL is an issue of utmost importance. In the US, more than 3.5 million women live with an history of diagnosis of invasive breast cancer.[56] In Europe, such number is estimated to be in the range of 2 million women. The field of survivorships deals with a large scope of topics, namely the surveillance for recurrence

and screening for second cancers, long-term and late side effects of cancer treatment, health behaviors (e.g. diet, weight management, physical exercise, as well as smoking and alcohol consumption) and promotion of psychosocial wellbeing (depression, anxiety, return-to-work and other financial issues).[55] Other topics pertaining to special populations include fertility issues and premature ageing in younger patients and a comprehensive geriatric assessment in the elderly. In the specific case of long-term or late side effects, these refer to adverse events persisting from treatment introduction to a date beyond treatment discontinuation or starting after treatment discontinuation, respectively. Some of the late side effects can have a long-lasting effect too. Examples of long-term effects include fatigue, chronic pain, sexual dysfunction, cognitive dysfunction, chemotherapy induced neuropathy or ovarian dysfunction. Examples of late side effects include osteoporosis (with corresponding risk of fractures), but also hematological malignancies and myelodysplastic syndromes.

While it is unquestionable that endocrine treatments present a favorable risk-benefit that support their regulatory approval and extensive clinical use, specific agents are known for specific side effects. Tamoxifen is associated with gynecological symptoms, thromboembolic events, cerebrovascular events and, in postmenopausal women, endometrial cancer.[57,58] Conversely, AI are associated with more vaginal dryness, joint symptoms, bone fractures and cardiovascular events.[57,58] The intensification of treatment with OFS also increases the risk for osteoporosis, musculoskeletal symptoms, vaginal dryness, hypertension and glucose problems.[52] Likewise, extending treatment duration further increases the risk of known deleterious adverse events, but without new safety signals.[59]

Beyond health professional reported outcomes, endocrine therapy seems also to impact health related quality of life (HRQOL) as measured by patient reported outcomes (PRO). The European Medicines Agency (EMA) defines PROs as “any outcome evaluated directly by the patient himself or herself.”[60] EMA further details that “it can be measured by self-report, generally in the form of a questionnaire, or by interview, provided that the interviewer records only the patient’s response.” PROs are measured using various tools available both for routine clinical practice and for clinical research. These person-centered instruments measure symptoms, functional status, treatment adherence or satisfaction with care, but the two most common uses in cancer research include the assessment of patients’ symptoms and HRQOL. The available instruments used to assess PROs in patients with breast cancer were critically reviewed elsewhere.[61] Some of the most used instruments include the European Organisation for Research and Treatment of Cancer (EORTC) instruments Quality of Life Questionnaire (QLQ)-C30 (designed for all types of cancer) with or without the complementing QLQ Breast Module 23 (QLQ BR23; currently being updated to the QLQ

BR45[62]) and the Functional Assessment of Chronic Illness Therapy (FACIT) Functional Assessment of Cancer Therapy (FACT)-B and FACT-Endocrine symptoms (FACT-ES). An emerging instrument for symptoms assessment is the US National Institutes of Health (NCI) PRO-CTCAE (Patient Reported Outcomes – Common Terminology Criteria for Adverse Events) inspired in the CTCAE instruments used for graduation of adverse event in clinical trials.[63]

In early breast cancer, the mind-body study was a prospective observational cohort study of 186 women that were receiving or not adjuvant endocrine therapy and for whom PROs are available.[64] Compared to patients not receiving endocrine therapy, the group receiving AI had more severe musculoskeletal issues, hot flashes and cognitive problems, while those receiving tamoxifen (also compared to no endocrine therapy) had more hot flashes, cognitive problems and bladder issues. In this setting, in the previously discussed ATAC trial of adjuvant tamoxifen, anastrazol or their sequence in postmenopausal women, 1021 patients were enrolled for the QoL substudy and thus had QoL metrics available as assessed by the Functional Assessment of Cancer Therapy – Breast Trial Outcome Index (FACT-B TOI) and FACT-ES (Functional Assessment of Cancer Therapy – Endocrine Subscale) scales. Despite the identification of small differences in the side effect profile, no overall significant differences in QoL were recorded between treatment arms.[65] Likewise, treatment intensification with OFS seems to impact side effects profile but QoL. In the previously discussed SOFT trial of 2045 premenopausal women randomized to tamoxifen with or without OFS, QoL PRO data as assessed by the International Breast Cancer Study Group QoL Core Form and a trial-specific module was available for 1722 patients.[66] Patients receiving combination endocrine therapy had more hot flashes at 6 and 24 months, loss of sexual interest and sleep issues at 6 months and vaginal dryness up to 60 months. However, changes in global QoL metrics were not substantial and did not differ between treatments. As adjuvant OFS is reserved for high risk patients, especially if younger than 35 and treated with adjuvant chemotherapy, the SOFT and TEXT trial investigators also looked at the QoL in the subgroup of women 35 years or younger. Vasomotor symptoms were the most prominent symptom, however, loss of sexual interest and difficulties in becoming aroused were also recorded and considered to be clinically meaningful. Even so, the symptomatic impact was similar to that identified in older premenopausal women and similar rates of early endocrine therapy discontinuation were documented (approaching to 1 in every 5 patients).

Despite the different impact in terms of side effects profile, QoL seems to be scarcely impacted in clinical trials of different strategies of adjuvant endocrine therapy. However, how different types of endocrine therapy and other systemic treatments modulate breast cancer survivors QoL in the real-world setting is still poorly characterized. The effect of different

treatments in QoL has several downstream implications namely by modulating the adherence to long-term adjuvant treatments which can impact cancer relapse and survival.

## 2.5 Adherence to oral adjuvant endocrine therapy

Oral adjuvant endocrine therapies were designed to be administered for periods of time spanning years. Moreover, such periods are being extended with most guidelines recommending between 5 to 10 years of treatment and considering 10 years in growing groups of patients.[45,67] In cardiovascular medicine, non-adherence to oral therapies for primary or secondary prevention of undesired health outcomes is in the range of 30 to 50%.[68] Likewise, non-adherence to breast cancer adjuvant endocrine therapies ranges from 20% at up to 2 years and to around 50% at 5 years.[69,70] These observations merit substantial clinical attention, given the impact of non-adherence on deleterious cancer outcomes (as recurrence and death), health care resources consumption/spending, the perception of drug activity/dose adjustments and patient-physician relationship.[71] Despite these objective estimates, it is important to consider that the setting in which adherence is measured (as e.g. in clinical trials or real-world practice) as well as the instruments used to measure adherence (as e.g. self-report, pills count, electronic monitoring systems or serum/urine assessment) might further add variability to the quantification of adherence.[72]

The identification of patients at higher risk for non-adherence, i.e. the identification of sociodemographic, behavioral and clinical features predicting for non-adherence, is an active field of research. Classic predictors of non-adherence include patient (as social support and family stability, health beliefs, previous adherence history and mental health problems), treatment (as regimen complexity, tolerability, duration and cost) and disease characteristics, but more complex models aiming to capture behavioral dimensions have also been developed.[72] These later models include e.g. the patients' perception of the risks posed by the disease and the efficacy/tolerability of treatment, as well as the individual set of beliefs concerning how her/his future is impacted by her/his own behaviors vs. by chance, but also the relationship between the patients and her/his health care providers.[72]

The real-world incidence of non-adherence to adjuvant endocrine therapy is scarcely characterized. In addition, it is unclear if more objective methods of assessment, e.g. serum assessment, will reveal consistent estimates of non-adherence when compared with more common methods of assessment as questionnaires.

## 2.6 Outcomes research as a tool to summarize real-world evidence

While well conducted clinical trials are the most robust tool to ensure precision and establish causality (thus overcoming bias and confounding) in studies comparing the efficacy of clinical interventions, a growing body of evidence reveals that the group of patients recruited for clinical trials might not represent the overall group of patients observed in routine clinical practice, both due to stringent selection criteria, small number of recruited patients (less than 5% of patients participate in clinical trials) and due to overconcentration of clinical trials in specific academic centers.[73] Overall, underrepresented groups in cancer clinical trials include e.g. elderly patients, patients with relevant comorbidities, ethnical minorities, pregnant women, patients living in remote areas and those with specific disease presentations, as brain metastases. Underrepresenting certain groups of patients harms the external validity of the study findings and thus the generalizability of the conclusions. Derived from this perception, some have proposed that outcomes as measured in clinical trials are at most a surrogate of the actual outcome captured in real-world practice/true outcome.[74] Another challenge applicable to randomized clinical trials comes from the increasing regulation applicable to interventional research and the limited resources available to fund large clinical studies with human participants, reasons that further limit the number of clinical questions that can be addressed using randomized clinical trials.[75]

To increase the number of patients included in clinical studies and with it the external validity of clinical research, but also to extend the scope of questions that can be addressed in a timely manner, a growing number of researchers are complementing clinical trials research with observational studies using real-world data.[73] Real-world data, i.e. observational data collected from routine clinical practice (e.g. through electronic medical records, registries and billing data) or directly from patients (e.g. through wearables and health applications) and not from clinical trials, when properly curated, allows the generation of real-world evidence.[76] As the overall group of patients seen in routine clinical practice compose the real-world data, real-world evidence maximizes the external validity of study findings. Conversely, while external validity is maximized, other methodological issues ensue namely the risk for confounding and bias. To deal with the methodological challenges of such research the field of real-world research matured to apply the methodological tools of epidemiology and in the process reach sound conclusions. This dynamic field of clinical research is commonly referred to as outcomes research. Outcomes research deals with the study of the end results, i.e. the outcomes, of different clinical interventions. In the United States national library of medicine medical subject heading (MeSH), outcomes research is considered a synonym of “outcome assessment (health care)” and defined as “research aimed at assessing the quality and effectiveness of health care as measured by the attainment of a specified

end result or outcome. Measures include parameters such as improved health, lowered morbidity or mortality, and improvement of abnormal states (such as elevated blood pressure)."[77] The outcomes that are measured can be collected both through the lens of health-professionals (e.g. breast cancer distant recurrence or common terminology criteria for adverse events [CTCAE]-defined diarrhea) or through the lens of patients (patient reported outcomes [PRO]). While health-professional and patient reported outcomes should report a similar perception of a clinical phenomenon, it is becoming clearer that these two lens to characterize clinical phenomena tell complementary stories thus making the case for the systematic collection of PROs in clinical research studies.[78]

One of the aims of epidemiology is to estimate unbiased causal associations. To attain that, several methodological optimizations were implemented to prevent, reduce and quantify bias and confounding.[79] While bias derives from inadequate study design and/or conduct, confounding reflects the rich interrelationships between factors and outcomes. Several design and analytic techniques are available to handle bias and confounding. The most effective tools to reduce or eliminate bias come from appropriate selection of patients, randomization and from several types of blinding of the research stakeholders (e.g. participants, researchers and statisticians). These strategies are widely implemented in interventional research/randomized clinical trials. Given the non-interventional nature of outcomes research, strategies to overcome bias come from thoughtful study design and careful patient selection. While impossible to exclude, bias needs to be proactively handled and be taken into consideration when interpreting results. Design strategies to reduce confounding include individual or group matching, while analytic strategies include stratification and adjustment. In the body of work presented here we took advantage of thoughtful study design and patient selection, we further adjusted analyses using standard statistical methods, including multivariable modelling. In cases where expected unmeasured patients' characteristics could affect both the decision to treat and the outcome we used propensity score risk adjustment (matching or inverse probability treatment weighting).

Throughout the present body of work, we made use of real-world evidence derived from national and international data sources to answer several clinical questions around treatment effectiveness, tolerability of interventions, among others. As outcomes, we used both health-professional reported outcomes and patient-reported outcomes, the former to measure efficacy and the later to assess tolerability and quality of life. While not a substitute for randomized clinical trials, our body of work using real-world data reveals relevant pieces of information that complement interventional research in the field of early breast cancer.

# 3. Objectives and thesis overview

The real-world use, efficacy, tolerability and adherence to adjuvant endocrine therapy is scarcely characterized. In this thesis we aimed to quantify such dimensions using quantitative research methods. Bellow we detail the specific objectives of this work.

**Specific objective 1 – Patterns of care of adjuvant endocrine therapy**

a) To detail patterns of use of endocrine therapy in the adjuvant treatment of pre and post-menopausal women with breast cancer.
    - Using data from ROS-Sul this specific objective contributed to generate manuscripts 1 (Ferreira et al, Breast 2018) and 3 (Ferreira et al, Clin Breast Cancer 2019).

**Specific objective 2 – Effectiveness of adjuvant endocrine therapy**

a) In post-menopausal women with HRe+ breast cancer, to assess the relative effectiveness of adjuvant AI vs. tamoxifen using a multi-institutional cohort;
    - To assess how histology and histologic differentiation modulates response to AI and tamoxifen;
        - Using data from *ROR-Sul* and DFCI through NCCN this specific objective contributed to generate manuscripts 1 (Ferreira et al, Breast 2018) and 2 (Metzger and Ferreira et al, Oncologist 2019).

b) In pre-menopausal women with HRe+ breast cancer, to assess the effectiveness of ovarian function suppression using a multi-institutional cohort;
    - Quantify OFS effectiveness in the pts <35 years of age and/or treated with adjuvant CT;
        - Using data from *ROR-Sul* this specific objective contributed to generate manuscript 3 (Ferreira et al, Clin Breast Cancer 2019).

**Specific objective 3 – Tolerability of adjuvant ET**

a) In women with HR+ BC receiving adjuvant ET, to describe safety and tolerability and its impact in QoL using a prospective multi-institutional cohort (CANTO).
    - Using data from CANTO this specific objective contributed to generate manuscript 4 (Ferreira et al, Annals of Oncology 2019).

**Specific objective 4 – Adherence to adjuvant ET**

a) In women with HR+ BC receiving adjuvant ET, to describe safety and tolerability and its impact in QoL using a prospective multi-institutional cohort (CANTO).
    - Using data from CANTO this specific objective contributed to generate manuscript 5 (Pistilli et al, JCO 2020).

# 4. Methods

Here we briefly detail the data sources used to complete the set of projects developed in this thesis. Specific methods are further detailed under each project.

To complete the set of projects in this thesis we used data from 3 independent data sources:

- *Registo Oncológico Regional do Sul* (Cancer Registry of Southern Portugal [ROR-Sul], currently included in *Registo Oncológico Nacional* [National Cancer Registry]). ROR-Sul was a population-based cancer registry collecting data from patients diagnosed and/or treated for invasive carcinomas in southern Portugal or Madeira island;
- Dana-Farber Cancer Institute institutional data obtained through the US national comprehensive cancer network (NCCN) database (currently breast cancer outcomes research database [BC-CORD]);
- CANTO cohort (NCT01993498), a nation-wide, multicenter, French, prospective, longitudinal study of breast cancer survivors [reviewed by Vaz-Luis et al in reference[80]].

# 5. Results

This current body of work generated 5 publications. These publications will be presented in the sub-sections ahead. In all studies Arlindo R. Ferreira participated in all steps of the project, from study design, data analysis, results interpretation and manuscript writing.

## 5.1 Treatment adoption and relative effectiveness of aromatase inhibitors compared to tamoxifen in early breast cancer: a multi-institutional observational study.

### 5.1.1 Introductory notes

This project details the introduction in clinical practice of adjuvant aromatase inhibitors for the adjuvant treatment of postmenopausal women with hormone receptor positive breast cancer and summarizes the real-world effectiveness of such intervention compared to tamoxifen. This project was published in The Breast. Arlindo R. Ferreira led the study design, data analysis, results interpretation and manuscript writing.

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### 5.1.3 Abstract

Background: Since 2005, aromatase inhibitors (AIs) have been the adjuvant treatment of choice for postmenopausal women with early breast cancer (BC). In this study we characterize the adoption of AIs in Portugal, variables associated with treatment administration, and compare its effectiveness (either in monotherapy or sequential therapy) to tamoxifen monotherapy (TAM).

Patients and methods: This was a retrospective cohort study that included postmenopausal women with stage I-III hormone receptor (HR) positive BC diagnosed from 2006-2008 and treated with adjuvant endocrine therapy in four participating institutions.

Results: Of the 1283 eligible patients, 527 (41%) received an AI (16% as monotherapy, 25% as sequential therapy) and 756 (59%) TAM. Patients treated with AI had less differentiated tumors, with higher TNM stage, and were more frequently HER2-positive. Use of AI also differed by center (use range from 33%-75%, p<0.001). With a median follow-up of 6.3 years and controlling for clinicopathological and treatment characteristics, treatment with AI had a better overall survival (OS) when compared with TAM (adjusted-HR 0.55, 95% CI 0.37-0.81).

Conclusion: AIs were successfully introduced as adjuvant treatment for HR-positive BC in Portuguese hospitals. Its use was influenced by tumor and patient characteristics, but also center of care. In this large cohort, AI use was associated with an OS benefit.

Keywords: early breast cancer, aromatase inhibitors, tamoxifen, treatment effectiveness.

### 5.1.4 Introduction

In developed countries, the majority of breast cancers (>80%) are diagnosed in early stages, and can be treated with curative intent.[81] Of these, more than 2/3 are hormone receptor-positive[82], for whom the prognosis is substantially improved by adjuvant endocrine therapy (ET). As compared to no endocrine therapy, adjuvant ET is associated with a reduction in the rates of disease recurrence of approximately 50%, and this translates into a reduction in breast cancer mortality of more than 1/3 in the first 15 years after diagnosis.[10] Since 2005, international guidelines have supported several adjuvant ET regimens for postmenopausal patients, including tamoxifen (TAM), aromatase inhibitors (AI) or a sequence of these agents.[83–85] Nevertheless, several clinical trials showed an advantage of regimens including an AI, an effect recently summarized in a large meta-analysis of the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) that estimated a lower 10-year breast cancer mortality in the AI *vs.* TAM group (RR = 0.85, 95% CI 0.75–0.96).[39] Therefore, given the absolute benefit of strategies with AIs, there is an overall consensus that the treatment

of high risk patients, such as those with nodal involvement, high grade or high Ki-67, should include an AI.[84]

Even so, the choice between different ETs also entails the choice of different safety, tolerability/adherence and cost profiles. While TAM is associated with an increased risk of thromboembolic events and endometrial cancer, AIs are associated with an increase in the risk of osteoporosis and bone fractures, as well as arthralgias and other musculoskeletal complaints.[58] Out-of-pocket and health system cost differences also exist (for example, in the United States, patients receiving AIs were more likely to experience financial hardship than those taking TAM only[86]).

Recently, a multi-institutional group of Portuguese centers, both public and private, started to collect granular information on clinicopathological features, patterns of care and clinical outcomes of their patients with breast cancer using a regional cancer registry platform.[87] In this study we characterize how real world providers introduced different ET strategies after 2005 (date of first consensus advocating the use of AI-based strategies for postmenopausal women[85]) and explore the comparative effectiveness of these interventions.

### 5.1.5 Patients and Methods

Study design and data source

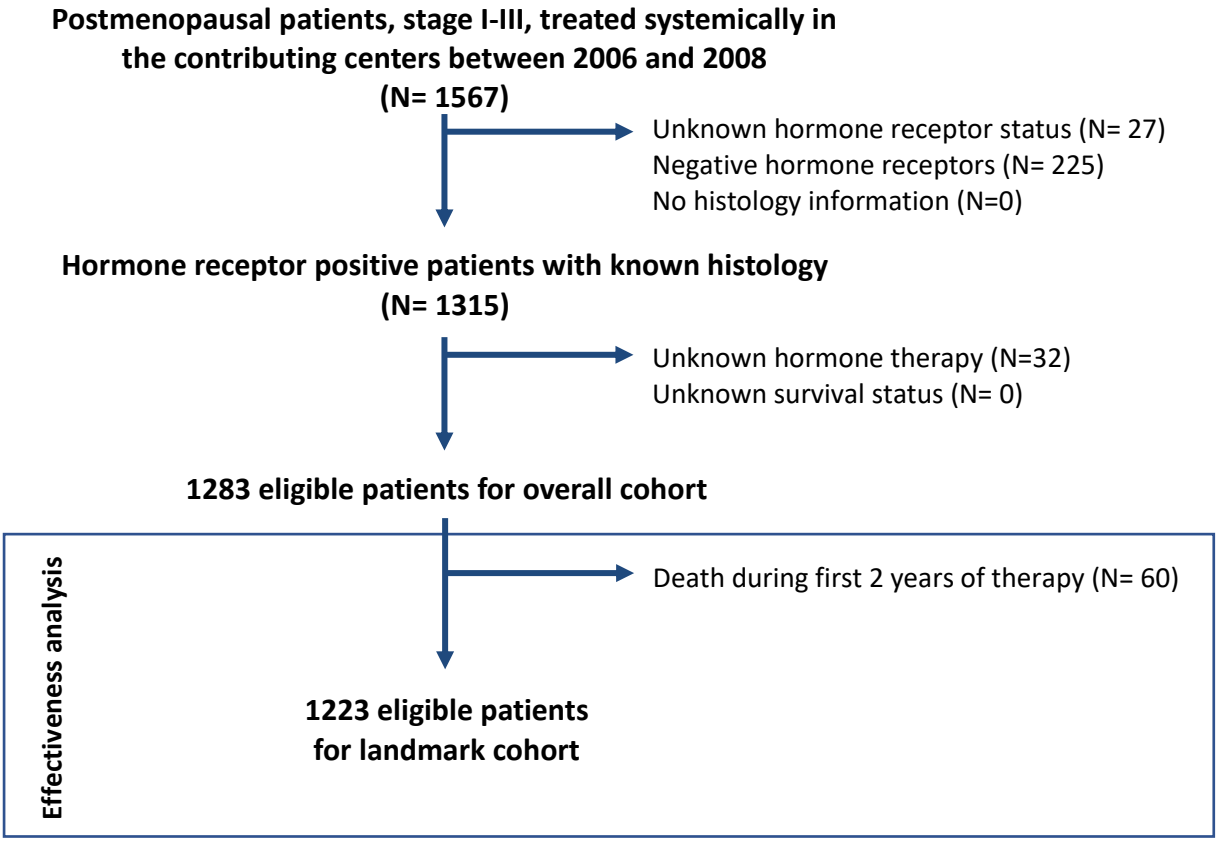
This is a retrospective cohort study. Data from four hospitals in the Lisbon area, Portugal, were retrieved from *Registo Oncológico Regional do Sul* (ROR-S; Southern Regional Oncology Registry). ROR-S is a population-based cancer registry. Data audits focused on 10% of cases were performed and variables had a higher than 95% concordance rate. Due to the observational nature of the study, treatments and follow-up were performed at patient-physician description. ROR-S institutional review board approved study protocol. Description of data collection and procedures were previously reported.[87] We followed the STROBE statement in reports of cohort studies.

Cohort definition

We selected all consecutive postmenopausal primary breast cancer patients with stage I-III disease, tumors expressing estrogen/progesterone receptor (≥1%) and diagnosed and treated systemically (i.e., treatments beyond local therapy as surgery or radiotherapy) at *Centro Hospitalar de Lisboa Norte, Hospitais CUF Lisboa, Hospital da Luz* or *Instituto Português de Oncologia Francisco Gentil de Lisboa* between 2006 and 2008. Follow-up details (treatment, new tumors and vital status) were available up to December 2013. We excluded patients who did not have surgery and patients with other concurrent primary tumors. A cohort of 1283 patients was identified (M1 Figure 1). Two

groups were further defined as a function of type of ET received: Group A) 756 (58.9%) patients treated with TAM monotherapy and Group B) 527 (41.1%) patients treated either with AI monotherapy or sequential TAM-AI/AI-TAM. In addition to this cohort of 1283 patients (overall cohort), a landmark cohort and propensity score matching cohorts were built specifically for the effectiveness analyses as a strategy to address confounding, and the details about their set-up are elaborated in the statistical analysis section.

**M1 Figure 1** – Study diagram.




Variables definition

*Outcomes*

Primary outcome was overall survival (OS). OS was defined as time from diagnosis to death of any cause. Follow-up was available until up to December 2013.

Menopausal status

Post-menopausal was defined as older than 52 at date of diagnosis. Previous studies of unselected Portuguese women showed that the median age of menopause for the Portuguese population is 48 years (interquartile range [IQR] 44 - 52) [88]. Given the treatment with (neo)adjuvant chemotherapy in approximately 50% of cases in the present cohort, we estimated a slightly lower

median age for menopause, thus selecting the upper IQR estimate for the definition of the cut-off for the age of menopause.

*Covariates*

Covariates included age, clinicopathological characteristics (UICC/AJCC TNM staging, histology, grade, and human epidermal growth factor receptor 2 [HER2] status); treatment characteristics (systemic therapy), center of care and year of diagnosis.

Statistical analysis

Baseline clinicopathological characteristics and treatment received were tabulated, and differences between groups tested using chi-square test or t-test, as appropriate. To examine treatment characteristics (type of ET used and the duration of therapy) we used descriptive statistics. Multivariate logistic regression was used to examine associations with AI prescription. Survival plots were built using Kaplan–Meier method. Effectiveness analysis between groups was completed using multivariate Cox proportional hazards models. To overcome immortal/guaranteed-time bias[89] in patients receiving ET switch, the effectiveness analysis was performed as a landmark analysis at 2 years, so that patients dying before that period were removed (close to median time to treatment switch in case of sequential therapy). The landmark analysis was used as primary analysis because the planed ET strategy was not available at baseline, and treatment group assignment was based on the actual prescription. Therefore, patients planning for a switch from tamoxifen to an aromatase inhibitor, but dying before the planned time for switch, would invariably be assigned to tamoxifen only cohort, thus disproportionally enriching one of those cohorts of patients with worse survival outcomes (immortal/guaranteed-time bias). As a sensitivity analysis, we repeated the analyses in the full cohort, which included all the patients removed from the landmark, to avoid the opposite bias. Since absolute benefit of AI is higher among high-risk patients, we also tested the interaction between type of ET and TNM stage. Finally, given the differences in demographic and clinicopathological features of the groups, and to further address confounding we performed a propensity score matching (with a 1:1 matching) to assess the effectiveness of tamoxifen when compared to AI exposure (n= 1019), AI monotherapy (n= 762) or AI sequencing (n=878). All patients with missing data in relevant variables were excluded from multivariate analysis. All analyses met proportional hazards assumption as assessed by the Schoenfeld residuals. Missing information was considered missing completely at random. The analyses were performed using Stata 12.3 (StataCorp LP). For propensity score matching, Stata ado-file psmatch2 was used.[90]

### 5.1.6 Results

Study sample and baseline characteristics

The overall study sample was composed of 1283 postmenopausal women with hormone receptor positive early breast cancer. Of those, 756 (58.9%) were treated with TAM monotherapy, while 527 (41.1%) were treated with an AI at some point in time (205 as monotherapy and 322 as sequential therapy). Baseline demographic and clinicopathological characteristics are shown in M1 Table 1.

**M1 Table 1** – Baseline demographic, clinicopathologic and treatment characteristics

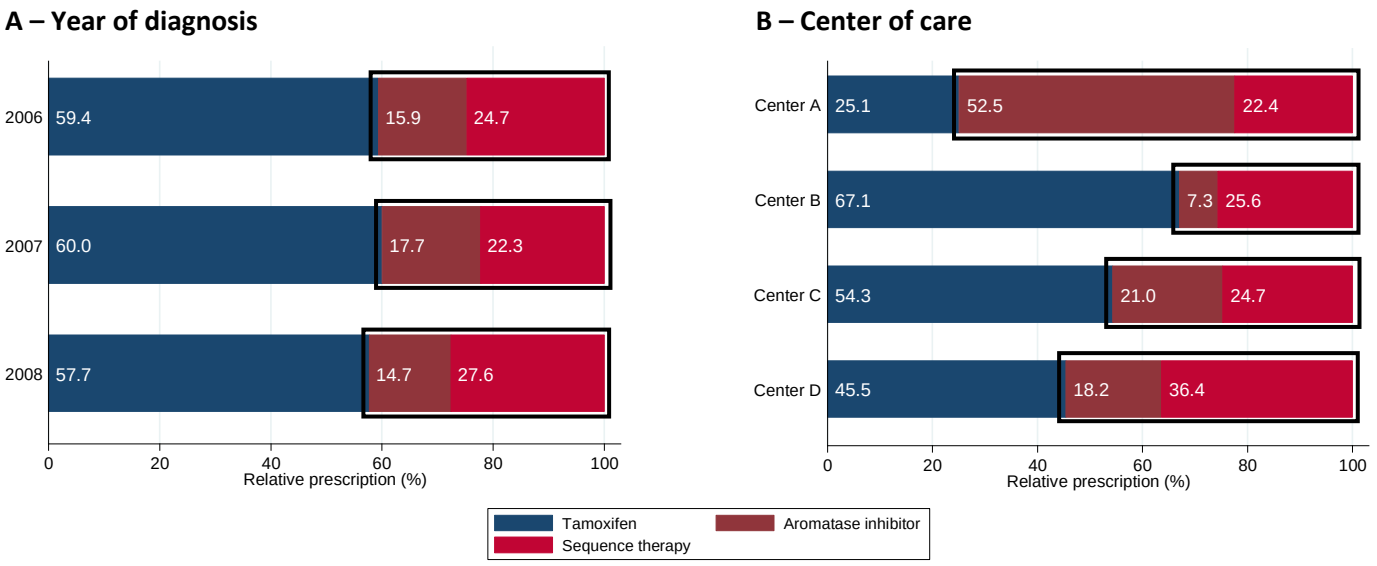
| | **Tamoxifen** | **Exposure to aromatase inhibitor** | | **Exposure to aromatase inhibitor** |
|---|---|---|---|---|
| | | **Sequential therapy** | **Aromatase inhibitor only** | |
| Number of patients (%) | 756 (58.92) | 322 (25.10) | 205 (15.98) | 527 (41.08) |
| OS follow-up (months) | | | | |
| Median | 75.62 | 78.48 | 72.46 | 75.77 |
| P25 – P75 | (65.39 – 85.25) | (70.85 – 87.61) | (62.16 – 84.23) | (67.15 – 86.98) |
| Age at diagnosis (mo.) | | | | |
| Median | 66.11 | 63.28 | 65.74 | 64.31 |
| P25 – P75 | (58.30 – 75.66) | (56.46 – 71.53) | (58.69 – 74.58) | (57.29 – 72.29) |
| Histologic type, % | | | | |
| IDC | 619 (81.88) | 273 (84.78) | 170 (82.93) | 443 (84.06) |
| ILC | 40 (5.29) | 14 (4.35) | 20 (9.76) | 34 (6.45) |
| Other | 97 (12.83) | 35 (10.87) | 15 (7.32) | 50 (9.49) |
| Simplified staging, % | | | | |
| Stage I | 413 (57.12) | 76 (24.68) | 42 (23.73) | 118 (24.33) |
| Stage II | 240 (33.20) | 160 (51.95) | 86 (48.59) | 246 (50.72) |
| Stage III | 70 (9.68) | 72 (23.38) | 49 (27.68) | 121 (24.95) |
| Unknown | 33 (4.37) | 14 (4.35) | 28 (13.66) | 42 (7.97) |
| Histologic grade, % | | | | |
| Grade I | 173 (25.26) | 43 (13.45) | 18 (10.78) | 61 (13.90) |
| Grade II | 448 (65.40) | 181 (56.21) | 124 (74.25) | 305 (69.48) |
| Grade III | 64 (9.34) | 48 (14.91) | 25 (14.97) | 73 (16.63) |
| NA/Unknown | 71 (9.39) | 50 (15.53) | 38 (18.54) | 88 (16.70) |
| Hormone receptors, % | | | | |
| Both ER and PgR positive | 577 (78.50) | 141 (70.85) | 221 (71.06) | 362 (70.98) |
| HER2 status, % | | | | |
| Positive | 62 (8.68) | 40 (21.16) | 39 (12.83) | 79 (16.02) |
| Negative | 652 (91.32) | 149 (78.84) | 265 (87.17) | 414 (78.56) |
| Unknown | 42 (5.56) | 16 (7.80) | 18 (5.59) | 34 (6.45) |
| (Neo)adjuvant CT, % | | | | |
| Yes | 222 (29.40) | 241 (74.84) | 133 (65.20) | 374 (71.10) |
| No | 533 (70.60) | 81 (25.16) | 71 (34.80) | 152 (28.90) |
| Unknown | 1 (0.13) | 0 (0) | 1 (0.49) | 1 (0.19) |

Patterns of endocrine therapy use

Patients receiving TAM were older (median 66 years, IQR 58 – 76 vs. 64 years, IQR 57 – 72 for AI treated), had lower stage disease (e.g., 57.1% had stage I disease vs. 24.3% for AI treated), had more differentiated tumors (e.g., 25.3% had histologic grade I disease vs. 13.9% for AI treated) and were less frequently HER2 positive (8.7% vs. 16.0% for AI treated) when compared with those treated with AI (all p<0.01). Furthermore, patients treated with TAM were less frequently treated with (neo)adjuvant chemotherapy (29.4% vs. 71.1% for AI treated; p<0.001).

In the overall cohort, more patients were treated with TAM (M1 Figure 2 – A). Use of TAM varied according to disease stage and center of care (M1 Table 1 and M1 Figure 2 – B). For example, 77.8% of patients with stage I disease received TAM monotherapy, while only 36.6% of patients with stage III did. Remarkably, treatment pattern differed markedly between centers, with some centers providing TAM to the majority of patients (67% in center B), while others providing TAM to a smaller proportion of patients (25% in center A; p<0.001).

**M1 Figure 2** – Relative use of tamoxifen, aromatase inhibitors or their sequence per year of diagnosis (A), and center of care (B).



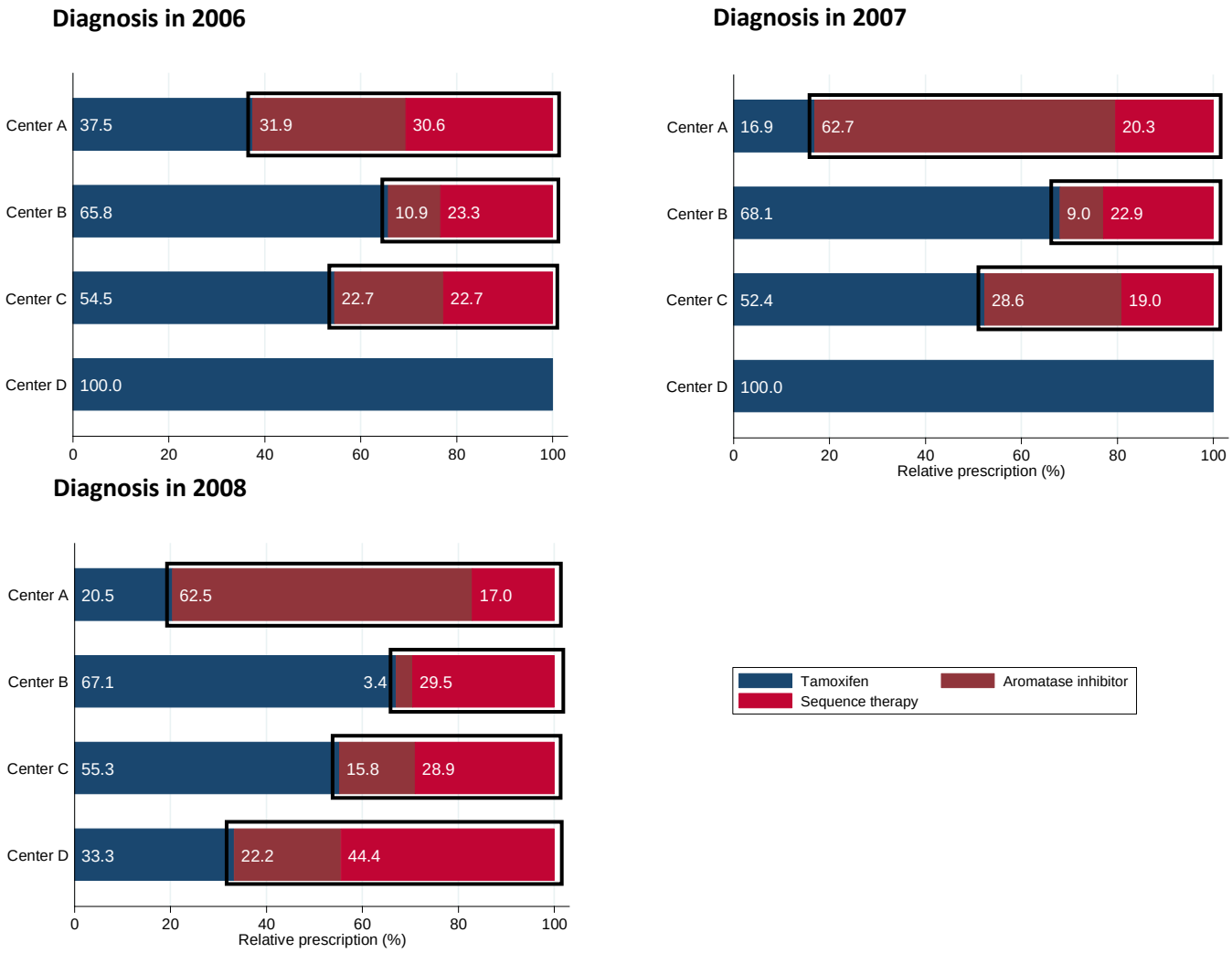

In the multivariable model, variables independently associated with prescription of AI-based strategies included younger age at diagnosis, higher TNM stage, less differentiated tumors, HER2 positivity and care at center A (M1 Supplementary Table A.1).

**M1 Supplementary Table A.1** – Multivariate model of features associated with the prescription of aromatase inhibitors based strategies. OR – Odds ratio.

| | Odds ratio | 95% Confidence Interval | p-value |
|---|---|---|---|
| **Age of diagnosis (years)** | | | |
| Between 50 and <60 | (reference) | (reference) | <0.001 |
| More or equal to 60 | 0.54 | 0.40 – 0.73 | |
| **TNM Stage** | | | |
| Stage I | (reference) | (reference) | <0.001 |
| Stage II | 3.71 | 2.72 – 5.04 | |
| Stage III | 5.82 | 3.79 – 8.95 | |
| **Histologic grade** | | | |
| Grade 1 | (reference) | (reference) | 0.013 |
| Grade 2 | 1.43 | 0.97 – 2.08 | |
| Grade 3 | 1.90 | 1.14 – 3.19 | |
| **HER2 positive** | 1.82 | 1.17 – 2.82 | 0.008 |
| **Center** | | | |
| Center A | (reference) | (reference) | <0.001 |
| Center B | 0.14 | 0.09 – 0.21 | |
| Center C | 0.20 | 0.10 – 0.39 | |
| Center D | 0.21 | 0.05 – 0.82 | |
| **Year of diagnosis** | | | |
| 2006 | (reference) | (reference) | 0.242 |
| 2007 | 0.85 | 0.59 – 1.23 | |
| 2008 | 1.18 | 0.84 – 1.66 | |

Among those exposed to AIs, the proportion of patients treated with monotherapy or sequential therapy differed between centers and year of diagnosis (M1 Supplementary Figure A.1). For example, the proportion of sequential therapy in center A decreased from 2006 to 2008 (sequential therapy of 49% in 2006, 24.5% in 2007 and 21.4% in 2008), while in center B sequential strategies were always preferred across the study period (sequential therapy of 68.1% in 2006, 71.8% in 2007 and 89.7% in 2008).

**M1 Supplementary Figure A.1 –** Relative use of tamoxifen, aromatase inhibitors or their sequence according to disease stage and year of diagnosis.




A detailed characterization of ET strategies and duration is shown in M1 Table 2. When analyzing the group of patients with available date of initiation and completion of ET as a monotherapy (33% for patients treated with TAM and 38% for those treated with an AI), the median time on either agent was close to 5 years, despite the IQR extending from as low as 38 months to as high as 61 months. Among patients treated with sequential therapy the majority were started with upfront TAM (95%). Median time on first agent TAM was 32 months (IQR from 20 to 44 months), while median time on second agent AI was 25 months (IQR from 13 to 33 months) completing close to 5 years of adjuvant ET. The reverse sequence (AI → TAM) was infrequent and, in this case, up-front AI was given for a shorter period than TAM (16 months; IQR 2 to 16 months) compared to a longer period of subsequent TAM (50 months; IQR from 43 to 61 months).

**M1 Table 2** – Duration of therapy.

| | | Tamoxifen | Exposure to aromatase inhibitor |
|---|---|---|---|
| **Monotherapy** | Time on treatment | | |
| | Median, months | 55.61 | 58.87 |
| | P25 – P75 | (37.61 – 59.87) | (41.80 – 61.41) |
| | Date of completion available, n (%) | 247 (32.67) | 78 (38.05) |
| **Sequential therapy** | Option as first agent, n (%) | 290 (95.39) | 14 (4.61) |
| | Time on first agent | | |
| | Median, months | 32.26 | 15.74 |
| | P25 – P75 | (19.84 – 44.00) | (2.00 – 16.33) |
| | Date of completion available, n (%) | 211 (72.76) | 7 (50) |
| | Time on second agent | | |
| | Median, months | 24.56 | 49.64 |
| | P25 – P75 | (13.25 – 32.56) | (43.47 – 61.18) |
| | Date of completion available, n (%) | 127 (43.79) | 5 (35.71) |

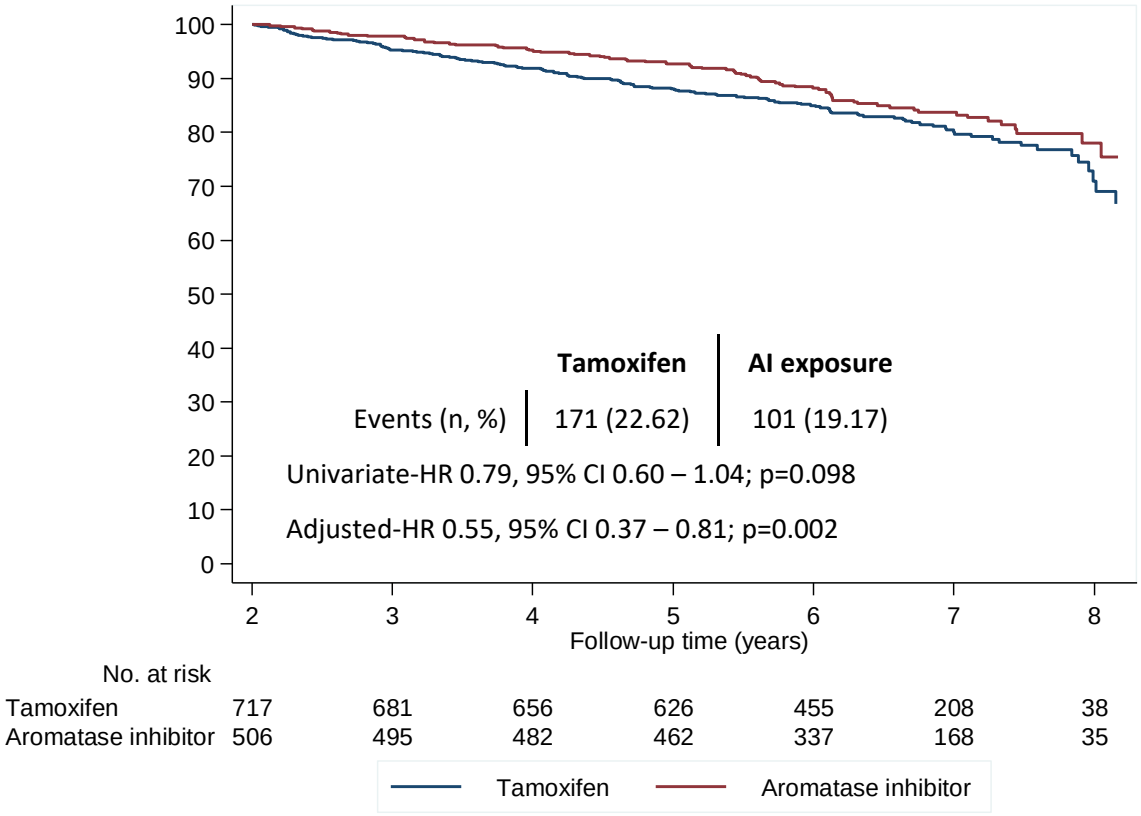
Relative effectiveness

Median follow-up for the entire population (overall cohort, n= 1283) was 6.3 years (IQR 5.5 – 7.2). Date of study enrollment and follow-up time was equal for both patients treated with TAM and exposed to an AI (p=0.705 and p=0.282, respectively).

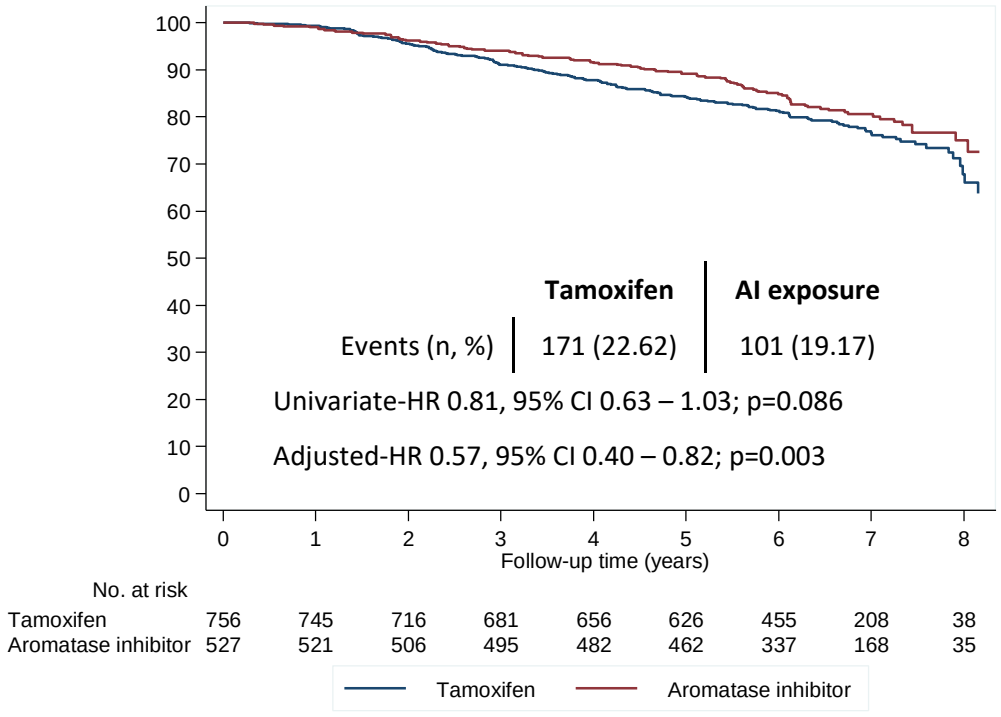
In the overall cohort, from the 527 patients treated with TAM, 171 (22.6%) died, while from the 474 patients exposed to AI, 101 (19.2%) died. OS was very favorable for both groups: for those treated with TAM, the 5 and 7 years OS proportion was of 83.8% (95% CI 81.0 – 86.3) and 76.2% (95% CI 72.5 – 79.4), respectively; while for those exposed to AI, 89.2% (95% CI 86.2 – 91.5) and 80.1% (95% CI 76.0 – 83.6), respectively.

In the landmark cohort (n=1223), when controlling for age, TNM stage, histologic grade, HER2 status, treatment with (neo)adjuvant chemotherapy and treatment center, exposure to an AI was associated with an improved OS (M1 Figure 3; HR 0.55, 95% CI 0.37 – 0.81). Of note, there is a consistent curve separation between groups until year 5. Other variables associated with survival included age at diagnosis and TNM stage (p<0.001), but not center of care (p=0.358). The same analysis performed in the overall cohort (n=1283) is consistent with the landmark cohort (M1 Supplementary Figure A.2).

**M1 Figure 3 –** Overall survival in the landmark cohort (n=1223) and according to treatment with tamoxifen or with exposure to aromatase inhibitors.

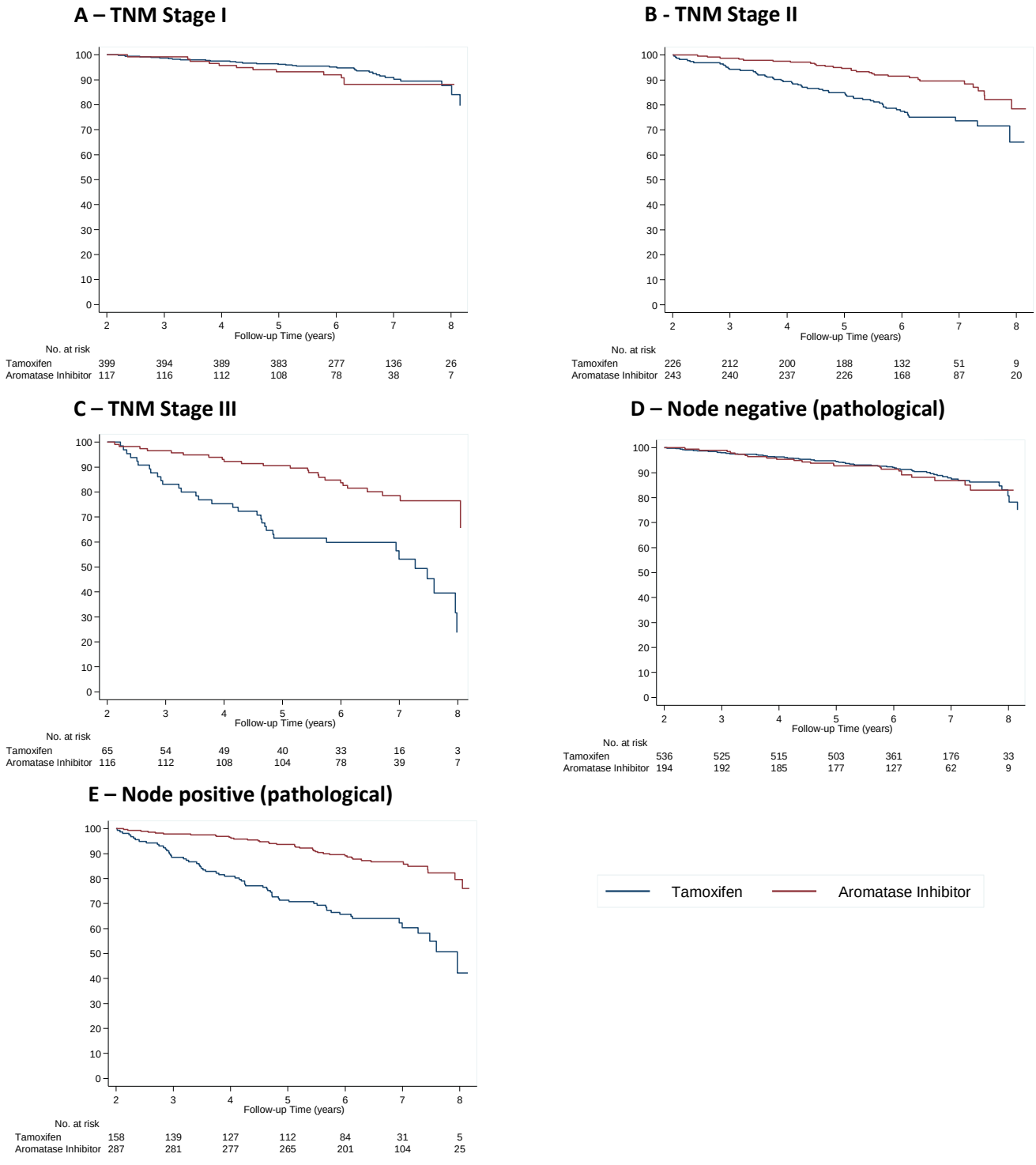



**M1 Supplementary figure A.2 –** Overall survival in the overall cohort (n=1283) and according to treatment with tamoxifen or with exposure to aromatase inhibitors.



Stratifying the relative effectiveness according to TNM stage, an incremental and consistent benefit was observed for stage II and III (stage II: HR 0.57, 95% CI 0.34 – 0.95; stage III: HR 0.32, 95% CI 0.17 – 0.60; M1 Figure 4). Interaction between ET and TNM was significant (p for interaction = 0.002).

**M1 Figure 4** – Overall survival according to the treatment with tamoxifen or with exposure to aromatase inhibitors and disease stage/nodal status in the landmark cohort (n=1223): A) stage I, B) stage II, C) stage III, D) node negative, and E) node positive disease.



Finally, we further conducted an exploratory comparison using a propensity score matching analysis of patients receiving 1) tamoxifen or aromatase inhibitors exposure, 2) tamoxifen or aromatase inhibitors as monotherapy, and 3) tamoxifen or aromatase inhibitors as sequencing therapy, all favoring the use of aromatase inhibitors (M1 Supplementary Table A.2).

**M1 Supplementary Table A.2** – Propensity score matching comparison of tamoxifen and different aromatase inhibitor-based strategies. A 1:1 matching was performed. Sample size varies according to the existence of a matched observation and the size of the smaller group used for comparison.

| | Number of patients included | Hazard ratio (95% Confidence Interval) | 5 years survival estimates | 7 years survival estimates |
|---|---|---|---|---|
| **Tamoxifen vs. AI exposure** | 1019 | 0.60 (0.43 – 0.85) | Tamoxifen: 85.9% AI: 93.5% | Tamoxifen: 79.6% AI: 85.1% |
| **Tamoxifen vs. AI monotherapy** | 762 | 0.80 (0.49 – 1.30) | Tamoxifen: 80.7% AI: 87.9% | Tamoxifen: 76.0% AI: 76.8% |
| **Tamoxifen vs. AI sequencing** | 878 | 0.42 (0.26 – 0.67) | Tamoxifen: 85.2% AI: 96.5% | Tamoxifen: 80.7% AI: 91.9% |

### 5.1.7 **Discussion**

Over the last 40 years breast cancer detection and treatment has evolved significantly, and this has translated into measurable improvements in OS, especially for hormone receptor positive/HER2-negative and HER2-positive breast cancers.[7] One typical example of treatments that are now widespread and which have had a major impact on clinical outcomes is ET, first with TAM and more recently with AIs. In postmenopausal women, multiple randomized trials have demonstrated that AIs decrease the risk of disease recurrence, which led international guidelines to support its use in early 2005.[85] However, implementation of such treatments guidelines is not optimal.[91]

In our study, which focused on the care delivered from 2006-2008 in four Portuguese institutions, we found a substantial adoption of the randomized trial data, with almost half of the patients being treated with AI by 2008. As expected, providers selected patients at higher risk of recurrence to receive AIs, and were more likely to prescribe TAM to those at lower risk of recurrence. Although the EBCTCG meta-analysis showed no substantial heterogeneity in the benefit of AI according to age, body-mass index, stage, grade, PR status, or HER2 status, the selection of an AI-based strategy for those at higher risk is reasonable, as these are the patients most likely to derive a larger absolute benefit of AI over TAM. In an era where the choices of extending therapy, or, in the case of premenopausal women, of treatment intensification with ovarian suppression in association with an AI or TAM are taking place[92], it is likely that these same risk-based decisions will be happening: more effective cancer therapies will have substantial adoption among those at

higher risk and, and less so for those at smaller risk, who may do well regardless of the treatment choice (e.g., 5 and 7 years survival for stage I patients were 93.7% and 87.8% for those taking tamoxifen, respectively, and 92.4% and 87.4% for those taking an AI, respectively). Patients and physicians will have the flexibility to more easily personalize choices according to treatment side-effects, patients' preferences and cost.

Nevertheless, in parallel with the higher level of prescription of AI therapy in high risk patients, it is also remarkable that introduction of AI was asymmetrical between centers, and center of care was a strong independent predictor for the receipt of AI. While some of the variation may be appropriate, the very wide absolute differences in uptake rates between centers point to reasons beyond tumor characteristics or patient preference as driving factors. Factors that might have contributed to these differences include: local challenges in access to treatment innovation, cultural differences in the weighting of the risk/benefit of interventions, or even cultural differences in application of treatment innovation. Therefore, obstacles in the access to innovations in cancer care should be studied to reduce disparities in cancer outcomes in Europe, both at the regional level, but also at the center level.[93] Empowered by evidence-based guidelines produced with great effort from international associations[83,84,92], locally, we need to be vigilant and make efforts to translate these recommendations into clinical practice thus contributing to overcome disparities in cancer care.

In this cohort, median time on ET was approximately 5 years, suggesting an adequate treatment duration (at time of treatment decision). However, some treatment duration disparities were noted, which highlights that there may have been patients that struggle with adherence. For example, in the monotherapy group 25% of patients received ET for less than 38 months, and in the sequencing group starting with an AI, median time on AI was only 16 months, suggesting an eventual premature switch due to reasons other than initial treatment decision (as tolerability).

Finally, our study also showed an OS advantage for patients taking AI vs. TAM alone. This is concordant with the EBCTCG meta-analysis that showed that, when compared to 5 years of adjuvant TAM alone, 5 years of AIs improved 10-year breast cancer mortality (12.1% vs. 14.2% for TAM).[39] Relatively similar results were obtained for sequential strategies. When accommodating all possible strategies, all-cause mortality also significantly favored AIs (RR 0.88, 95% CI 0.82–0.94). Our data are consistent with these findings, however a more pronounced benefit in terms of OS was noted (adjusted-HR 0.55, 95% CI 0.37 – 0.81; proportion alive at year 7 of 76.2% and 80.1% for TAM and AI, respectively). Our data provides real-world confirmation of the benefits of AI therapy, whether given as monotherapy or as part of a sequential approach, as compared to TAM alone, outside of a controlled clinical trial setting.

Although this study provides interesting insights, it also has several limitations. It is a retrospective observational study, thus despite statistical rigor we cannot exclude residual confounding. To this end, we did not have access to patients' co-morbidities, educational level nor Ki67; their distribution between arms is unknown, which might contribute to residual confounding. This cohort includes data from four large centers from a single region in Portugal, which might not reflect practices in smaller centers and/or other regions. Treatment effectiveness was measured as OS, not cancer specific survival (due to cancer registry specifications), a limitation in a cohort of postmenopausal women with other competing causes of death. No data on actual reasons for treatment discontinuation or drug non-adherence was available (actual drug intake), and patients receiving consecutive prescription were considered to be active takers of the respective drug. Studies on patients' preferences are needed. Of note, a high proportion of cases did not have information regarding definitive treatment stop date. Lastly, our study did not examine quality of life nor pharmacoeconomic metrics.

### 5.1.8 Conclusion

AIs were effectively introduced as adjuvant treatment of early breast cancer in a group of Portuguese centers, particularly among patients with high stage disease. However, its use relative to TAM was not only influenced by tumor and patient characteristics, but also center of care. In accordance to guidelines at the time of diagnosis (2006 to 2008), treatment was provided for approximately 5 years. Finally, exposure to an AI was associated with a strong OS benefit.

### 5.1.9 Conflicts of interest

The authors declare no conflict of interest.

### 5.1.10 Funding

This work was supported by *Fundação para a Ciência e a Tecnologia* (FCT) with grants HMSP-ICS/0004/2011 and HMSP-ICJ/0007/2013 under the Harvard Medical School Portugal program. FCT did not interfere in any step of the study.

## 5.2 Mixed Invasive Ductal and Lobular Carcinoma of the Breast: Prognosis and the Importance of Histologic Grade

### 5.2.1 Introductory notes

This project details the relative effectiveness of aromatase inhibitors and tamoxifen for the adjuvant treatment of postmenopausal women with hormone receptor positive breast cancer within the lobular family of tumors, in specific in pure invasive lobular carcinoma and mixed ductal and lobular carcinoma. It further dissects its prognostic implications and summarizes the prognostic role of histologic grade according to histology. This project was published in The Oncologist. Arlindo R. Ferreira led the study design, data analysis, results interpretation and manuscript writing.

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### 5.2.3 Abstract

Background: The diagnosis of mixed invasive ductal and lobular carcinoma (IDC-L) in clinical practice is often associated with uncertainty related to its prognosis and response to systemic therapies. With the increasing recognition of invasive lobular carcinoma (ILC) as a distinct disease subtype, questions surrounding IDC-L become even more relevant. In this study, we took advantage of a detailed clinical database to compare IDC-L and ILC regarding clinicopathologic and treatment characteristics, prognostic power of histologic grade and survival outcomes.

Methods: In this retrospective cohort study, we identified 811 patients diagnosed with early-stage breast cancer with IDC-L or ILC. Descriptive statistics were performed to compare baseline clinicopathologic characteristics and treatments. Survival rates were subsequently analyzed using the Kaplan-Meier method and compared using the Cox proportional hazards model.

Results: Patients with ILC had more commonly multifocal disease, low to intermediate histologic grade and HER2-negative disease. Histologic grade was prognostic for patients with IDC-L, but had no significant discriminatory power in patients with ILC. Among postmenopausal women,

those with IDC-L had significantly better outcomes when compared to those with ILC: disease-free survival (DFS) and overall survival (OS; adjusted-HR 0.54, 95% CI 0.31 – 0.95). Finally, postmenopausal women treated with an AI had more favorable DFS and OS than those treated with tamoxifen-only (OS adjusted-HR 0.50, 95% CI 0.29 – 0.87), which was similar for both histologic types (p=0.212).

Conclusions: IDC-L tumors have a better prognosis than ILC tumors, particularly among postmenopausal women. Histologic grade is an important prognostic factor in IDC-L, but not in ILC.

Keywords: breast cancer, early; carcinoma, lobular; invasive ductal carcinoma, breast; tumor grading; outcomes research.

### 5.2.4 Introduction

Breast cancer is morphologically classified as either invasive breast carcinoma of no special type (NST), also known as invasive ductal carcinoma (IDC), or as a "special subtype" of breast cancer.[94] Special subtypes account for an array of different histological features, with invasive lobular carcinoma (ILC) being the most common subtype.[95] In addition, certain breast carcinomas present with varying proportions of NST and other types of breast cancers and are classified as *carcinomas of mixed type*. This category is defined as tumors in which at least 50% of the tumor has a specialized pattern and a non-specialized pattern in 10% to 49% of the tumor.[94] Mixed invasive ductal and lobular carcinomas (IDC-L) account for approximately 5% of all breast cancers and, side with ILC, present a growing incidence.[95–97]

ILC has long been distinguished from other types of breast cancer for its unique clinicopathologic features and more recently genomic landscape.[98–100] When compared to IDC, ILC tend to lack the cell adhesion molecule e-cadherin, is more frequently multifocal, hormone receptor-positive/HER2-negative, lower grade (I or II), presents reduced response rates to preoperative chemotherapy and may benefit differently from adjuvant endocrine therapies.[101–104] In contrast, studies characterizing IDC-L are currently scarce and limited by cohort size, lack of granular clinicopathological/treatment data or short follow-up.[95,105–109] It is thus unclear how patients with these tumors perform in terms of survival outcomes and whether known classic prognostic features of IDC, as histologic grade, apply to IDC-L.

In this retrospective analysis, we took advantage of a large, detailed and curated single center database to compare clinicopathologic features and outcomes between ILC and IDC-L. We further focused on the prognostic implications of histological grade taking into consideration differences in systemic therapies.

### 5.2.5 Patients and Methods

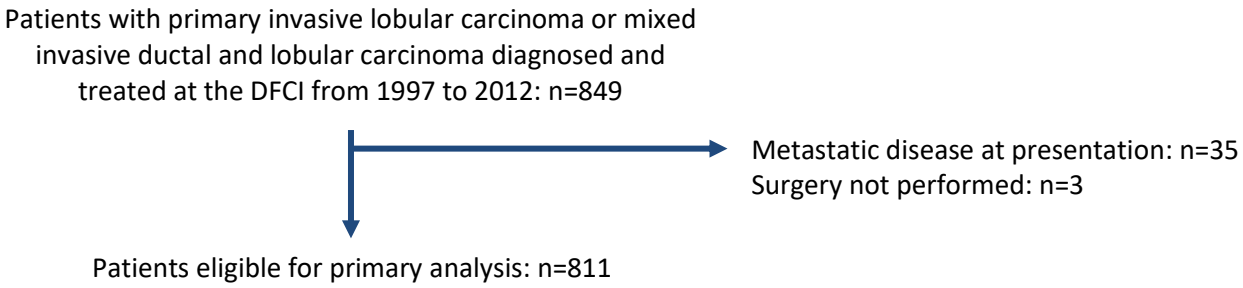
Study design and data source

This is a retrospective cohort study using prospectively collected data from the Dana-Farber Cancer Institute (DFCI) and stored in the National Comprehensive Cancer Network (NCCN) Oncology Outcomes Database. The current study was approved by the DFCI Institutional Review Board and complies with all national regulations. We applied the STROBE statement in reports of cohort studies (http://www.strobe-statement.org/).

Patient selection and extracted information

We identified all patients who were older than 18 years of age, and were diagnosed and treated at DFCI for stage I-III breast cancer of ILC or IDC-L histology from 1997 to 2007. IDC-L was defined as tumors in which at least 50% of the tumor is of lobular pattern and 10% to 49% of non-specialized pattern. Follow-up details (disease recurrence, new primaries and death) were available up to January 2012 and analyzed as per registry specifications. Dates of study entry were balanced between groups. We excluded patients with metastatic disease at presentation, patients who received neoadjuvant therapy, patients who did not have surgery and patients with other concurrent primary tumors. A cohort of 811 patients was identified for the analysis (M2 Figure 1).

**M2 Figure 1** - Study Consort diagram. DFCI – Dana-Farber Cancer Institute.




Overall survival (OS), disease-free survival (DFS), and time to specific relapse were defined as time from diagnosis to death, time from diagnosis to any relapse or death, and time from diagnosis to local, regional or distant relapse, respectively.

Statistical analysis

Descriptive statistics of baseline demographic, clinicopathologic and treatment characteristics were performed. Differences between groups were tested using chi-squared test or t-test where applicable. Time-to-event data was analyzed using the Kaplan–Meier method and compared using Cox proportional hazards models. All patients with missing data in relevant variables were excluded from the multivariate analysis. All the presented analyses successfully met

proportional hazards assumption as assessed by the Schoenfeld residuals. Missing information was considered as missing at random, as per study design. The analyses were completed using Stata 12.3 (StataCorp LP).

### 5.2.6 Results

Study population and baseline characteristics

The study population included 811 patients, 337 (41.6%) with ILC and 474 (58.4%) with IDC-L (M2 Table 1). When compared to patients with IDC-L, patients with ILC were slightly older, had larger tumors (11.0% had tumors > 5 cm vs. 3.0% for IDC-L; p<0.001), more positive nodes (16.9% had ≥4 nodes vs. 9.7% for IDC-L; p=0.002) and less frequently poorly differentiated tumors (8.3% vs. 19.8% for IDC-L; p<0.001). In addition, ILC was less likely to be HER2-positive (3.9% vs. 8.6%; p=0.02). Finally, multifocal disease was also more common in patients with ILC (36.2% vs. 26.6%; p=0.004).

**M2 Table 1** – Patient demographics, clinicopathologic characteristics and treatments overall and by histologic type.

| **Variable list** | **Total sample** (n= 811) | **ILC** (n= 337) | **IDC-L** (n= 474) | P-value (ILC vs. IDC-L) |
|---|---|---|---|---|
| **Demographic and clinicopathologic characteristics** | | | | |
| Age (years) | | | | |
| Median | 53.79 | 54.55 | 53.02 | **0.030** |
| IQR | 46.90 – 62.16 | 47.85 – 64.17 | 46.17 – 60.97 | |
| Menopausal status, n (%) | | | | |
| Premenopausal | 349 (43.0) | 133 (39.5) | 216 (45.6) | 0.084 |
| Postmenopausal | 462 (57.0) | 204 (60.5) | 258 (54.4) | |
| Multifocal tumor present (in pathology report), n (%) | 248 (30.5) | 122 (36.2) | 126 (26.6) | **0.004** |
| Missing | 66 (8.1) | 26 (7.7) | 40 (8.4) | |
| pT (tumor size, pathological) , n (%) | | | | |
| ≤ 2 cm | 482 (59.4) | 184 (54.6) | 298 (62.9) | **<0.001** |
| > 2 – 5 cm | 200 (24.7) | 89 (26.4) | 111 (23.4) | |
| > 5 cm | 51 (6.3) | 37 (11.0) | 14 (3.0) | |
| Missing | 78 (9.6) | 27 (8.0) | 51 (10.7) | |
| Dissected nodes | | | | |
| Median | 9 | 10 | 9 | 0.235 |
| Range | 3 – 15 | 4 – 15 | 3 – 14 | |
| Positive nodes, n (%) | | | | |
| Negative | 408 (50.3) | 174 (51.6) | 234 (49.4) | **0.002** |
| 1 – 3 positive | 198 (24.4) | 67 (19.9) | 131 (27.6) | |
| 4 – 9 positive | 66 (8.1) | 33 (9.8) | 33 (7.0) | |
| 10 or more | 37 (4.6) | 24 (7.1) | 13 (2.7) | |
| Missing | 102 (12.6) | 39 (11.6) | 63 (13.3) | |
| Simplified TNM staging, n (%) | | | | |
| Stage I | 351 (43.2) | 144 (42.7) | 207 (43.7) | **0.026** |
| Stage II | 355 (43.8) | 137 (40.7) | 218 (46.0) | |
| Stage III | 105 (13.0) | 56 (16.6) | 49 (10.3) | |

| | | | | |
|---|---|---|---|---|
| Lymphovascular invasion, n (%) | | | | |
| Yes | 219 (27.0) | 55 (16.3) | 164 (34.6) | **<0.001** |
| No | 592 (73.0) | 282 (83.7) | 310 (65.4) | |
| Histologic grade, n (%) | | | | |
| Grade I | 182 (22.4) | 113 (33.5) | 69 (14.6) | **<0.001** |
| Grade II | 497 (61.3) | 188 (55.8) | 309 (65.2) | |
| Grade III | 122 (15.1) | 28 (8.3) | 94 (19.8) | |
| Missing | 10 (1.2) | 8 (2.4) | 2 (0.4) | |
| Hormone receptor, n (%) | | | | |
| ER and/or PR positive | 776 (95.7) | 323 (95.9) | 453 (95.6) | **0.548** |
| ER and PR negative | 33 (4.1) | 12 (3.5) | 21 (4.4) | |
| Missing | 2 (0.2) | 2 (0.6) | 0 (0) | |
| ER positive only | 771 (95.1) | 321 (95.3) | 450 (94.9) | **0.558** |
| HER2 receptor, n (%) | | | | |
| Positive | 54 (6.7) | 13 (3.9) | 41 (8.6) | **0.02** |
| Negative | 680 (83.8) | 273 (81.0) | 407 (85.9) | |
| Missing | 77 (9.5) | 51 (15.1) | 26 (5.5) | |
| Molecular type, n (%) | | | | |
| ER or PR+ and HER2- | 659 (81.3) | 268 (79.5) | 391 (82.5) | **0.01** |
| ER or PR+ and HER2+ | 44 (5.4) | 8 (2.4) | 36 (7.6) | |
| ER- and PR- and HER2+ | 10 (1.2) | 5 (1.5) | 5 (1.0) | |
| ER- and PR- and HER2- | 21 (2.6) | 5 (1.5) | 16 (3.4) | |
| Missing | 77 (9.5) | 51 (15.1) | 26 (5.5) | |
| **Treatment characteristics** | | | | |
| Surgery, n (%) | | | | |
| Mastectomy | 414 (51.0) | 192 (57.0) | 222 (46.8) | **0.004** |
| Breast conservation | 397 (49.0) | 145 (43.0) | 252 (53.2) | |
| Radiotherapy, n (%) | | | | |
| Yes | 577 (71.2) | 233 (69.1) | 344 (72.6) | 0.287 |
| No | 234 (28.8) | 104 (30.9) | 130 (27.4) | |
| Adjuvant endocrine therapy, n (%) | | | | |
| Yes | 722 (89.0) | 302 (89.6) | 420 (88.6) | 0.371 |
| No | 56 (6.9) | 20 (5.9) | 36 (7.6) | |
| Missing | 33 (4.1) | 15 (4.5) | 18 (3.8) | |
| Adjuvant chemotherapy, n (%) | | | | |
| Yes | 451 (55.6) | 170 (50.5) | 281 (59.3) | **0.021** |
| No | 306 (37.7) | 141 (41.8) | 165 (34.8) | |
| Missing | 54 (6.7) | 26 (7.7) | 28 (5.9) | |

ER – estrogen receptor; HER2 – human epidermal growth factor receptor 2; IDC-L – mixed invasive ductal and lobular carcinoma; ILC – invasive lobular carcinoma; IQR – interquartile range; PR – progesterone receptor.

Treatment

Patients with ILC underwent mastectomy more frequently than those with IDC-L (57.0% vs. 46.8%; p=0.004; M2 Table 1). Yet, no significant differences were found in the frequency of radiotherapy (69.1% vs. 72.6% for IDC-L; p=0.287). Nevertheless, despite the higher tumor burden at diagnosis, patients with ILC received chemotherapy less frequently (50.5% vs. 59.3% than IDC-L; p=0.021).

Outcomes

The median follow-up for the entire cohort was 7.9 years, and was similar for both histologic types (p=0.190). Among 337 patients with ILC, 73 (21.7%) developed a DFS event and 62 (18.4%) developed an OS event; while among 474 patients with IDC-L, 70 (14.8%) developed a DFS event and 59 (12.5%) developed an OS event. For patients with ILC, the 5- and 10-year proportion of patients free of a DFS event was 89.3% (95% confidence interval [CI] 85.3 – 92.2) and 74.2% (95% CI 67.8 – 79.6), respectively; while for IDC-L, the 5- and 10-year rates were 90.4% (95% CI 87.3 – 92.8) and 81.0% (95% CI 75.7 – 85.3), respectively.

In a multivariate model, variables associated with DFS included year of diagnosis, TNM stage and histologic grade; whereas variables associated with OS included age at diagnosis, TNM staging and histologic grade (M2 Table 2). Overall, the differences in DFS and OS outcomes by histologic type were not statistically significant, despite a trend towards an improved outcome for IDC-L when compared to ILC. Specifically, the hazard ratio for DFS was 0.72 (95% CI 0.49 – 1.08; p=0.114) (M2 table 2 and M2 figure 2) and the hazard ratio for OS was 0.77 (95% CI 0.50 – 1.20; p=0.244) (M2 table 2 and M2 figure A-1).

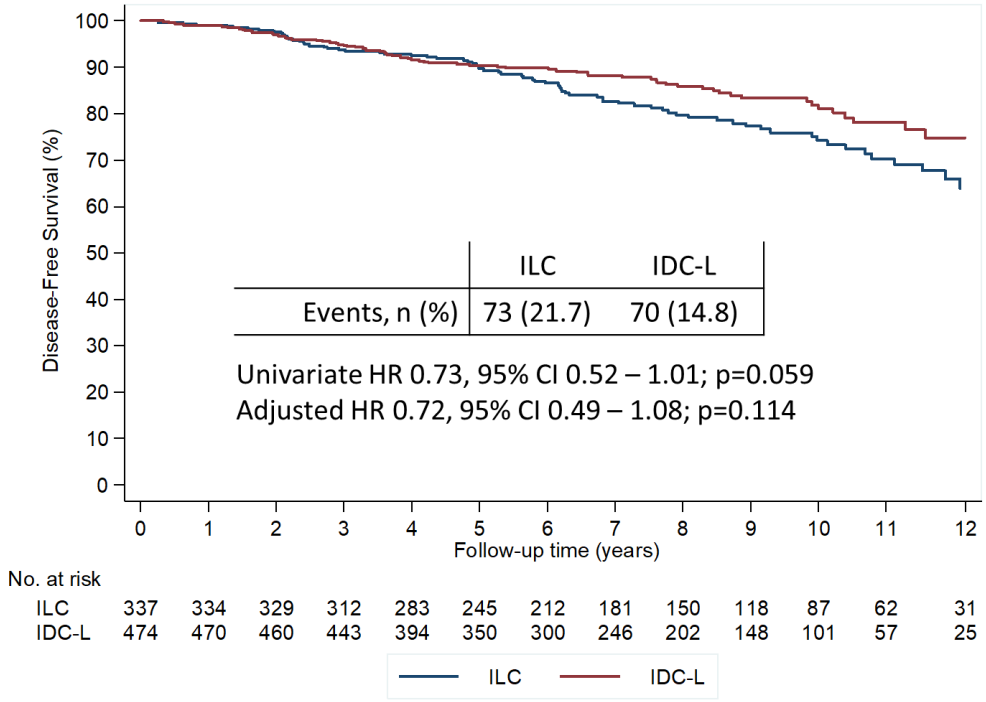
**M2 Table 2 –** Multivariate Cox proportional hazards model for overall survival and disease-free survival.

| Variable list | **Disease-Free Survival** (No. patients: 681/811; Events 110/143) | | | **Overall Survival** (No. patients: 681/811; Events 93/121) | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | p-value | HR | 95% CI | p-value |
| **Main multivariate model (estimates without interaction terms)** | | | | | | |
| ILC (vs. IDC-L) | 0.72 | 0.49 – 1.08 | 0.114 | 0.77 | 0.50 – 1.20 | 0.244 |
| Age at diagnosis (per year increase) | 1.01 | 0,99 – 1.04 | 0.091 | 1.03 | 1.00 – 1.06 | **0.034** |
| Menopausal status | 1.08 | 0.60 – 1.96 | 0.791 | 1.14 | 0.59 – 2.21 | 0.703 |
| TNM staging<br>Stage I<br>Stage II<br>Stage III | <br>(reference)<br>2.43<br>13.57 | <br>(reference)<br>1.37 – 4.31<br>6.71 – 27.44 | <br><br>**0.002**<br>**<0.001** | <br>(reference)<br>2.309<br>12.117 | <br>(reference)<br>1.213 – 4.393<br>5.714 – 25.698 | <br><br>**0.011**<br>**<0.001** |
| Lymphovascular invasion | 1.06 | 0.67 – 2.11 | 0.815 | 1.18 | 0.72 – 1.93 | 0.519 |
| Histologic grade<br>Grade I<br>Grade II<br>Grade III | <br>(reference)<br>1.24<br>2.20 | <br>(reference)<br>0.73 – 2.12<br>1.14 – 4.23 | <br><br>0.423<br>**0.018** | <br>(reference)<br>1.639<br>2.450 | <br>(reference)<br>0.879 – 3.057<br>1.138 – 5.276 | <br><br>0.120<br>**0.022** |
| ER positive | 0.70 | 0.22 – 2.22 | 0.543 | 0.46 | 0.13 – 1.59 | 0.222 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| HER2 receptor positive | | 0.76 | 0.39 – 1.46 | 0.406 | 0.96 | 0.49 – 1.88 | 0.906 |
| Adjuvant chemotherapy use | | 0.66 | 0.37 – 1.17 | 0.159 | 0.87 | 0.46 – 1.65 | 0.673 |
| Adjuvant hormone therapy use | | 0.58 | 0.18 - 1.84 | 0.360 | 0.72 | 0.20 - 2.57 | 0.613 |
| Year of diagnosis | | 0.91 | 0.83 – 0.99 | **0.034** | 1.04 | 0.93 – 1.16 | 0.489 |
| **Interaction term 1 in the main multivariate model (see accompanying figure 3)** | | | | | | | |
| Histologic type x menopausal status | | 0.52 | - | 0.118 | 0.31 | - | **0.020** |
| **Premenop.** | Histologic type<br>ILC<br>IDC-L | (reference)<br>1.06 | (reference)<br>0.52 – 2.17 | 0.875 | (reference)<br>1.52 | (reference)<br>0.62 – 3.74 | 0.356 |
| **Postmenop.** | Histologic type<br>ILC<br>IDC-L | (reference)<br>0.58 | (reference)<br>0.34 – 0.97 | **0.039** | (reference)<br>0.53 | (reference)<br>0.30 – 0.94 | **0.028** |
| **Interaction term 2 in the main multivariate model (see accompanying figure 4)** | | | | | | | |
| Histologic type x grade | | 2.08 | - | **0.022** | 2.11 | - | **0.033** |
| ILC | Histologic grade<br>Grade I<br>Grade II<br>Grade III | (reference)<br>1.06<br>1.27 | (reference)<br>0.57 – 1.97<br>0.44 – 3.64 | (reference)<br>0.859<br>0.657 | (reference)<br>1.17<br>1.07 | (reference)<br>0.58 – 2.35<br>0.32 – 3.49 | (reference)<br>0.660<br>0.917 |
| IDC-L | Histologic grade<br>Grade I<br>Grade II<br>Grade III | (reference)<br>2.34<br>5.65 | (reference)<br>0.70 – 7.80<br>1.58 – 20.2 | (reference)<br>0.166<br>0.008 | (reference)<br>6.26<br>11.36 | (reference)<br>0.84 – 46.76<br>1.44 – 89.90 | (reference)<br>0.074<br>0.021 |

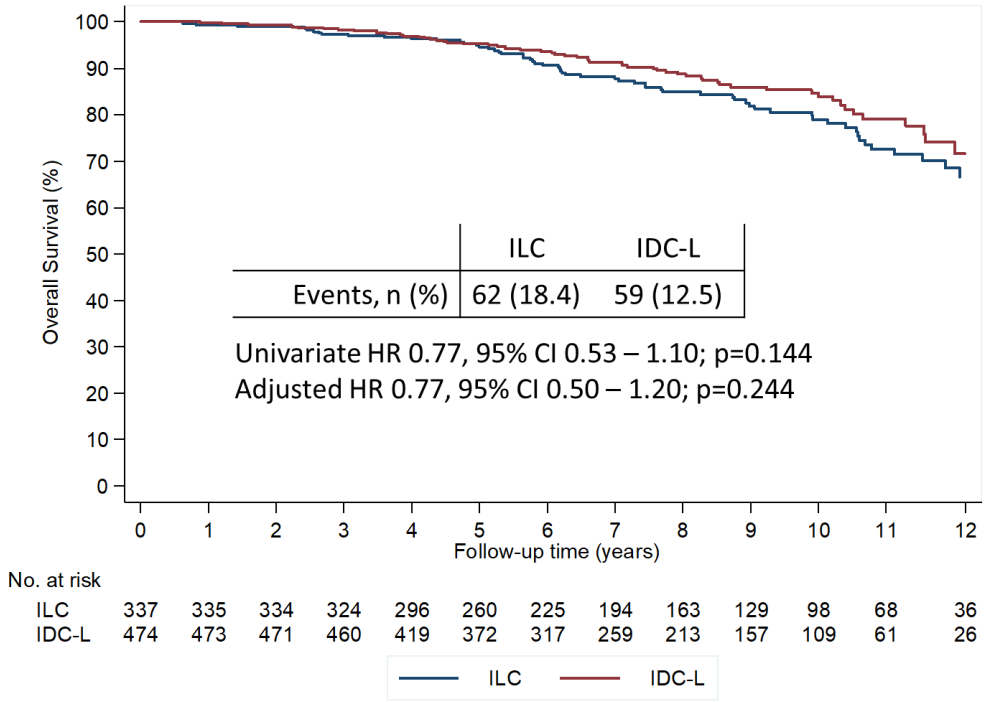
CI – confidence interval; ER – estrogen receptor; HER2 – human epidermal growth factor receptor 2; HR – hazard ratio; IDC-L – mixed invasive ductal and lobular carcinoma; ILC – invasive lobular carcinoma; x – interaction between terms.

**M2 Figure 2** – Disease-free survival in ILC and IDC-L.




CI – confidence interval; IDC-L – mixed invasive ducal and lobular carcinoma; ILC – invasive lobular carcinoma; HR – hazard ratio.

**M2 Figure A-1** – Overall survival in ILC and IDC-L.

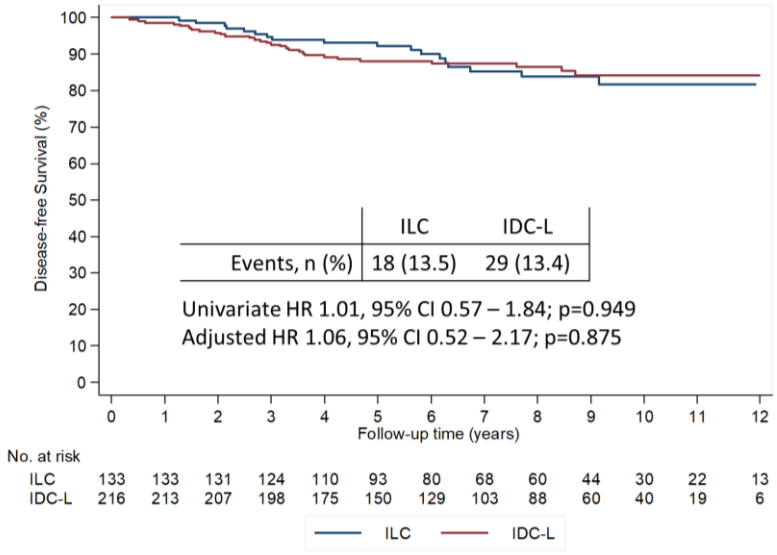



CI – confidence interval; IDC-L – mixed invasive ducal and lobular carcinoma; ILC – invasive lobular carcinoma; HR – hazard ratio.

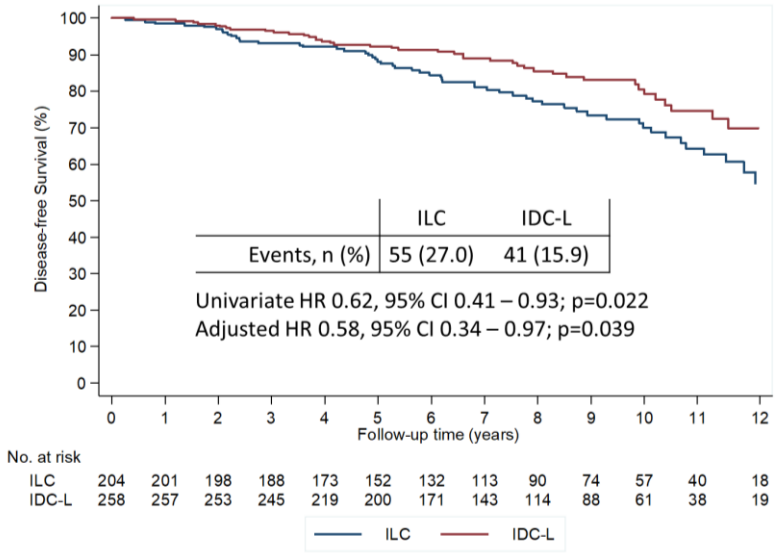
Given the hormone dependent nature of lobular carcinomas, we tested whether menopausal status modified outcomes according to histologic type. The interaction between histology and menopausal status was statistically significant for OS and a trend in the same direction was noted for DFS (M2 Table 2). When stratifying the analysis by menopausal status, no difference in DFS or OS was seen in premenopausal patients (adjusted hazard ratio [HR] for DFS 1.06, 95% CI 0.52 – 2.17; p=0.875), but superior outcome is evident for postmenopausal patients with IDC-L, compared to ILC (adjusted HR for DFS 0.58, 95% CI 0.34 – 0.97; p=0.039) (M2 Figure 3 and M2 Figure A-2).

**M2 Figure 3** – Disease-free survival in ILC and IDC-L in premenopausal (A) and postmenopausal (B) patients.

**A**



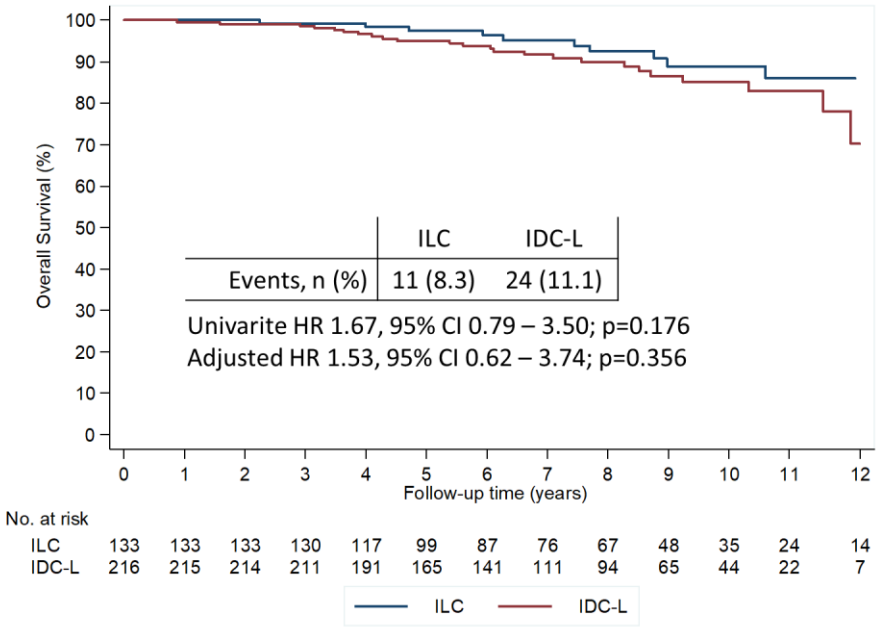

**B**



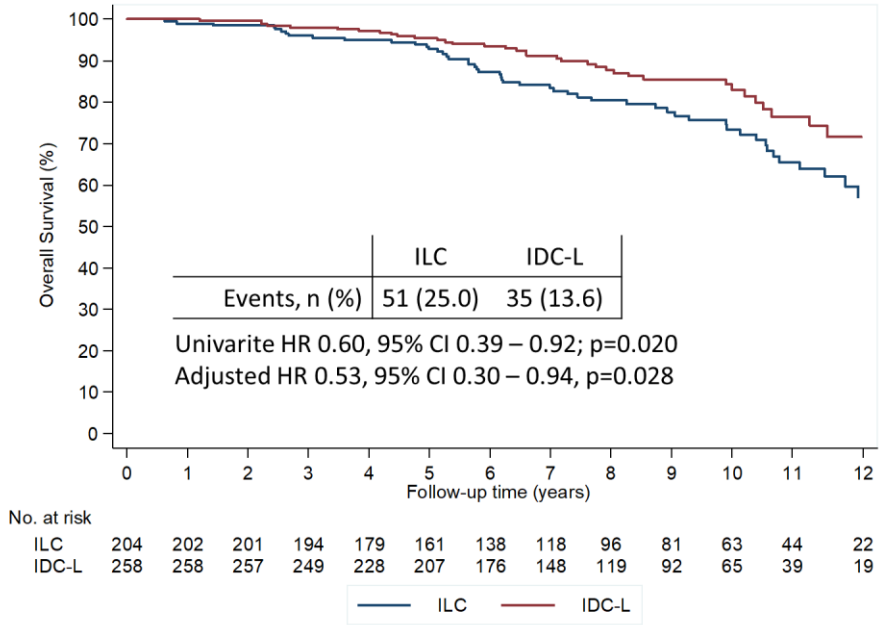

CI – confidence interval; IDC-L – mixed invasive ducal and lobular carcinoma; ILC – invasive lobular carcinoma; HR – hazard ratio.

**M2 Figure A-2** – Overall survival in ILC and IDC-L in premenopausal (A) and postmenopausal (B) patients.

**A**




**B**

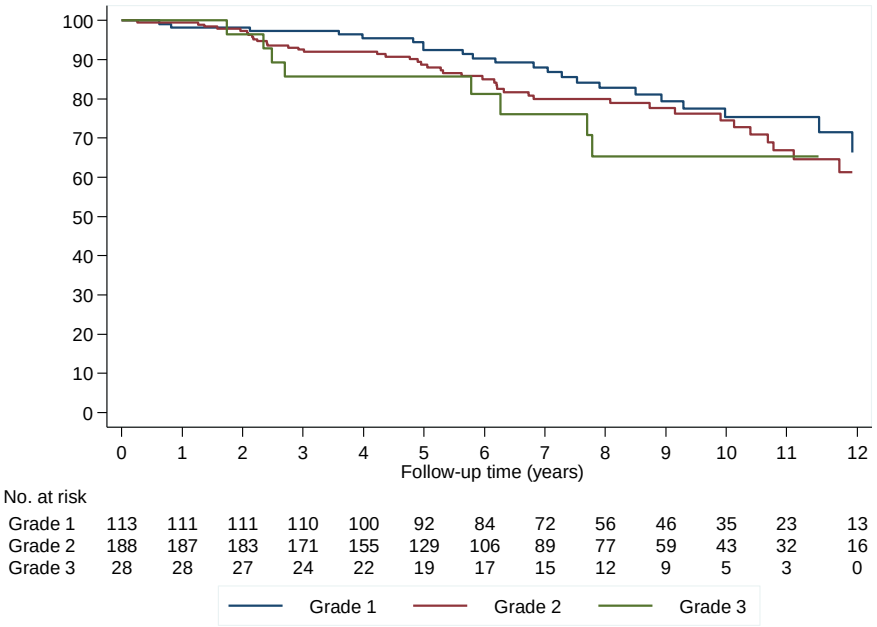



CI – confidence interval; IDC-L – mixed invasive ducal and lobular carcinoma; ILC – invasive lobular carcinoma; HR – hazard ratio.

To explore the prognostic role of histologic grade, we performed an interaction analysis between histology and grade, which was statistically significant (M2 Table 2). While histologic grade was unable to discriminate the prognosis of patients with ILC, it was an effective tool to discriminate the prognosis of those with IDC-L (M2 Figure 4 and M2 Figure A-3).

**M2 Figure 4** – Disease-free survival based on tumor grade in ILC (A) and IDC-L (B).

**A**

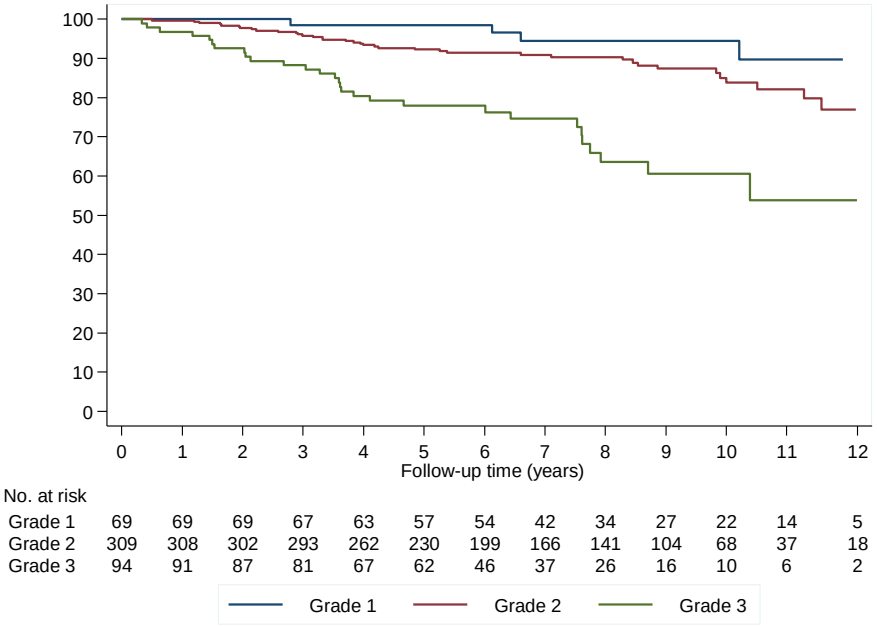



No. at risk

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Grade 1 | 113 | 111 | 111 | 110 | 100 | 92 | 84 | 72 | 56 | 46 | 35 | 23 | 13 |
| Grade 2 | 188 | 187 | 183 | 171 | 155 | 129 | 106 | 89 | 77 | 59 | 43 | 32 | 16 |
| Grade 3 | 28 | 28 | 27 | 24 | 22 | 19 | 17 | 15 | 12 | 9 | 5 | 3 | 0 |




**B**

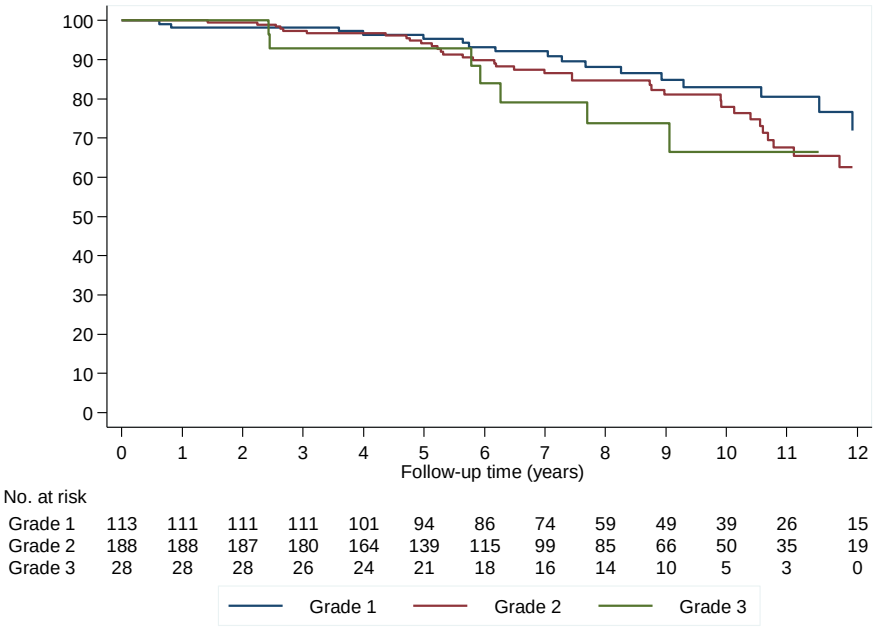



No. at risk

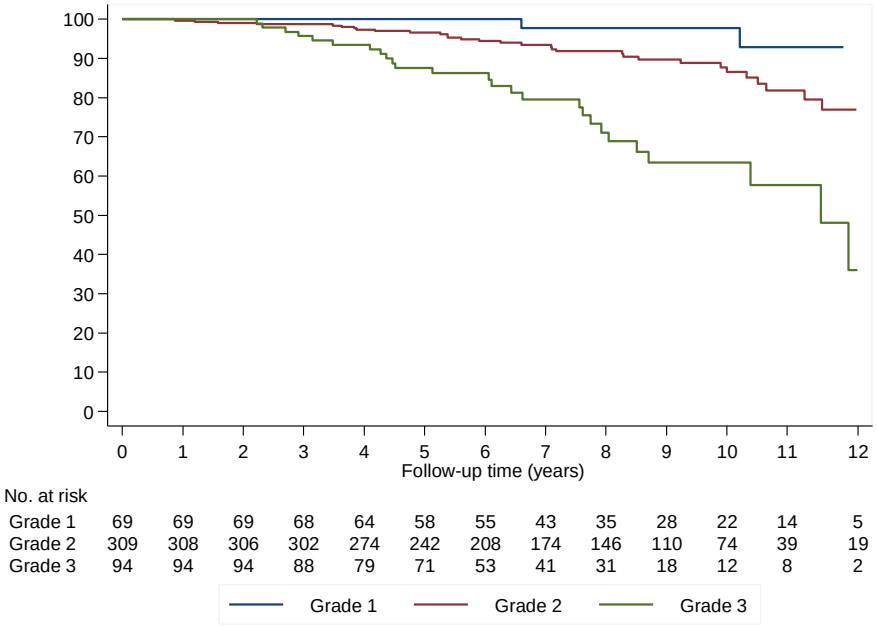
| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Grade 1 | 69 | 69 | 69 | 67 | 63 | 57 | 54 | 42 | 34 | 27 | 22 | 14 | 5 |
| Grade 2 | 309 | 308 | 302 | 293 | 262 | 230 | 199 | 166 | 141 | 104 | 68 | 37 | 18 |
| Grade 3 | 94 | 91 | 87 | 81 | 67 | 62 | 46 | 37 | 26 | 16 | 10 | 6 | 2 |

**M2 Figure A-3** – Overall survival according to tumor grade in ILC (A) and IDC-L (B).

**A**




**B**




We further explored the relative effectiveness of tamoxifen versus AI among postmenopausal patients with hormone receptor-positive tumors. Patients treated with an AI (as monotherapy or sequentially with tamoxifen) had more favorable outcomes than those treated with tamoxifen-only, both in terms of DFS (HR 0.36, 95% CI 0.21 – 0.61; p<0.001) and OS (HR 0.50, 95% CI 0.29 – 0.87; p=0.015) (M2 Table A-1). The magnitude of benefit was similar for both histologic types (p=0.212). Similar results for both analyses were obtained after the introduction of

a landmark analysis including only patients free of recurrence at 24 months (which would be a reasonable date of endocrine therapy switch from tamoxifen to an AI in clinical practice).

**M2 Table A-1** – Efficacy of therapy in postmenopausal hormone receptor-positive patients. Adjusted-HR controlling for age, stage, tumor grade, LVI and HER2 expression.

| **Type of therapy** | **DFS model** (No. patients: 358/441; Events 65/86) | | | **OS model** (No. patients: 358/441; Events 60/77) | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | P-value | HR | 95% CI | P-value |
| Type of hormone therapy | | | | | | |
| Tamoxifen | (reference) | (reference) | | (reference) | (reference) | |
| AI or sequential therapy | **0.36** | **0.20 – 0.65** | **0.001** | **0.39** | **0.22 – 0.74** | **0.003** |
| Chemotherapy | | | | | | |
| None | (reference) | (reference) | | (reference) | (reference) | |
| Any | 1.54 | 0.72 – 3.27 | 0.341 | 1.55 | 0.70-3.46 | 0.283 |

AI – aromatase inhibitor; CI – confidence interval; DFS – disease-free survival; HR – hazard ratio; OS – overall survival.

Disease recurrence

A total of 91 patients had a disease recurrence: 44 (48.35%) patients with ILC and 47 (51.65%) patients with IDC-L (p=0.163) (M2 Table A-2). When considering the specific site of disease recurrence, bone was the most frequent site in both histologic types (14 [37.84%] vs. 17 cases [53.12%], for ILC and IDC-L, respectively). Nevertheless, intra-abdominal recurrences (excluding liver) were only identified in ILC (7 [18.9%]).

**M2 Table A-2** – Recurrence and second primary tumor characterization.

| **Disease relapse** | **Total sample** | **ILC** | **IDC-L** | **P-value** |
|---|---|---|---|---|
| Patients with disease relapse, n (%) | 91 (11.2) | 44 (13.1) | 47 (9.9) | 0.163 |
| Type of first relapse, n (%) | | | | |
| Local relapse | 13 (14.3) | 4 (9.1) | 9 (19.2) | 0.203 |
| Regional relapse | 9 (9.9) | 3 (6.8) | 6 (12.7) | |
| Distant metastasis | 69 (75.8) | 37 (84.1) | 32 (68.1) | |
| Type of first relapse, n (%) | | | | |
| Local and regional relapses | 22 (24.2) | 7 (15.9) | 15 (31.9) | 0.075 |
| Distant metastasis | 69 (75.8) | 37 (84.1) | 32 (68.1) | |
| Types of distant relapse, n (%) | | | | |
| Bone | 31 (44.9) | 14 (37.8) | 17 (53.1) | 0.101 |
| Intra-abdominal NOS | 7 (10.1) | 7 (18.9) | 0 (0) | |
| Liver | 6 (8.7) | 3 (8.1) | 3 (9.4) | |
| Pleural effusion | 6 (8.7) | 2 (5.4) | 4 (12.5) | |
| Nodes, non-local/regional | 7 (10.2) | 5 (13.5) | 2 (6.3) | |
| CNS | 5 (7.3) | 2 (5.4) | 3 (9.4) | |
| Other | 7 (10.1) | 4 (10.8) | 3 (9.4) | |

CNS – central nervous system; IDC-L – mixed invasive ducal and lobular carcinoma; ILC – invasive lobular carcinoma; NOS – not otherwise specified.

Finally, using a multivariate analysis model (controlling for the same variables detailed in M2 Table 2) we found no significant differences according to histologic type for other outcomes, namely locoregional recurrence (HR 0.97, 95% CI 0.36 – 2.60; p=0.944), distant recurrence (HR 0.69, 95% CI 0.41 – 1.18; p=0.174), bone recurrence (HR 0.80, 95% CI 0.35 – 1.80; p=0.584) and second breast cancers (HR 1.81, 95% CI 0.65 – 5.05; p=0.258).

### 5.2.7 Discussion

In this retrospective analysis, we took advantage of a clinical database including 811 patients to compare clinicopathologic features, management, and survival outcomes between IDC-L and ILC. Patients with ILC were older, had more multifocal disease, larger tumors, more positive nodes, HER2-negative tumors, and received less frequently adjuvant chemotherapy than patients with IDC-L. When compared to ILC, IDC-L had superior survival outcomes, particularly for women in the postmenopausal setting. Histologic grade was an important prognostic factor for IDC-L, but not for ILC. These observations resemble differences between hormone receptor-positive IDC and ILC.[105,110–112]

Previous retrospective studies have failed to identify meaningful differences in survival outcomes in patients with ILC compared to patients with IDC-L.[106–109,113,114] By contrast, in a retrospective series including 140 patients with IDC-L, Rakha et al reported worse outcomes for patients with IDC-L than those with ILC (n=380).[105] The interpretation of previous results is impaired by cohort size, limited multivariate adjustment or short follow-up. In this study, the overall results suggested similar survival outcomes between patients with ILC and IDC-L, but when stratifying by menopausal status, we noticed superior survival outcomes for patients with IDC-L. These observations were corroborated by a large analysis of SEER database including a total of 209,109 patients.[115] In the SEER analysis, Xiao et al compared survival outcomes based on histology including 172,379 IDC, 17,503 ILC and 19,227 IDC-L patients. The survival analysis performed pointed to better breast cancer-specific survival (BCSS) for patients with IDC-L than IDC and ILC. The evaluation of HR over time using Scaled Schoenfeld residual plots revealed interesting findings: the HR of IDC-L versus IDC increased over time indicating a continuous long-term risk of relapse, which could be attributed to the lobular component of mixed tumors. By contrast, the HR for the comparison of IDC-L versus ILC decreased over time indicating better long-term prognosis for IDC-L versus ILC. When evaluating the differences in outcomes between IDC-L and ILC, patients > 50 years diagnosed with IDC-L had superior outcomes.[115] While the larger sample size from the SEER analysis provided robust prognostic information, the lack of detailed clinicopathologic information (e.g. HER2 status) and treatment information is an important limitation.

Our results complement the findings from the SEER analysis, given that we were able to interpret survival outcomes correcting for important clinicopathologic variables (e.g. adjuvant systemic therapy). Taken together, available data suggests that patients diagnosed with IDC-L have a better survival outcome when compared to patients with ILC, which is probably explained by the continuous long-term risk of relapse associated with ILC. Furthermore, patients with IDC-L generally did not develop intra-abdominal relapses that characterize ILC. The TCGA research network recently published results of genomic characterization of 490 IDC, 127 ILC, 88 IDC-L, and 112 other breast cancer cases.[116] As expected, ILC-like tumors were enriched for luminal A subtype, *CDH1* mutations and loss of e-cadherin by mRNA expression. Among the 88 cases of IDC-L, there did not appear to be a distinct genomic profile; rather, the IDC-L cases segregated into IDC-like (n=64) or ILC-like (n=24) tumors. The overrepresentation of molecular IDC-like tumors in the clinical IDC-L cases in the TCGA is consistent with our findings – IDC-L (as assessed by pathological evaluation) diverged from ILC in histologic grade, frequency of HER2 status, and survival outcomes, among other differences, which would be expected if most clinical IDC-L are molecular IDC-like. Further research is needed to investigate whether there is any clinical utility of molecularly classifying IDC-L for the purpose of prognostic evaluation and/or treatment planning.

In our cohort, histologic grade was prognostic for IDC-L, but not for ILC. In a previous retrospective series pooling outcomes from 707 classic ILC, 102 special subtypes of ILC and 44 mixed tumors, Talman et al. found a significant difference in OS and DFS between grade II and III tumors, but not between grade I and II tumors.[117] In addition, a subsequent study including 517 ILC patients[118] reported a significant prognostic value for histologic grade. However, approximately one third of cases in the series were special subtypes of ILC often characterized by tubule formation, and when tubule formation was removed from the analysis, the remaining histologic grade variables (i.e., mitotic count and nuclear pleomorphism) were no longer associated with outcome. Collectively, our findings and those of others suggest that the current grading system may be limited for ILC, but useful for IDC-L.

In an exploratory analysis from our cohort, postmenopausal patients receiving adjuvant AI, either as monotherapy or sequentially after tamoxifen, had better outcomes when compared to patients treated with tamoxifen monotherapy independently of the histologic subtype. These results are in agreement with the updated aromatase inhibitor (AI) overview meta-analysis.[39] Of interest, two retrospective studies have compared the effectiveness of AI versus tamoxifen among patients diagnosed with ILC or IDC: the BIG1-98 study and the ABCSG-8 study.[103,104] In the BIG1-98 study, patients with ILC derived a greater benefit to letrozole when compared to tamoxifen[103] and in the ABCSG-8 study, patients diagnosed with ILC had better survival outcomes when treated with

a sequential regimen (tam-AI) than tamoxifen monotherapy.[104] Our current study is limited in its ability to determine a difference between AI and tamoxifen for patients diagnosed with ILC and IDC-L and further investigation into this topic is needed.

We acknowledge a number of limitations to our study. Despite the methodological rigor, as a retrospective observational study, it is amenable to residual confounding. While pathologic review was, in most cases, performed by an academic pathologist at Brigham & Women's Hospital, central pathology review and additional immunohistochemical studies, such as E-cadherin/p120[119] to further characterize these tumors, were not performed. Tumor classifications were taken from the diagnostic pathology reports and likely reflect both individual pathologist preferences as well as changing tumor classification practices over the period of this study. Finally, the relative effectiveness of tamoxifen versus AIs results are based on observational data, and not a randomized trial.

Despite these limitations, we report several important findings: 1) patients diagnosed with IDC-L have a better prognosis than patients with ILC, particularly for postmenopausal women; 2) histologic grade is an imperfect tool for patients with ILC, but provides relevant information for patients with IDC-L; 3) consistent with data from phase III studies, where AIs have shown a DFS advantage over tamoxifen that appeared greatest in the ILC subset, these improvements also held true for patients with IDC-L. Taken together, our work adds to the literature pointing to significant differences in survival outcomes for patients with IDC-L when compared to patients with ILC. Patients with IDC-L have more favorable outcomes, particularly for those in the postmenopausal setting; the unfavorable outcomes associated with ILC are likely to be explained by its continuous pattern of relapse beyond year 5.

### 5.2.8 Implications for Practice

We compared mixed invasive ductal and lobular carcinoma (IDC-L) to invasive lobular carcinomas (ILC) to assess the overall prognosis, the prognostic role of histologic grade, and response to systemic therapy. We found that patients with IDC-L tumors have a better prognosis than ILC, particularly among postmenopausal women, which may impact follow-up strategies. Moreover, while histologic grade failed to stratify the risk of ILC, it showed an important prognostic power in IDC-L, thus highlighting its clinical utility to guide treatment decisions of IDC-L. Finally, the DFS advantage of adjuvant aromatase inhibitors over tamoxifen in ILC was consistent in IDC-L.

### 5.2.9 Funding and role of funding source

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### 5.2.10 Conflicts of interest statement

All the authors declare that they have no conflict of interest.

## 5.3 Effectiveness of adjuvant ovarian function suppression in premenopausal women with early breast cancer: a multicenter cohort study

### 5.3.1 Introductory notes

This project details the introduction in clinical practice of adjuvant ovarian function suppression for the adjuvant treatment of premenopausal women with hormone receptor positive breast cancer and summarizes the real-world effectiveness of such intervention compared to no OFS. This project was published in Clinical Breast Cancer. Arlindo R. Ferreira led the study design, data analysis, results interpretation and manuscript writing.

### 5.3.2 Authors

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### 5.3.3 Abstract

Background: Ovarian function suppression (OFS) with tamoxifen or aromatase inhibitors (AI) improves disease-free survival in premenopausal women with breast cancer (BC), mostly in those at higher risk of recurrence. However, its real-world use and impact remain poorly understood.

Methods: This is a multicenter retrospective cohort study of premenopausal women with stage I-III hormone receptor-positive BC diagnosed from 2006-2015 aimed to look at the uptake and effectiveness of the addition of OFS to backbone endocrine therapy (tamoxifen or AI). To deal with confounding, we used both multivariate modelling and propensity score matching.

Results: Of 1717 eligible patients, 17.1% were treated with OFS. There was a substantial increase of use of OFS over time, especially from 2014 onward (16% vs 25% after 2014), particularly for the combination with AI (0.4% vs 8% after 2014). In a multivariate model, only younger age and year of diagnosis ≥2014 were associated with OFS utilization (both $p<0.001$).

With a median follow-up of 38 months (P25-P75 19.6-66.4) patients receiving OFS had a better OS than those not receiving OFS (adjusted-HR 0.44, 95% confidence interval 0.19-0.96, absolute

benefit at 5 years: 2.1% (95.3% vs. 93.2% in those not receiving OFS). A similar benefit was identified using propensity score matching.

Conclusions: In the real-world setting, there was an increase in the use of OFS after 2014. After 2014 a quarter of premenopausal women received adjuvant OFS, of which more than 30% in combination with an AI. In this study, use of adjuvant OFS was associated with an OS benefit.

Keywords: Gonadotropin-releasing hormone agonist, tamoxifen, aromatase inhibitor, treatment effectiveness, breast cancer.

### 5.3.4 Introduction

Breast cancer (BC) is the most frequently diagnosed and the most common cause of women cancer related death in the European Union (EU), with an estimated incidence and death rate of approximately 108.8 and 22.4 cases per 100 000 women/year, respectively.[2] The generalization of screening and the introduction of incrementally more efficacious adjuvant treatments contributed substantially to improve outcomes of patients diagnosed with BC at early stages. For the two thirds of patients with BC expressing the estrogen and/or progesterone receptors, collectively referred as hormone receptor (HR)-positive, it is well established the clinical utility of the use of hormone-related therapies.[10]

In the subset of premenopausal women, tamoxifen has been the mainstay of adjuvant endocrine therapy for more than 30 years.[25,120] However, recent studies showed that intensifying treatment with the combined use of ovarian function suppression (OFS) to either tamoxifen or aromatase inhibitors (AI) further improves cancer outcomes.[51,52,54] Particularly, results of the SOFT trial suggested that, after a median follow-up of 8 years and compared to tamoxifen alone, the addition of OFS to tamoxifen (OFS-T) improved overall survival (OS; a similar strong trend was also recorded for the association between OFS and an AI [OFS-AI]), especially in those patients judged to have a risk of recurrence justifying the use of adjuvant chemotherapy and among the very young patients (less than 35 years old).[52] In the group of women who were treated with chemotherapy, the 8-year OS estimates were 89.4% vs. 87.2% vs. 85.1% for the OFS-T, OFS-AI and the tamoxifen-only arm, respectively (hazard ratio [HR] 0.59, 95% confidence interval [CI] 0.42-0.84 for OFS-T vs. tamoxifen and HR 0.79, 95% CI 0.57-1.09 for OFS-AI vs. tamoxifen). In addition, for the group of women younger than 35, the 8-year DFS estimates (OS data not reported) were 80.0 vs. 74.6% vs. 64.9% for the OFS-AI, OFS-T and tamoxifen only arm, respectively. Furthermore, a consistent DFS advantage was also found for the combinations OFS-AI and OFS-T when compared to tamoxifen in the overall cohort.

Since 2006, the *Registo Oncológico Regional do Sul* (Portuguese southern cancer registry; ROR-S) collects detailed tumor and treatment data on a large cohort of women with newly diagnosed breast cancer. In this study of premenopausal women treated with adjuvant endocrine therapy we aim to 1) characterize real-world prescription of OFS and particularly to describe how recent data from clinical trials modified routine endocrine therapy practice, and 2) examine the short-term OS impact of OFS.

### 5.3.5 Patients and Methods

Study design and data source

This is an observational retrospective cohort study. Clinical data concerning five large centers located in Lisbon, Portugal was retrieved from ROR-S. ROR-S is a population-based cancer registry that serves as the unifying framework for variables definition, data registry and quality assurance. Due to the observational nature of the study, treatments and follow-up were performed at patient-physician description. ROR-S institutional review board (IRB) approved study protocol and ROR-S performed the oversight of study conduct. Description of data collection and procedures were previously reported.[87] We followed the STROBE statement in reports of cohort studies.

Patient selection

All consecutive premenopausal women diagnosed with hormone receptor (HR)-positive, non-metastatic breast cancer between January 2006 and December 2015, and treated at participating institutions (*Centro Hospitalar de Lisboa Norte, Instituto CUF de Oncologia, Hospital da Luz, Hospital de Beatriz Ângelo* and *Instituto Português de Oncologia de Lisboa*) were included. Patients with no information about surgery and with incomplete or missing information on adjuvant therapy were excluded. For this study, two cohorts of patients were defined: those patients treated with adjuvant OFS and those not treated with adjuvant OFS.

*Menopausal status and hormone receptor status*

ROR-S does not collect menopausal status. For this study, pre-menopausal status was defined as age at date of diagnosis younger than 50, a reference age adjusted to the Portuguese population.[88] Hormone receptor positivity was defined as either estrogen receptor positive and/or progesterone receptor positive with positivity defined as ≥1% of tumor cell nuclei immunoreactivity or tumor classified as “HRe-positive” in the patient medical records.

Study outcomes and variables

*Outcomes*

The primary study outcome was OS, defined as time from tumor diagnosis to death from any cause. Vital status, as register on ROR-S, is obtained from a centralized and electronic platform of national death certificates (*Sistema de Informação dos Certificados de Óbito* [SICO] managed by *Direção Geral de Saúde*). Follow-up was available up to December 2016. Given the nature of the data source, recurrences were not available.

As secondary outcomes we examined use of OFS and duration of OFS treatment. Administration of OFS was defined as the prescription of any OFS agent started after surgery and for at least two consecutive prescriptions. Duration of therapy was defined as the time from first to last treatment prescription plus 1 month (to account for treatment action). Four patients had oophorectomy shortly after introduction of adjuvant OFS and here considered as continuing OFS. No patient had upfront oophorectomy.

*Other covariates*

Study covariates included age at diagnosis, tumor characteristics (American Joint Committee on Cancer [AJCC] TNM staging, histology, grade and human epidermal growth factor receptor 2 [HER2] status); treatment characteristics (local and systemic) and year of diagnosis.

Statistical analysis

Descriptive statistics of patient, disease and treatment characteristics were performed. Differences of these features by use of OFS were tested using chi-squared test or Wilcoxon rank-sum test, as appropriate and univariate and multivariate logistic regression models. Variables included in the multivariate logistic model included year of diagnosis, age at diagnosis, histologic type, grade, HER2 status, type of surgery, radiotherapy, and (neo)adjuvant chemotherapy. Time-to-event outcomes were estimated and plotted using the Kaplan-Meier method. Survival rates were compared using Cox proportional hazards models. To deal with confounding, both multivariate Cox proportional hazards models and propensity score (PS) matching with a 1:1 matching were performed. Variables included both in the multivariate model and PS matching included: age at diagnosis, stage, histologic grade, HER2 status, use of (neo)adjuvant chemotherapy, type of surgery and year of diagnosis. The patient characteristics of the matched samples are shown in M3 Supplementary Table A.1. Two sensitivity analysis were performed: 1) to deal with eventual immortal-time bias, we performed a sensitivity analysis including only patients alive 1 year after surgery and 2) to test the robustness of findings in patients with longer follow-up, we completed a sensitivity analysis including only patients with a minimum follow-up of 3 and 5 years. All time-to-event analyses met proportional hazards assumption as assessed by the Schoenfeld residuals. We performed a complete full data analysis. The dataset had 100% completion data for survival

outcomes and for other variable, missing values did not exceed 8%. Missing information was considered missing at random. All tests were 2-sided and p-values of ≤ 0.05 were considered statistically significant. The analyses were performed using Stata 13.1 (StataCorp LP). For propensity score matching, Stata ado-file psmatch2 was used.[90]

**M3 Supplementary table A.1 –** Patients demographics, tumor characteristics and type of concomitant treatment by type of adjuvant endocrine therapy in the matching samples

| Variable list | PS matching (1:1) | | |
|---|---|---|---|
| | No ovarian function suppression | Ovarian function suppression | P-value (No OFS vs. OFS) |
| Number of patients (%) | 250 (50.0) | 250 (50.0) | NA |
| Follow-up, months | | | |
| Median | 34.8 | 36.5 | 0.419 |
| IQR | 14.6 – 63.7 | 16.1 – 62.6 | |
| **Demographic and clinicopathological characteristics** | | | |
| Age (years) | | | |
| ≤35 | 68 (27.2) | 84 (33.6) | 0.291 |
| >35 to ≤40 | 103 (41.2) | 92 (36.8) | |
| >40 to ≤50 | 79 (31.6) | 74 (29.6) | |
| Year of diagnosis | | | |
| 2006 – 2009 | 73 (29.2) | 64 (25.6) | 0.445 |
| 2010 – 2012 | 67 (26.8) | 79 (31.6) | |
| 2013 – 2015 | 110 (44.0) | 107 (42.8) | |
| pT (tumor size, pathological), n (%) | | | |
| pT0/1 | 174 (69.6) | 168 (67.7) | 0.103 |
| pT2 | 71 (28.4) | 66 (26.6) | |
| pT3/4 | 5 (2.0) | 14 (5.7) | |
| pN (nodes, pathological), n (%) | | | |
| Negative | 150 (60.0) | 148 (59.2) | 0.948 |
| pN1 | 66 (26.4) | 64 (25.6) | |
| pN2 | 23 (9.2) | 27 (10.8) | |
| pN3 | 11 (4.4) | 11 (4.4) | |
| Simplified TNM staging, n (%) | | | |
| Stage I | 115 (46.0) | 229 (91.6) | 0.331 |
| Stage II | 98 (39.2) | 13 (5.2) | |
| Stage III | 37 (14.8) | 8 (3.2) | |
| Histology, n (%) | | | |
| Invasive ductal carcinoma | 225 (90.0) | 229 (91.6) | 0.331 |
| Invasive lobular carcinoma | 20 (8.0) | 13 (5.2) | |
| Other | 5 (2.0) | 8 (3.2) | |
| Histological grade, n (%) | | | |
| Grade 1 | 39 (15.6) | 41 (16.4) | 0.694 |
| Grade 2 | 155 (62.0) | 146 (58.4) | |
| Grade 3 | 56 (22.4) | 63 (25.2) | |
| Hormone receptor status, n (%) | | | |
| ER and PR positive | 200 (85.5) | 202 (86.3) | 0.791 |
| ER or PR positive | 34 (14.5) | 32 (13.7) | |
| HER2 receptor, n (%) | | | |
| Negative | 207 (82.8) | 201 (80.4) | 0.489 |

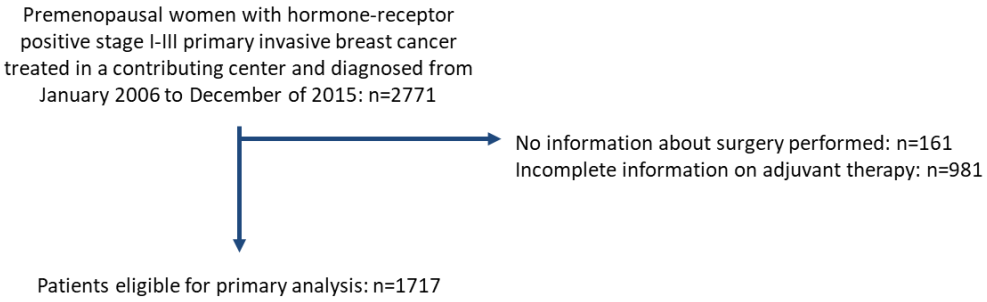
| | | | |
|---|---|---|---|
| Positive | 43 (17.2) | 49 (19.6) | |
| **Treatment characteristics** | | | |
| Surgery, n (%) | | | |
| Breast-conserving surgery | 120 (48.0) | 110 (44.0) | 0.370 |
| Mastectomy | 130 (52.0) | 140 (56.0) | |
| Radiotherapy, n (%) | | | |
| Yes | 176 (70.4) | 175 (70.0) | 0.922 |
| No | 74 (29.6) | 75 (30.0) | |
| (Neo)adjuvant chemotherapy, n (%) | | | |
| Yes | 187 (74.8) | 198 (79.2) | 0.242 |
| No | 63 (25.2) | 52 (20.8) | |

### 5.3.6 Results

Study sample and baseline characteristics

A total of 1717 consecutive eligible patients were included in the study analysis (M3 Supplementary Figure A.1), of which 294 (17.1%) received adjuvant OFS (goserelin in almost all cases) and 1423 (82.9%) did not.

**M3 Supplementary figure A.1 –** Patients flowchart




Baseline demographic and clinicopathological characteristics, as well as treatments received are summarized in M3 Table 1. Patients treated with OFS were younger (34.7% vs. 6.5% ≤35 years) and had less differentiated tumors (grade 3 in 24.8% vs. 16.4%), but similar TNM stage. Treatments also differed, with patients treated with OFS receiving more frequently mastectomy (56.6% vs. 48.4%) and (neo)adjuvant chemotherapy (78.9% vs. 72.7%). OFS was more commonly administered in combination with tamoxifen than with an AI (detailed bellow), and patients receiving tamoxifen (compared to those treated with an AI) tended to have more often node negative tumors (62.0% vs. 48.5%), less frequently histological grade 3 tumors (24.1% vs. 30.3%) and received less frequently adjuvant chemotherapy (administered in 77.8% vs. 87.9%).

**M3 Table 1 –** Patients demographics, tumor characteristics and type of concomitant treatment by type of adjuvant endocrine therapy

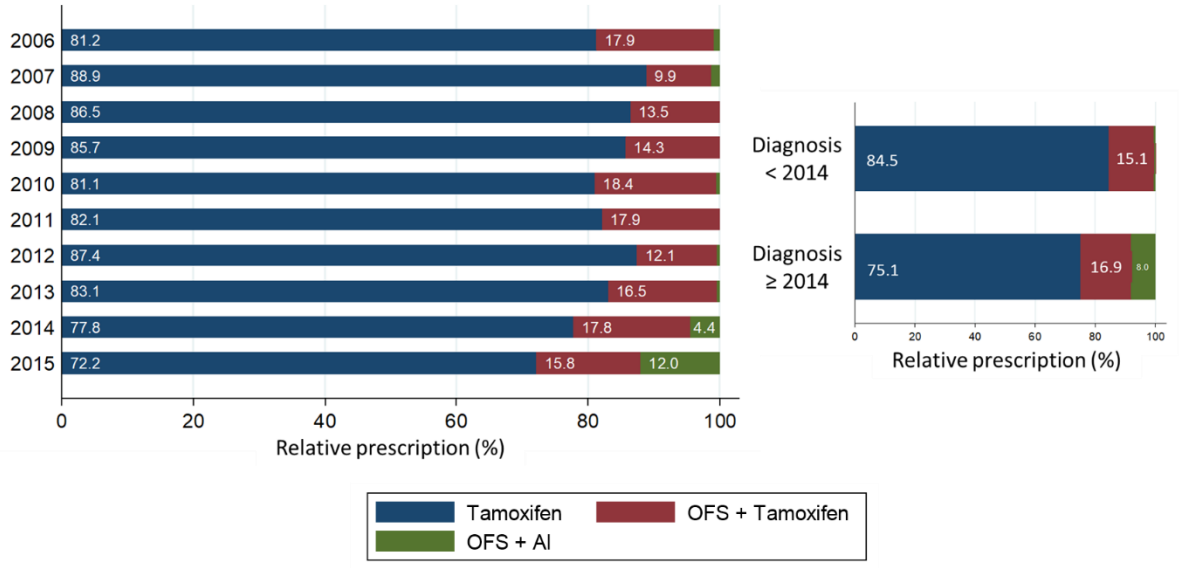
| Variable list | No ovarian function suppression (Tamoxifen only) | Ovarian function suppression | Ovarian function suppression | | P-value (No OFS vs. OFS) |
|---|---|---|---|---|---|
| | | | OFS + Tamoxifen | OFS + AI | |
| Number of patients (%) | 1423 (82.9) | 294 (17.1) | 261 (15.2) | 33 (1.9) | - |
| **Demographic and clinicopathologic characteristics** | | | | | |
| Age (years) | | | | | |
| ≤35 | 93 (6.5) | 102 (34.7) | 92 (32.3) | 10 (30.3) | **<0.001** |
| >35 to ≤40 | 233 (16.4) | 109 (37.1) | 100 (38.3) | 9 (27.3) | |
| >40 to ≤50 | 1097 (77.1) | 83 (28.2) | 69 (26.4) | 14 (42.4) | |
| Year of diagnosis | | | | | |
| 2006 – 2009 | 468 (32.9) | 77 (26.2) | 74 (28.4) | 3 (9.1) | **0.003** |
| 2010 – 2012 | 487 (34.2) | 90 (30.6) | 88 (33.7) | 2 (6.1) | |
| 2013 – 2015 | 468 (32.9) | 127 (43.2) | 99 (37.9) | 28 (84.8) | |
| pT (tumor size, pathological), n (%)[1] | | | | | |
| pT0/1 | 913 (65.9) | 190 (66.9) | 169 (67.3) | 21 (63.6) | 0.320 |
| pT2 | 422 (30.5) | 79 (27.8) | 70 (27.9) | 9 (27.3) | |
| pT3/4 | 50 (3.6) | 15 (5.3) | 12 (4.8) | 3 (9.1) | |
| Missing | 38 (2.7) | 10 (3.4) | 10 (3.8) | 0 | |
| pN (nodes, pathological), n (%) | | | | | |
| Negative | 845 (60.2) | 176 (60.5) | 160 (62.0) | 16 (48.5) | 0.847 |
| pN1 | 380 (27.0) | 74 (25.4) | 64 (24.8) | 10 (30.3) | |
| pN2 | 133 (9.5) | 29 (10.0) | 25 (9.7) | 4 (12.1) | |
| pN3 | 46 (3.3) | 12 (4.1) | 9 (3.5) | 3 (9.1) | |
| Missing | 19 (1.3) | 3 (1.0) | 3 (1.2) | 0 | |
| Simplified TNM staging, n (%) | | | | | |
| Stage I | 603 (43.6) | 129 (45.1) | 117 (46.3) | 12 (36.4) | 0.703 |
| Stage II | 583 (42.1) | 113 (39.5) | 100 (39.5) | 13 (39.4) | |
| Stage III | 198 (14.3) | 44 (15.4) | 36 (14.2) | 8 (24.2) | |
| Missing | 39 (2.7) | 8 (2.7) | 8 (3.1) | 0 | |
| Histology, n (%) | | | | | |
| Invasive carcinoma of NST | 1217 (85.5) | 268 (91.2) | 238 (91.2) | 30 (90.9) | **0.024** |
| Invasive lobular carcinoma | 126 (8.9) | 13 (4.4) | 11 (4.2) | 2 (6.1) | |
| Other | 80 (5.6) | 13 (4.4) | 12 (4.6) | 1 (3.1) | |
| Histological grade, n (%) | | | | | |
| Grade 1 | 268 (20.1) | 44 (15.6) | 37 (14.9) | 7 (21.2) | **0.002** |
| Grade 2 | 849 (63.6) | 168 (59.6) | 152 (61.0) | 16 (48.5) | |
| Grade 3 | 219 (16.4) | 70 (24.8) | 60 (24.1) | 10 (30.3) | |
| Missing | 87 (6.1) | 12 (4.1) | 12 (4.6) | 0 | |
| Hormone receptor status, n (%) | | | | | |
| ER and PR positive | 1159 (89.0) | 240 (86.6) | 210 (85.7) | 30 (93.7) | 0.259 |
| ER or PR positive | 143 (11.0) | 37 (13.4) | 35 (14.3) | 2 (6.3) | |
| HER2 receptor, n (%) | | | | | |
| Negative | 1120 (85.6) | 227 (81.1) | 202 (81.5) | 25 (78.1) | 0.058 |
| Positive | 189 (14.4) | 53 (18.9) | 46 (18.6) | 7 (21.9) | |
| Missing | 114 (8.0) | 14 (4.8) | 13 (5.0) | 1 (3.0) | |
| **Treatment characteristics** | | | | | |
| Surgery, n (%) | | | | | |
| Breast-conserving surgery | 701 (51.6) | 121 (43.4) | 105 (42.5) | 16 (50.0) | **0.012** |
| Mastectomy | 658 (48.4) | 158 (56.6) | 142 (57.5) | 16 (50.0) | |

| | | | | | |
|---|---|---|---|---|---|
| Missing | 64 (4.5) | 15 (5.1) | 14 (5.4) | 1 (3.0) | |
| Radiotherapy, n (%) | | | | | |
| Yes | 1065 (74.8) | 204 (69.4) | 80 (30.7) | 10 (30.3) | **0.053** |
| No | 358 (25.2) | 90 (30.6) | 181 (69.3) | 23 (69.7) | |
| (Neo)adjuvant chemotherapy, n (%) | | | | | |
| No | 388 (27.3) | 62 (21.1) | 58 (22.2) | 4 (12.1) | **0.028** |
| Yes | 1035 (72.7) | 232 (78.9) | 203 (77.8) | 29 (87.9) | |
| Yes, neoadjuvant | 276 (19.4) | 83 (28.2) | 68 (26.1) | 15 (45.5) | |

Patterns of endocrine therapy use

In this cohort of premenopausal women, only a minority of patients received OFS as part of the adjuvant endocrine therapy strategy (294 patients, 17.1%). Median time to introduction of OFS was 5.1 months (IQR 1.4 – 8.6; max. 14.0). Of those receiving OFS, 261 (15.2%) received it in combination with tamoxifen, while 33 (1.9%) in combination with an AI. There was evidence of OFS use since the beginning of the cohort in 2006, but in 2014 there was a significant increase in the use of OFS: 15.5% received OFS before 2014, while approximately 25% received OFS in or after 2014 (M3 Figure 1).

**M3 Figure 1 –** Patterns of prescription of adjuvant endocrine therapy over time




A similar trend was noted for the combination with AI, with 0.4% receiving OFS in combination with an AI before 2014, and 8% from 2014 onwards; in contrast, the combination with tamoxifen was relatively stable (15.1% before 2014 and 16.9% from 2014 onwards). Prescription of OFS over time and according to age and disease stage is depicted in M3 Figure 2. A consistent trend

for OFS use in younger patients was clear, reaching 87.5% of patients in those with ≤35 years old in 2015 in contrast with 13.2% in those >40 years old in the same year.

**M3 Figure 2 –** Patterns of prescription of adjuvant endocrine therapy over time and according to age at diagnosis and UICC/AJCC TNM staging


In the univariate analysis, features associated with the use of OFS included age at diagnosis, year of diagnosis, histologic type, grade, type of surgery, and treatment with radiotherapy and (neo)adjuvant chemotherapy. However, in the multivariate model, only age at diagnosis (reference > 40 and ≤50 years; OR 14.7, 95% CI 9.7 – 22.1 for ≤35 and OR 6.1, 95% CI 4.3 – 8.7 for > 35 and ≤40 years) and year of diagnosis after 2014 (OR 1.9, 95% CI 1.3-2.7) were associated with the use of OFS (M3 Table 2). Despite the predominant use of combination with tamoxifen, 32% of patients received an AI in the interval from 2014 to 2015.

**M3 Table 2 –** Patient and tumor features associated with OFS prescription

| Variable list | **Predictors of OFS prescription** | | |
|---|---|---|---|
| | OR | 95% CI | p-value |
| **Univariate analysis** | | | |
| Age at diagnosis<br><35<br>35 to <40<br>40 to <50 | <br>14.5<br>6.2<br>Reference | <br>10.1 – 20.8<br>4.5 – 8.5<br>Reference | **<0.001** |
| Year of diagnosis (for each added year since 2006) | 1.08 | 1.03 – 1.13 | **0.002** |
| Year of diagnosis<br>Before 2014<br>In or after 2014 | <br>Reference<br>1.84 | <br>Reference<br>1.38 – 2.45 | **< 0.001** |
| Pathologic staging<br>Stage I | <br>Reference | <br>Reference | 0.920 |

| | | | |
|---|---|---|---|
| Stage II | 0.91 | 0.69 – 1.20 | |
| Stage III | 1.04 | 0.71 – 1.52 | |
| Histology | | | |
| Invasive carcinoma of NST | Reference | Reference | **0.039** |
| Invasive lobular carcinoma | 0.47 | 0.26 – 0.84 | |
| Other | 0.74 | 0.40 – 1.35 | |
| Histologic grade | | | |
| Grade 1 | Reference | Reference | **0.001** |
| Grade 2 | 1.21 | 0.84 – 1.73 | |
| Grade 3 | 1.95 | 1.28 – 2.95 | |
| HER2 status | | | |
| Negative | Reference | Reference | 0.059 |
| Positive | 1.38 | 1.99 – 1.94 | |
| Type of surgery | | | |
| Breast conserving surgery | Reference | Reference | **0.013** |
| Mastectomy | 1.39 | 1.07 – 1.80 | |
| Radiotherapy | | | |
| No | Reference | Reference | **0.053** |
| Yes | 0.76 | 0.58 – 1.00 | |
| (Neo)adjuvant chemotherapy | | | |
| No | Reference | Reference | **0.029** |
| Yes | 1.40 | 1.04 – 1.90 | |
| **Multivariate model** (full model shown) | | | |
| Age at diagnosis | | | |
| Less or equal to 35 | 14.7 | 9.74 – 22.1 | **< 0.001** |
| More than 35 to less than 40 | 6.12 | 4.32 – 8.67 | |
| More than 40 to less than 50 | Reference | Reference | |
| Year of diagnosis | | | |
| Before 2014 | Reference | Reference | **< 0.001** |
| In or after 2014 | 1.89 | 1.34 – 2.67 | |
| Histology | | | |
| Invasive carcinoma of NST | Reference | Reference | 0.171 |
| Invasive lobular carcinoma | 0.75 | 0.39 – 1.43 | |
| Other | 0.63 | 0.27 – 1.43 | |
| Histologic grade | | | |
| Grade 1 | Reference | Reference | 0.156 |
| Grade 2 | 1.04 | 0.68 – 1.59 | |
| Grade 3 | 1.44 | 0.85 – 2.43 | |
| HER2 status | | | |
| Negative | Reference | Reference | 0.969 |
| Positive | 0.99 | 0.66 – 1.48 | |
| Type of surgery | | | |
| Breast-conserving surgery | Reference | Reference | 0.526 |
| Mastectomy | 1.11 | 0.78 – 1.56 | |
| Radiotherapy | | | |
| No | Reference | Reference | 0.399 |
| Yes | 0.85 | 0.58 – 1.24 | |
| (Neo)adjuvant chemotherapy | | | |
| No | Reference | Reference | 0.243 |
| Yes | 0.79 | 0.53 – 1.18 | |

CI – Confidence interval; HG – Histologic grade; HER2 – Human epidermal growth factor receptor 2; OR – Odds ratio.

Among patients treated with OFS and with available date of treatment status, approximately 6% were still receiving OFS at the time of analysis. In those with available date of

treatment completion (35.2%), median duration of OFS was of approximately 25 months (IQR 20 – 27; M3 Table 3).

**M3 Table 3 –** Adjuvant endocrine treatment description

| | Overall | Before 2014 | In or after 2014 | P-value (<2014 vs. ≥2014) |
|---|---|---|---|---|
| Receiving OFS, n (%) | 294 (17.1) | 210 (15.2) | 84 (24.9) | **< 0.001** |
| Concomitant therapy to OFS, n (%) | | | | |
| Tamoxifen | 261 (88.8) | 204 (97.1) | 57 (67.9) | **< 0.001** |
| Aromatase inhibitor | 33 (11.2) | 6 (2.9) | 27 (32.1) | |
| Time on OFS† | | | | |
| Median, months | 24.7 | 24.9 | NR | NA |
| P25 – P75 | 20.4 – 26.9 | 21.5 – 26.9 | | |
| Min. – Max. | 1.6 – 65.9 | 1.6 – 65.9 | | |
| Date of completion available, n (%) | 99 (33.6) | 78 (43.8) | | |
| Ongoing treatment, n (%) | 17 (5.8) | 4 (2.2) | | |

†Time on OFS excludes patients with ongoing treatment with goserelin at time of data cut-off and those with prescription in of after 2013. NR–not reported; OFS–ovarian function suppression.

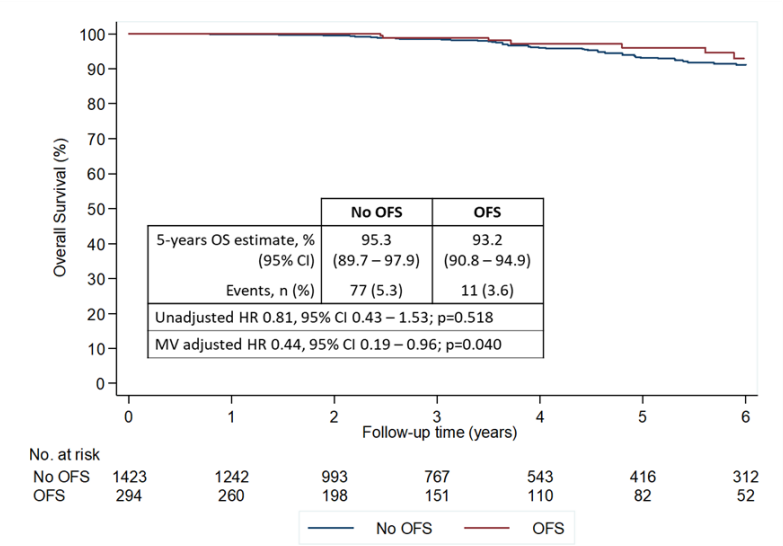
Effectiveness of ovarian function suppression

After a median follow-up of 38.3 months (interquartile range [IQR] 19.6 – 66.4; minimum – maximum 1.8 - 125), 88 deaths were registered, 11 (3.6%) in the OFS cohort and 77 (5.3%) in the no OFS cohort. The median follow-up is balanced between treatment cohorts, with 38.9 (IQR 20.5 - 67.7) months in the no OFS cohort and 36.5 (15.9 - 62.6) months in the OFS cohort (p=0.231). The proportion of patients alive at 5 years was 95.3% (95% CI 89.7 – 97.9) in the OFS cohort and 93.2% (95% CI 90.8 – 94.9%) in the no OFS cohort (M3 Figure 3-A). Overall survival by treatment arm and according to age at diagnosis and staging is shown in M3 Supplementary figure A.2. In a multivariate model controlling for age at diagnosis, stage, histologic grade, HER2 status, use of (neo)adjuvant chemotherapy, type of surgery and year of diagnosis, patients receiving adjuvant OFS had a 56% decrease in the risk of death (HR 0.44, 95% CI 0.19-0.96; p=0.04). Similar results were observed when performing a sensitivity analysis including only patients alive at 1 year (adjusted-HR 0.44, 95% CI 0.20 – 0.97) and with a minimum follow-up of 3 years (adjusted-HR 0.43, 95% CI 0.18 – 1.01) and 5 years (adjusted-HR 0.39, 95% CI 0.12 – 1.23). With a 2.1% absolute difference in survival at five years, the number needed to treat to avoid one death was of 48.

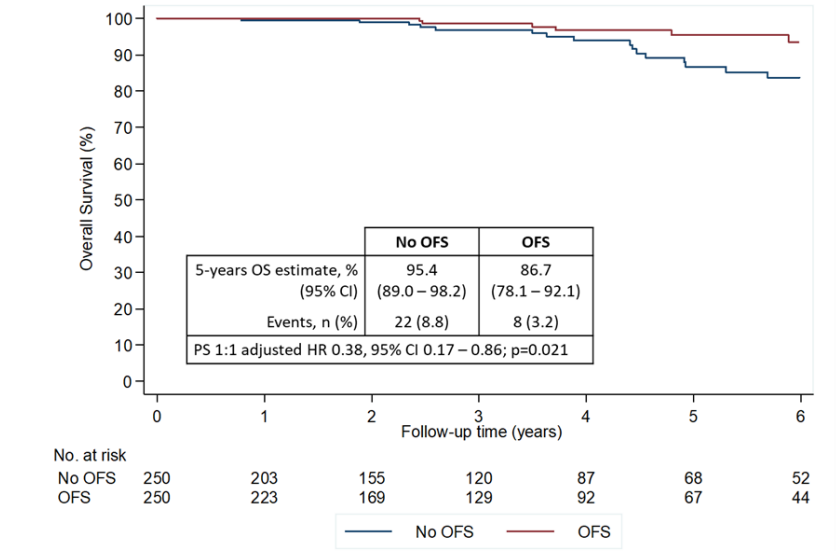
The propensity score matching cohort results were consistent with those of Cox proportional hazards multivariate analysis (M3 Figure 3-B). While 8 patients died in the OFS cohort (3.2%), 22 died in the no OFS cohort (8.8%). Proportion of patients alive at 5 years was 95.4% (95% CI 89.0 – 98.2) in the OFS cohort and 86.7% (95% CI 78.1 – 92.1%) in the no OFS cohort. Patients receiving adjuvant OFS had a 62% decrease in the risk of death (HR 0.38, 95% CI 0.17-0.86; p=0.021).

**M3 Figure 3 –** Overall survival in the overall cohort (A) and propensity score matching (1:1 matching) cohort (B). Variables included both in the multivariate CM and PS matching included: age at diagnosis, stage, histologic grade, HER2 status, use of (neo)adjuvant chemotherapy, type of surgery and year of diagnosis.

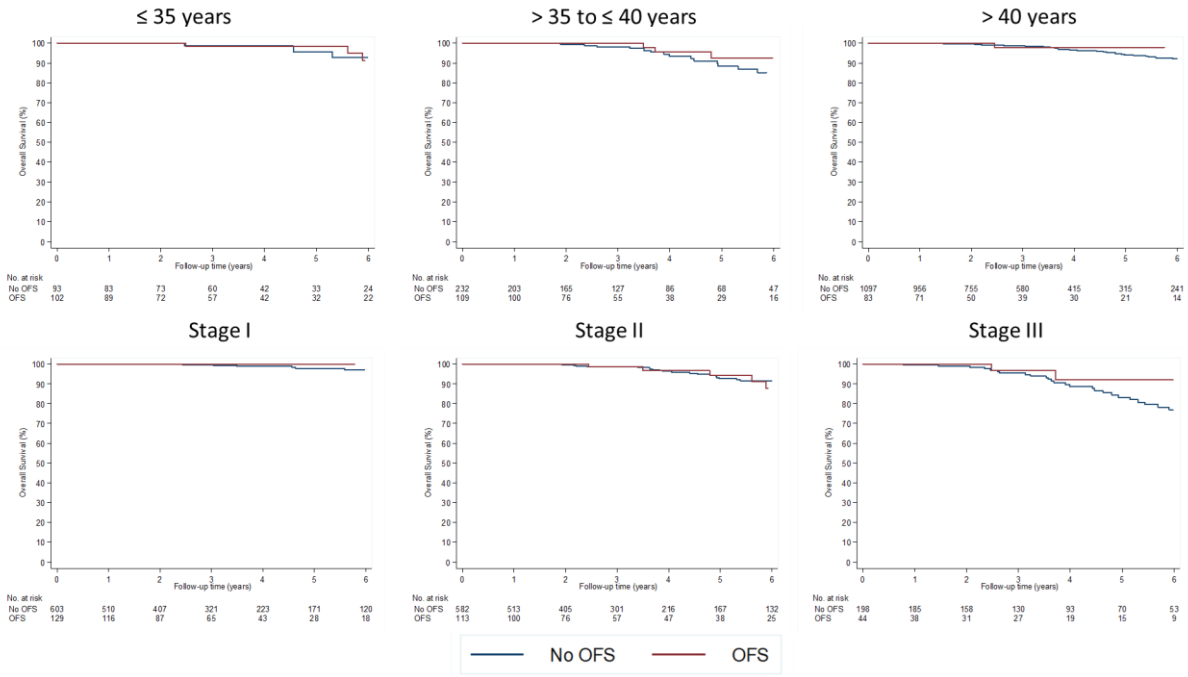
A




B



**M3 Supplementary Figure A.2 –** Overall survival by treatment arm in subgroups defined by age at diagnosis and UICC/AJCC TNM staging.




## 5.3.7 Discussion

In this large real-world cohort of premenopausal women with breast cancer receiving adjuvant endocrine therapy we observed an increment in the use of OFS in recent years. Moreover, young age at diagnosis (≤ 35 years old) was strongly associated with the use of adjuvant OFS. Albeit the short follow-up time, treatment with OFS combined with either tamoxifen or AI improved short-term OS.

Over the last decades, several randomized trials and meta-analyses examined the impact of adding OFS to backbone endocrine therapy (ET) (M3 Supplementary Table A.2). Overall, it emerged from these trials that specific populations, such as premenopausal women with enough risk of recurrence to be eligible for adjuvant chemotherapy, as well as younger women, may derive benefit from adding OS to ET.[51–53,121–124]. In addition, the most recent SOFT trial results further revealed an overall survival advantage for the combination OFS-T.[52]

**M3 Supplementary Table A.2 –** Summary of adjuvant trials and meta-analyses comparing tamoxifen with or without OFS and OFS with either tamoxifen or an AI†

| Study (year of last results update) | Treatment arms | Sample size | Follow-up | Disease-free survival | Overall survival |
|---|---|---|---|---|---|
| **Clinical trials: tamoxifen vs. tamoxifen + OFS** | | | | | |
| SOFT (2018)[51,52] | Tamoxifen alone vs. tamoxifen + OFS (vs. exemestane + OFS) | 2033 | 8.0 | HR 0.76, 95% CI 0.62 – 0.93; p=0.009 | HR 0.67, 95% CI 0.48 – 0.92; p=0.01 |
| ASTRRA (2018)[53] | Tamoxifen alone vs. tamoxifen + OFS | 1282 | 5.3 | HR 0.686, 95% CI 0.48 - 0.97; p=0.033 | HR 0.310, 95% CI 0.10 - 0.94; p=0.029 |
| E-3193/INT-0142 (2014)[125] | Tamoxifen alone vs. tamoxifen + OFS | 345 | 9.9 | HR 1.16, 95% CI 0.64 - 2.08; p=0.62 | HR 1.19, 95% CI 0.52 - 2.70; 0.67 |
| ABC/OAS (2007)[126] | Tamoxifen alone vs. tamoxifen + OFS/ablation | 2144 | 5.9 | HR 0.95, 95% CI 0.81 - 1.12; p=0.56 | HR 0.94, 95% CI 0.78 - 1.13; p = 0.44 |
| ZIPP (2005)[127] | Tamoxifen alone vs. tamoxifen + OFS | 2710 | 5.5 | HR 0.80, 95% CI 0.69 - 0.92; p=0.002 | HR 0.81, 95% CI 0.67 - 0.99; p = 0.038 |
| INT 0101/E5188 (2005)[128] | CAF vs. CAF + OFS vs. CAF + OFS + tamoxifen | 1503 | 9.6 | HR 0.93, 95% CI 0.76 - 1.12; p=0.22 | HR 0.88, 95% CI 0.70 - 1.11; p=0.14 |
| **Meta-analyses: tamoxifen vs. tamoxifen + OFS** | | | | | |
| Zhang (2017)[122] | OFS + tamoxifen vs. tamoxifen | 7331 | NR | HR 0.94, 95% CI 0.88 – 1.01; p=0.09 | HR 0.92, 95% CI 0.82 – 1.03; p=0.13 |
| Qiu (2016)[123] | OFS + tamoxifen vs. tamoxifen‡ | 12292 | NR | RR 0.86, 95% CI 0.75 – 0.96; p=NR | RR 0.79, 95% CI 0.70 – 0.89; p=NR |
| Yan (2015)[124] | OFS + tamoxifen vs. tamoxifen | 6279 | NR | RR 0.87, 95% CI 0.71 – 1.06; p=0.16 | RR 0.84, 95% CI 0.66 – 1.07; p=0.16 |
| Cuzick (2007)[121] | OFS + tamoxifen vs. tamoxifen | 1013 | NR | HR 0.85, 95% CI 0.67 – 1.09; p=0.20 | HR 0.84, 95% CI 0.59 – 1.19; p=0.33 |
| **Clinical trials: OFS + tamoxifen vs. OFS + AI** | | | | | |
| SOFT/TEXT (2018)[52,54] | OFS + tamoxifen vs. OFS + exemestane (vs. tamoxifen alone) | 4690 | 9.0 | HR 0.77, 95% CI 0.67 – 0.90; p<0.001 | HR 0.98, 95% CI 0.79 – 1.22; p=0.84 |
| ABCSG-12 (2015)[129] | OFS + tamoxifen vs. OFS + anastrazol | 1803 | 7.9 | HR 1.13, 95% CI 0.88 – 1.45; p=0.335 | HR 1.63, 95% CI 1.05 – 1.45; p=0.030 |
| HOBOE-2 (2018)[130] | OFS + tamoxifen vs. OFS + letrozole (vs. OFS + letrozole + ZA) | 710ǂ | 5.4 | HR 0.72, 95% CI 0.48 – 1.07; p=0.06 | NR |

†NSABP-30 and ABCSG 13-93 studies further showed that patients achieving chemotherapy-induced amenorrhea had improved survival. The 2005 EBCTCG meta-analysis compared adjuvant OFS/ablation to no further ET showing a significant effect of OFS on both DFS and OS. ‡Tamoxifen or other ET beyond OFS was not provided in all trials included in this meta-analysis. ǂ1065 patients if including the OFS + letrozole + ZA arm. AI–aromatase inhibitor; CI–confidence interval; ET–endocrine therapy; HR–hazard ratio; NR–not reported; OFS–ovarian function suppression; RR–relative risk; Vs–versus; ZA – zoledronic acid.

The first results of the combined analysis of SOFT and TEXT trials in 2014, suggesting the benefit of OFS in some populations, led to the incorporation of this recommendation in several breast cancer treatment guidelines.[25,120] In fact, in the present study, although there is evidence of utilization of OFS since 2006, there was a substantial increment of its use after 2014. As expected, in this study of patients with HR-positive tumors diagnosed between 2006 and 2015, of whom more than 70% treated with (neo)adjuvant chemotherapy, we observed that patients at a higher risk of relapse receive OFS more frequently. We further observed that adjuvant OFS added to tamoxifen

or AI led to a statistically significant reduction in the risk of death, with an absolute magnitude in line with previous achievements in adjuvant ET and to the updated results of SOFT trial. Although these results might be influenced by unmeasured confounders, short median follow-up and time bias (given the increase in use of OFS overtime), the treatment effect was consistently present when using different methods to deal with confounding. Thus, this study adds real-world evidence to clinical trials data, supporting the decision of patients and physicians to incorporate OFS in the ET of premenopausal women at higher risk of recurrence.

These results must be put in context of the tolerability implications of OFS. In the SOFT trial, patients randomized to tamoxifen plus OFS had more frequently hot flashes, loss of sexual interest and sleep disturbance, as well as vaginal dryness, with early discontinuation of oral ET close to 20%.[66] Interestingly, after 6 months of therapy, symptom-specific treatment differences were less evident in those patients previously treated with chemotherapy. Other studies further showed a detrimental effect of OFS in self-reported health-related quality of life.[125] However, no particular changes in global cognitive function, nor depression or anxiety scores were noted.[131,132]

The use of OFS further opens the possibility of using AIs in premenopausal women. The incremental efficacy of OFS and AI (versus OFS-T) is not definitely established (M3 Supplementary Table A.2). While the ABCSG-12 trial did not document any DFS advantage of OFS-AI over OFS-T and even found a statistically significant detrimental impact of OFS-AI compared to OFS-T (of note, this trial also tested the role of adjuvant zoledronic acid), the analyses of SOFT and TEXT trials showed that patients treated with chemotherapy who remained premenopausal and those with <35 years (higher risk patients) are the ones obtaining the most benefit from AIs (absolute breast cancer-free interval reduction ranging from 5 to 15%).[129,133,134] However, no OS differences were noted in the overall SOFT/TEXT cohort (HR 0.98, 95% CI 0.79–1.22). In terms of tolerability, both the toxicity profile and their evolution over time differ: patients taking tamoxifen plus OFS had more hot flashes and sweats that improve over time, while those on exemestane plus OFS had more vaginal dryness, greater loss of sexual interest, and difficulties becoming aroused that persist over time.[135] No major differences in quality of life over time were captured with the instruments used. Of note, current guidelines consider both AI and tamoxifen reasonable alternatives when added to OFS, even though ASCO guidelines favor the use of AI in women <35 years.[25,120]

OFS is also being increasingly used as an approach to reduce the likelihood of chemotherapy-induced ovarian insufficiency and thus as a complementary strategy to improve future fertility without impacting survival.[136–138] Such use is reflected in current international guidelines.[139–141] While we had access to the exact date of OFS initiation, in our cohort the date of (neo)adjuvant chemotherapy introduction was not thoroughly available beyond the knowledge of

its administration before or after surgery. This limited the possibility of describing the use of OFS as a fertility preservation strategy in our cohort of premenopausal women.

Despite the large sample size and the methodological rigor, this study has limitations. It is a retrospective observational study, thus susceptible to residual confounding. ROR-S does not collect menopausal status, both at diagnosis and after primary treatment, that was estimated for local patterns. Also, ROR-S does not accurately collect co-morbidities, educational level, type of (neo)adjuvant chemotherapy, patients' preferences or Ki67 that can be unbalanced between arms; nevertheless, we used different modelling strategies to address confounding with consistent findings. Therapy administration was measured by drug prescription, not actual drug administration, and a substantial proportion of patients did not have information available concerning treatment stop date. Also, median follow-up is short for a HR-positive population and there might exist a time bias associated with an increase in use of OFS over time. The follow-up is impacted by the inclusion of patients up to the date of censoring, but this was done to extract the most information possible from the analysis focusing on the patterns of use of OFS. In addition, the sensitivity analysis restricting to patients with 5 or more years of follow-up showed consistent results. Moreover, the follow-up is balanced between the two groups. While unexpected, the fact that the use of OFS is present since the beginning of the cohort, the fact that the increased uptake of OFS is predominantly achieved in the very later years of the cohort, and the fact that the overall absolute number of patients receiving OFS is lower than those not receiving this treatment explain the balanced follow-up and add to the robustness of the analysis. Finally, treatment effectiveness was measured as OS, not cancer-specific survival, and DFS was not available. While cancer-specific survival could add some extra robustness to our study, the relatively young age of this group of patients might increase the likelihood of the identification of mostly cancer-specific deaths. While ROR-S exhaustively collects OS through its electronic connection to the national death certificates database, as a population-based registry, recurrence events need to be proactively reported by contributing centers leading to a relevant proportion of patients with missing DFS status and thus rendering this outcome not useful for clinical research at this point in time.

### 5.3.8 Conclusion

Now that intensification of ET with OFS in pre-menopausal women with HR-positive breast cancer at high risk of relapse is becoming standard of care, this large cohort of premenopausal women receiving adjuvant ET shows real-world evidence that supports these guidelines. Since 2014, a quarter of patients were treated with adjuvant OFS, of which more than 30% in combination with an AI. Use of adjuvant OFS showed an OS benefit.

### 5.3.9 Funding and role of funding source

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## 5.4 Differential impact of endocrine therapy and chemotherapy on quality of life of breast cancer survivors: a prospective patient reported outcomes analysis.

### 5.4.1 Introductory notes

This project details several QoL metrics of breast cancer survivors 2 years after diagnosis. It further dissects the QoL implications according to the use of adjuvant endocrine therapy and/or chemotherapy and by menopausal status. This project was published in Annals of Oncology. Arlindo R. Ferreira led the study design, data analysis, results interpretation and manuscript writing.

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### 5.4.3 Abstract

Background: In early breast cancer (BC) there has been a trend to escalate endocrine therapy (ET) and to de-escalate chemotherapy (CT). However, the impact of ET versus CT on the quality of life (QoL) of early BC patients is unknown. Here we characterize the independent contribution of ET and CT on patient-reported outcomes (PROs) at 2-years after diagnosis.

Patients and methods: we prospectively collected PROs in 4262 eligible patients using the European Organization for Research and Treatment of Cancer QLQ-C30/BR23 questionnaires inside CANTO trial (NCT01993498). The primary outcome was the C30 summary score (C30-SumSc) at 2-years after diagnosis.

Results: From eligible patients, 37.2% were premenopausal and 62.8% postmenopausal; 81.9% received ET and 52.8% CT. In the overall cohort, QoL worsened by 2-years after diagnosis in multiple functions and symptoms; exceptions included emotional function and future perspective, which improved over time. ET ($p_{int}$=0.004), but not CT ($p_{int}$=0.924), had a persistent negative impact on the C30-SumSc. In addition, ET negatively impacted role and social function, pain, insomnia, systemic therapy side effects, breast symptoms and further limited emotional function and future perspective recovery. Although CT had no impact on the C30-SumSc at 2-years it was associated with deteriorated physical and cognitive function, dyspnea, financial difficulties, body image and breast symptoms. We found a differential effect of treatment by menopausal status; in premenopausal patients, CT, despite only a non-significant trend for deteriorated C30-SumSc ($p_{int}$=0.100), was more frequently associated with QoL domains deterioration than ET, whereas in postmenopausal patients, ET was more frequently associated with QoL deterioration, namely using the C30-SumSc ($p_{int}$=0.004).

Conclusion(s): QoL deterioration persisted at 2-years after diagnosis with different trajectories by treatment received. ET, but not CT, had a major detrimental impact on C30-SumSc, especially in postmenopausal women. These findings highlight the need to properly select patients for adjuvant ET escalation.

Keywords: early breast cancer; quality of life; endocrine therapy; chemotherapy; patient-reported outcome.

### 5.4.4 Introduction

Due to improvements in early detection and treatment achieved over the last decades, 80-90% of women diagnosed with early-stage breast cancer (BC) in developed countries can expect long-term disease-free survival. With the growing number of women with history of BC, it is becoming increasingly important to address the potential long-term and late effects of treatments that survivors will face.[56]

There have been remarkable changes in the pattern of treatment of early BC in the last few years. Notable is the recent trend to escalate ET in patients with hormone receptor (HR)-positive early BC by extending the duration of treatment and/or by treatment intensification with the

addition of ovarian function suppression (OFS) for premenopausal patients.[25] Concurrently, there has been a trend to de-escalate chemotherapy (CT), driven by a desire to avoid short and long-term toxicities and the results of prospective trials that identified genomically low-risk patients who could be spared CT and treated with endocrine therapy (ET) alone.[142]

Despite their proven efficacy in improving BC outcomes, both ET and CT have the potential to negatively impact survivors' QoL.[143–145] ET strategies such as tamoxifen, aromatase inhibitors (AI) and OFS have well described and persistent side effects that may facilitate deterioration of QoL, although most clinical trials data indicate that the impact of ET on QoL of BC patients is only modest.[66] The deterioration in QoL might further negatively impact adherence and persistence to ET leading to early treatment discontinuation.[146,147] CT also worsens QoL, and this effect is well demonstrated through active treatment and in the immediate post-CT phase. However, there are few data on the long-term independent effect of CT on QoL. In addition, the differential impact of ET versus CT on QoL has not been fully characterized, especially among cohorts treated with modern adjuvant regimens using validated and modern tools to measure patient reported outcomes (PROs).[148] Such information could provide objective guidance for patients and physicians to weight the impact of each of these treatments on QoL and to define future research priorities in this evolving field.

We therefore compared the impact of different classes of treatment (CT and ET) on European Organization for Research and Treatment of Cancer (EORTC)-defined QoL instruments using CANTO (NCT01993498), a multicenter, nationwide, prospective cohort study of 12,012 women with stage I-III BC, of which 5,801 women available for research, that aims to quantify the toxicities of cancer treatment for up to 5 years after the end of primary treatment. We hypothesized that exposure to different classes of treatment, namely ET and/or CT, would have different impact on QoL 2-years after diagnosis. Moreover, we hypothesized that such impact would differ by menopausal status, given the different class of ET agents used (mostly tamoxifen in premenopausal and AIs in postmenopausal women) and the different *sequelae* of CT (with possible early loss of ovarian function in premenopausal women) by menopausal status.

### 5.4.5 Patients and methods

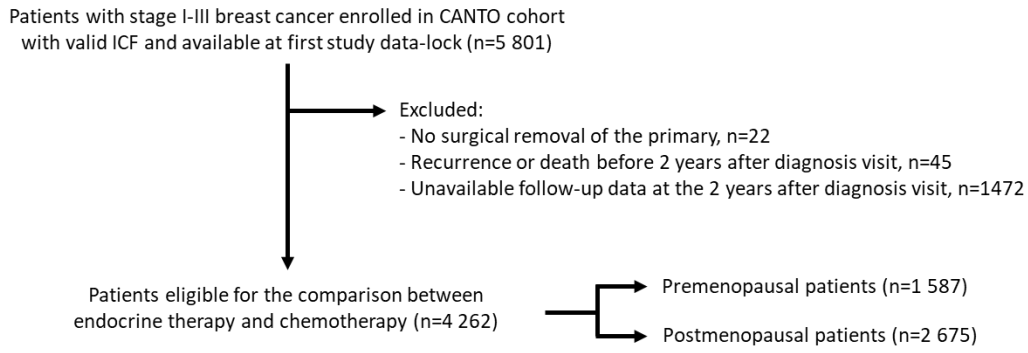
**Study design and patient selection**

This was a prospective, longitudinal cohort study. We used data collected at diagnosis, end of primary treatment, which include completion of BC surgery, chemotherapy, or radiation therapy, whatever ended last (median time from diagnosis=10.4 months, interquartile range [IQR], 8.0-12.3)

and at 2-years after diagnosis (median time from diagnosis=22.6 months, IQR 20.1-24.8; patients receiving ET were at a median of 16.3 months, IQR 14.9-17.9, into ET).

We included 4262 patients with stage I-III BC enrolled in CANTO cohort from March-2012 to January-2015, corresponding to the first data lock of CANTO. M4 Supplementary Figure 1 details exclusion and inclusion criteria. All patients provided written informed consent.

**M4 Supplementary Figure 1 -** Study consort diagram




To assess the potential bias introduced by the exclusion of patients with missing evaluation 2-years after diagnosis, the characteristics of such patients were compared to those of participating patients. Patients missing evaluation tended to be older, smokers, less educated, living alone, have lower income, present higher TNM stage or triple-negative BC, have undergone mastectomy and be more frequently depressed (M4 Supplementary Table 1).

**M4 Supplementary Table 1 –** Demographic, clinical and pathological characteristics at baseline and treatment details of study participants, overall non-participants and non-participants due to missing PRO questioners.

| | Overall cohort | | | | | |
|---|---|---|---|---|---|---|
| | Non-participants | | Missing PRO questioners | | Participants | |
| **Number, %** | 1539 | 26.5 | 1472 | 25.4 | 4262 | 73.5 |
| **Age, n (%)** | | | | | | |
| ≤35 | 59 | 3.8 | 57 | 3.9 | 124 | 2.9 |
| >35 to ≤40 | 65 | 4.2 | 58 | 3.9 | 221 | 5.2 |
| >40 to ≤50 | 368 | 23.9 | 358 | 24.3 | 1077 | 25.3 |
| >50 to ≤60 | 418 | 27.2 | 398 | 27.0 | 1211 | 28.4 |
| >60 to ≤70 | 392 | 25.5 | 376 | 25.5 | 1212 | 28.4 |
| >70 | 237 | 15.4 | 225 | 15.3 | 417 | 9.8 |
| **Charlson's score, n (%)** | | | | | | |
| 0 | 1079 | 77.8 | 1030 | 77.8 | 3127 | 80.1 |
| ≥1 | 307 | 22.2 | 294 | 22.2 | 779 | 19.9 |
| Missing | 153 | 9.9 | 148 | 10.1 | 356 | 8.4 |
| **BMI, n (%)** | | | | | | |
| Underweight | 43 | 2.8 | 42 | 2.9 | 96 | 2.3 |
| Normal | 716 | 47.3 | 683 | 47.2 | 2124 | 50.0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Overweight | 436 | 28.8 | 416 | 28.7 | 1225 | 28.8 |
| Obese | 319 | 21.1 | 306 | 21.1 | 804 | 18.9 |
| Missing | 25 | 1.6 | 25 | 1.7 | 13 | 0.3 |
| **Smoking status, n (%)** | | | | | | |
| No/previous smoker | 1186 | 78.8 | 1136 | 78.8 | 3511 | 84.0 |
| Smoker | 319 | 21.2 | 306 | 21.2 | 670 | 16.0 |
| Missing | 34 | 2.2 | 30 | 2.0 | 81 | 1.9 |
| **Education, n (%)** | | | | | | |
| Primary school | 264 | 20.6 | 252 | 20.6 | 587 | 14.6 |
| High school | 602 | 46.9 | 574 | 46.9 | 1903 | 47.2 |
| College or higher | 418 | 32.6 | 399 | 32.6 | 1539 | 38.2 |
| Missing | 255 | 16.6 | 247 | 16.8 | 233 | 5.5 |
| **Income, n (%)** | | | | | | |
| <1500 | 269 | 21.6 | 263 | 22.1 | 529 | 13.5 |
| ≥1500 to <3000 | 566 | 45.4 | 533 | 44.8 | 1665 | 42.5 |
| ≥3000 | 413 | 33.1 | 395 | 33.2 | 1726 | 44.0 |
| Missing | 291 | 18.9 | 281 | 19.1 | 342 | 8.0 |
| **Marital status, n (%)** | | | | | | |
| Living alone | 333 | 25.8 | 323 | 26.3 | 850 | 21.0 |
| Living as couple | 956 | 74.2 | 905 | 73.7 | 3200 | 79.0 |
| Missing | 250 | 16.2 | 244 | 16.6 | 212 | 5.0 |
| **Histology, n (%)** | | | | | | |
| Invasive carc., NST | 1224 | 79.9 | 1171 | 79.9 | 3310 | 77.7 |
| Invasive lobular carc. | 173 | 11.3 | 166 | 11.3 | 566 | 13.3 |
| Mixed NST/lobular | 39 | 2.5 | 37 | 2.5 | 129 | 3.0 |
| Others | 96 | 6.3 | 91 | 6.2 | 254 | 6.0 |
| Missing | 7 | 0.5 | 7 | 0.5 | 3 | 0.1 |
| **TNM stage, n (%)** | | | | | | |
| I | 651 | 43.1 | 635 | 44.0 | 2192 | 51.5 |
| II | 693 | 45.9 | 656 | 45.5 | 1675 | 39.3 |
| III | 165 | 10.9 | 151 | 10.5 | 393 | 9.2 |
| Missing | 30 | 1.9 | 30 | 2.0 | 2 | 0.0 |
| **Histologic grade, n (%)** | | | | | | |
| 1 | 274 | 18.0 | 265 | 18.2 | 776 | 18.4 |
| 2 | 761 | 50.1 | 732 | 50.3 | 2254 | 53.3 |
| 3 | 485 | 31.9 | 458 | 31.5 | 1197 | 28.3 |
| Missing | 19 | 1.2 | 17 | 1.2 | 35 | 0.8 |
| **IHC-defined subtype of breast cancer, n (%)** | | | | | | |
| HR+/HER2- | 1143 | 74.3 | 1096 | 74.5 | 3317 | 77.8 |
| HR+/HER2+ | 168 | 10.9 | 161 | 10.9 | 435 | 10.2 |
| HR-/HER2+ | 49 | 3.2 | 48 | 3.3 | 173 | 4.1 |
| HR-/HER2- | 179 | 11.6 | 167 | 11.3 | 337 | 7.9 |
| **Surgery type, n (%)** | | | | | | |
| BCS | 1078 | 71.1 | 1056 | 72.8 | 3145 | 73.8 |
| Mastectomy | 417 | 27.5 | 394 | 27.2 | 1117 | 26.2 |
| No surgery | 22 | 1.5 | 22 | 1.5 | 0 | 0.0 |
| **Axillary management, n (%)** | | | | | | |
| Axillary dissection | 660 | 43.5 | 628 | 43.3 | 1674 | 39.3 |
| Sentinel ganglia/none | 856 | 56.5 | 822 | 56.7 | 2587 | 60.7 |
| **Radiotherapy, n (%)** | | | | | | |
| Yes | 1365 | 88.7 | 1323 | 89.9 | 3881 | 91.1 |
| No | 174 | 11.3 | 149 | 10.1 | 381 | 8.9 |
| **(Neo)adjuvant CT type, n (%)** | | | | | | |
| Anthracyclines-taxanes | 697 | 46.3 | 659 | 45.8 | 1931 | 45.4 |
| Anthracyclines-based | 52 | 3.5 | 50 | 3.5 | 96 | 2.3 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Taxanes-based | 75 | 5.0 | 74 | 5.1 | 218 | 5.1 |
| Other | 0 | 0.0 | 0 | 0.0 | 1 | 0.0 |
| No | 681 | 45.2 | 655 | 45.5 | 2010 | 47.2 |
| Missing regimen | 34 | 2.2 | 34 | 2.3 | 6 | 0.1 |
| **HER2-directed therapy, n (%)** | | | | | | |
| Yes | 173 | 11.2 | 168 | 11.4 | 477 | 11.2 |
| No | 1366 | 88.8 | 1304 | 88.6 | 3785 | 88.8 |
| **Adjuvant endocrine therapy type, n (%)** | | | | | | |
| Tamoxifen ± LHRH | 392 | 26.7 | 381 | 27.2 | 1334 | 31.3 |
| AI ± LHRH | 669 | 45.6 | 635 | 45.4 | 1997 | 46.9 |
| LHRH | 4 | 0.3 | 4 | 0.3 | 10 | 0.2 |
| Tamoxifen → AI ± LHRH | 43 | 2.9 | 42 | 3.0 | 144 | 3.4 |
| No | 358 | 24.4 | 337 | 24.1 | 772 | 18.1 |
| Missing agent | 73 | 4.7 | 73 | 5.0 | 5 | 0.1 |
| **Anxiety, n (%)** | | | | | | |
| Normal | 516 | 39.7 | 488 | 39.4 | 1613 | 39.4 |
| Borderline | 366 | 28.2 | 352 | 28.4 | 1067 | 26.1 |
| Anxiety | 418 | 32.2 | 398 | 32.1 | 1412 | 34.5 |
| Missing | 239 | 15.5 | 234 | 15.9 | 170 | 4.0 |
| **Depression, n (%)** | | | | | | |
| Normal | 1027 | 79.0 | 980 | 79.2 | 3378 | 82.6 |
| Borderline | 175 | 13.5 | 165 | 13.3 | 442 | 10.8 |
| Depression | 98 | 7.5 | 93 | 7.5 | 272 | 6.6 |
| Missing | 239 | 15.5 | 234 | 15.9 | 170 | 4.0 |

AI – aromatase inhibitors; BCS – Breast conserving surgery; BMI – Body mass index; carc. – carcinoma; CT – Chemotherapy; HR – hormone receptors; HER2 – Human epidermal growth factor receptor 2; LHRH - luteinizing hormone-releasing hormone antagonist or agonist; n – number; NST – no special type; PRO – patient reported outcomes. Missing values do not add to the percentage count of non-missing categories. Missing values are not included as a category in the statistical tests. All p-values refer to Pearson's chi-squared test.

**Variables assessment**

*PROs Assessments*

PROs were assessed using the EORTC QoL Core 30 (EORTC QLQ-C30, version 4.0) and its BC specific module (QLQ-BR23).[149] Higher scores reflect a better level of QoL and function for global health and functional scales, respectively, and greater severity for symptoms. The primary endpoint of the study was the QLQ-C30 summary score (C30-SumSc) and specific domains were secondary endpoints.[149] Anxiety and depression were assessed using Hospital Anxiety and Depression Scale (HADS).

*Assessments of other variables*

Information on age, Charlson's comorbidity index, body mass index (BMI), smoking, marital status, education level, income, disease staging, center volume, type of surgery, axillary management, receipt of ET, CT, trastuzumab and radiotherapy was collected at diagnosis by medical record review.

**Statistical analysis**

First, we described QoL over time and by treatment, examining the C30-SumSc and dichotomizing QoL scores by clinical severity. Severe impairment was defined as function impairment or symptom intensity meeting a predefined clinically meaningful level. Clinically meaningful levels were defined using as reference the mean score of the validation cohort of EORTC QLQ-C30/B23, specific to patients with stage I-II BC, plus a detrimental variation to the level of the lower boundary of medium clinically meaningful differences according to evidence-based guidelines for C30 domains[150], or 10-points for B23 domains (a variation previously considered of clinical value)[151]. Functional scores below such thresholds defined "poor function", while symptom scores above threshold values defined "severe symptoms".

Then, repeated measurements of QoL scores collected from diagnosis to the 2-year post-diagnosis visit were analyzed as continuous outcomes using multivariate generalized estimating equations (GEE) with independent correlation structure. Model-derived least square mean values for QoL scores and respective mean least square (MLS) differences between diagnosis and the 2-year post diagnosis visit by ET and/or CT (used as independent variables) were obtained. To test the hypothesis that the population-averaged domain scores differ over time by treatment with ET/CT, p-values for the interaction of ET/CT by time were computed ($p_{int}$). Models included as covariates all variables previously described ("other variables" plus anxiety and depression), all of which were collected at diagnosis.

An exploratory analysis was also conducted to determine the effect of treatment on QoL across four treatment groups: CT-only, ET-only, CT plus ET and no CT/ET. Similarly, MLS changes from diagnosis were estimated from GEE.

All tests were two-sided with a 95% confidence interval (CI) and a p-value of <0.05 was considered significant. All analyses were conducted using Stata 15.1 (StataCorp, Texas, U.S.A.).

### 5.4.6 Results

**Patient characteristics**

Of the 4262 women available for the analysis, 1587 (37.2%) were premenopausal and 2675 (62.8%) postmenopausal. Patient characteristics are shown in M4 Table 1 and M4 Supplementary Table 2.

**M4 Table 1 –** Demographic, clinical and pathological characteristics at baseline and treatment details according to receipt of CT/ET.

| | Overall cohort | | | | | |
|---|---|---|---|---|---|---|
| | All | | Chemotherapy | | Endocrine therapy | |
| **Number, %** | 4262 (100) | | 2252 (52.8) | | 3490 (81.9) | |
| **Age, median (IQR)** | 56 (48 - 65) | | 52 (44.5 - 61) | | 56 (48 - 65) | |
| **Age, n (%)** | | | | | | |
| ≤35 | 124 | 2.9 | 118 | 5.2 | 83 | 2.4 |
| >35 to ≤40 | 221 | 5.2 | 193 | 8.6 | 166 | 4.8 |
| >40 to ≤50 | 1077 | 25.3 | 700 | 31.1 | 915 | 26.2 |
| >50 to ≤60 | 1211 | 28.4 | 645 | 28.6 | 979 | 28.1 |
| >60 to ≤70 | 1212 | 28.4 | 477 | 21.2 | 1003 | 28.7 |
| >70 | 417 | 9.8 | 119 | 5.3 | 344 | 9.9 |
| **Charlson's score, n (%)** | | | | | | |
| 0 | 3127 | 80.1 | 1678 | 81.5 | 2559 | 80.0 |
| ≥1 | 779 | 19.9 | 382 | 18.5 | 638 | 20.0 |
| Missing | 356 | 8.4 | 192 | 8.5 | 293 | 8.4 |
| **BMI, n (%)** | | | | | | |
| Underweight | 96 | 2.3 | 53 | 2.4 | 83 | 2.4 |
| Normal | 2124 | 50.0 | 1146 | 51.0 | 1736 | 49.9 |
| Overweight | 1225 | 28.8 | 617 | 27.5 | 978 | 28.1 |
| Obese | 804 | 18.9 | 429 | 19.1 | 682 | 19.6 |
| Missing | 13 | 0.3 | 7 | 0.3 | 11 | 0.3 |
| **Smoking status, n (%)** | | | | | | |
| No/previous smoker | 3511 | 84.0 | 1834 | 82.8 | 2874 | 83.8 |
| Smoker | 670 | 16.0 | 382 | 17.2 | 556 | 16.2 |
| Missing | 81 | 1.9 | 36 | 1.6 | 60 | 1.7 |
| **Education, n (%)** | | | | | | |
| Primary school | 587 | 14.6 | 258 | 12.2 | 498 | 15.1 |
| High school | 1903 | 47.2 | 955 | 45.1 | 1550 | 46.9 |
| College or higher | 1539 | 38.2 | 905 | 42.7 | 1257 | 38.0 |
| Missing | 233 | 5.5 | 134 | 6.0 | 185 | 5.3 |
| **Income, n (%)** | | | | | | |
| <1500 | 529 | 13.5 | 274 | 13.2 | 441 | 13.7 |
| ≥1500 to <3000 | 1665 | 42.5 | 849 | 40.8 | 1374 | 42.8 |
| ≥3000 | 1726 | 44.0 | 957 | 46.0 | 1397 | 43.5 |
| Missing | 342 | 8.0 | 172 | 7.6 | 278 | 8.0 |
| **Marital status, n (%)** | | | | | | |
| Living alone | 850 | 21.0 | 410 | 19.2 | 708 | 21.4 |
| Living as couple | 3200 | 79.0 | 1730 | 80.8 | 2608 | 78.6 |
| Missing | 212 | 5.0 | 112 | 5.0 | 174 | 5.0 |
| **Histology, n (%)** | | | | | | |
| Invasive carc., NST | 3310 | 77.7 | 1825 | 81.1 | 2645 | 75.8 |
| Invasive lobular carc. | 566 | 13.3 | 227 | 10.1 | 541 | 15.5 |
| Mixed NST/lobular | 129 | 3.0 | 69 | 3.1 | 117 | 3.4 |
| Others | 254 | 6.0 | 128 | 5.7 | 186 | 5.3 |
| Missing | 3 | 0.1 | 3 | 0.1 | 1 | 0.0 |
| **TNM stage, n (%)** | | | | | | |
| I | 2192 | 51.5 | 640 | 28.4 | 1788 | 51.3 |
| II | 1675 | 39.3 | 1235 | 54.9 | 1361 | 39.0 |
| III | 393 | 9.2 | 376 | 16.7 | 339 | 9.7 |
| Missing | 2 | 0.0 | 1 | 0.0 | 2 | 0.1 |
| **Histologic grade, n (%)** | | | | | | |
| 1 | 776 | 18.4 | 94 | 4.2 | 646 | 18.6 |
| 2 | 2254 | 53.3 | 1055 | 47.1 | 2078 | 59.7 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 3 | 1197 | 28.3 | 1093 | 48.8 | 758 | 21.8 |
| Missing | 35 | 0.8 | 3 | 0.1 | 8 | 0.2 |
| **IHC-defined subtype of breast cancer, n (%)** | | | | | | |
| HR+/HER2- | 3317 | 77.8 | 1397 | 62.0 | 410 | 11.7 |
| HR+/HER2+ | 435 | 10.2 | 373 | 16.6 | 3075 | 88.1 |
| HR-/HER2+ | 173 | 4.1 | 170 | 7.5 | 4 | 0.1 |
| HR-/HER2- | 337 | 7.9 | 312 | 13.9 | 1 | 0.0 |
| **Surgery type, n (%)** | | | | | | |
| BCS | 3145 | 73.8 | 1428 | 63.4 | 2575 | 73.8 |
| Mastectomy | 1117 | 26.2 | 824 | 36.6 | 915 | 26.2 |
| **Axillary management, n (%)** | | | | | | |
| Axillary dissection | | | | | | |
| Sentinel node/none | 1674 | 39.3 | 1328 | 59.0 | 1373 | 39.4 |
| | 2587 | 60.7 | 923 | 41.0 | 2116 | 60.6 |
| **Radiotherapy, n (%)** | | | | | | |
| Yes | 3881 | 91.1 | 2086 | 92.6 | 3182 | 91.2 |
| No | 381 | 8.9 | 166 | 7.4 | 308 | 8.8 |
| **(Neo)adjuvant CT type, n (%)** | | | | | | |
| Anthracyclines-taxanes | | | | | | |
| Anthracyclines-based | 1931 | 45.3 | 1931 | 86.0 | 1455 | 41.8 |
| Taxanes-based | 96 | 2.3 | 96 | 4.3 | 80 | 2.3 |
| Other | 218 | 5.1 | 218 | 9.7 | 171 | 51.0 |
| Missing regimen | 1 | 0.0 | 1 | 0.0 | 1 | 0.0 |
| No | 6 | 0.1 | 6 | 0.3 | 5 | 0.1 |
| | 2010 | 47.2 | 0 | 0.0 | 1778 | 4.9 |
| **HER2-directed therapy, n (%)** | | | | | | |
| Yes | | | | | | |
| No | 477 | 11.2 | 475 | 21.1 | 300 | 8.6 |
| | 3785 | 88.8 | 1777 | 78.9 | 3190 | 91.4 |
| **Adjuvant endocrine therapy type, n (%)** | | | | | | |
| Tamoxifen ± LHRH | 1334 | 31.2 | 797 | 35.4 | 1334 | 38.3 |
| AI ± LHRH | 1997 | 50.0 | 831 | 37.0 | 1997 | 57.3 |
| LHRH | 10 | 0.2 | 7 | 0.3 | 10 | 0.3 |
| Tamoxifen → AI ± LHRH | 144 | 3.3 | 74 | 3.3 | 144 | 4.2 |
| Missing agent | 5 | 0.1 | 3 | 0.1 | 5 | 0.1 |
| No | 772 | 18.1 | 540 | 24.0 | 0 | 0.0 |
| **HADS-defined anxiety, n (%)** | | | | | | |
| Normal | 1613 | 39.4 | 792 | 36.6 | 1326 | 39.6 |
| Borderline | 1067 | 26.1 | 580 | 26.8 | 881 | 26.3 |
| Anxiety | 1412 | 34.5 | 793 | 36.6 | 1144 | 34.1 |
| Missing | 170 | 4.0 | 87 | 3.9 | 139 | 4.0 |
| **HADS-defined depression, n (%)** | 3378 | 82.6 | 1765 | 81.5 | 2763 | 82.5 |
| Normal | 442 | 10.8 | 242 | 11.2 | 362 | 10.8 |
| Borderline | 272 | 6.6 | 158 | 7.3 | 226 | 6.7 |
| Depression | 170 | 4.0 | 87 | 3.9 | 139 | 4.0 |
| Missing | | | | | | |

BCS –

Breast conserving surgery; BMI – Body mass index; CT – Chemotherapy; HADS – Hospital Anxiety and Depression Scale; IQR – Interquartile range; n – number; Missing values do not add to the percentage count of non-missing categories.

**M4 Supplementary table 2 –** Demographic, clinical and pathological characteristics at baseline and treatment details according to menopausal status and receipt of CT/ET.

| | Premenopausal | | | | | | Postmenopausal | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | All | | Chemo | | Endocrine therapy | | All | | Chemo | | Endocrine therapy | |
| **Number, %** | 1587 (37.2) | | 1087 (68.4) | | 1317 (83.0) | | 2675 (62.8) | | 1165 (43.6) | | 2173 (81.2) | |
| **Age, median (IQR)** | 46 (41 - 49) | | 44 (40 - 48) | | 46 (42 - 50) | | 63 (57 - 68) | | 61 (56 - 66) | | 63 (58 - 68) | |
| **Age, n (%)** | | | | | | | | | | | | |
| ≤35 | 124 | 7.8 | 118 | 10.9 | 83 | 6.3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| >35 to ≤40 | 221 | 13.9 | 193 | 17.8 | 166 | 12.6 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| >40 to ≤50 | 999 | 62.9 | 654 | 60.2 | 855 | 64.9 | 78 | 2.9 | 46 | 3.9 | 60 | 2.8 |
| >50 to ≤60 | 243 | 15.3 | 122 | 11.2 | 213 | 16.2 | 968 | 36.2 | 523 | 44.9 | 766 | 35.3 |
| >60 to ≤70 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1212 | 45.3 | 477 | 40.9 | 1003 | 46.2 |
| >70 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 417 | 15.6 | 119 | 10.2 | 344 | 15.8 |
| **Charlson's score, n (%)** | | | | | | | | | | | | |
| 0 | 1285 | 87.6 | 870 | 87.1 | 1078 | 87.9 | 1842 | 75.5 | 808 | 76.2 | 1481 | 75.1 |
| ≥1 | 182 | 12.4 | 129 | 12.9 | 148 | 12.1 | 597 | 24.5 | 253 | 23.8 | 490 | 24.9 |
| Missing | 120 | 7.6 | 88 | 8.1 | 91 | 6.9 | 236 | 8.8 | 104 | 8.9 | 202 | 9.3 |
| **BMI, n (%)** | | | | | | | | | | | | |
| Underweight | 57 | 3.6 | 40 | 3.7 | 49 | 3.7 | 39 | 1.5 | 13 | 1.1 | 34 | 1.6 |
| Normal | 968 | 61.1 | 644 | 59.5 | 803 | 61.1 | 1156 | 43.4 | 502 | 43.2 | 933 | 43.1 |
| Overweight | 353 | 22.3 | 252 | 23.3 | 287 | 21.8 | 872 | 32.7 | 365 | 31.4 | 691 | 31.9 |
| Obese | 205 | 13.0 | 147 | 13.6 | 175 | 13.3 | 599 | 22.5 | 282 | 24.3 | 507 | 23.4 |
| Missing | 4 | 0.3 | 4 | 0.4 | 3 | 0.2 | 9 | 0.3 | 3 | 0.3 | 8 | 0.4 |
| **Smoking status, n (%)** | | | | | | | | | | | | |
| No/previous smoker | 1225 | 78.5 | 839 | 78.2 | 1020 | 78.6 | 2286 | 87.3 | 995 | 87.1 | 1854 | 87.0 |
| Smoker | 336 | 21.5 | 234 | 21.8 | 278 | 21.4 | 334 | 12.7 | 148 | 12.9 | 278 | 13.0 |
| Missing | 26 | 1.6 | 14 | 1.3 | 19 | 1.4 | 55 | 2.1 | 22 | 1.9 | 41 | 1.9 |
| **Education, n (%)** | | | | | | | | | | | | |
| Primary school | 62 | 4.1 | 43 | 4.2 | 55 | 4.4 | 525 | 20.8 | 215 | 19.7 | 443 | 21.6 |
| High school | 663 | 43.9 | 425 | 41.5 | 558 | 44.4 | 1240 | 49.2 | 530 | 48.5 | 992 | 48.4 |
| College or higher | 784 | 52.0 | 557 | 54.3 | 644 | 51.2 | 755 | 30.0 | 348 | 31.8 | 613 | 29.9 |
| Missing | 78 | 4.9 | 62 | 5.7 | 60 | 4.6 | 155 | 5.8 | 72 | 6.2 | 125 | 5.8 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Income, n (%)** | | | | | | | | | | | | |
| <1500 | 140 | 9.4 | 105 | 10.4 | 117 | 9.5 | 389 | 16.0 | 169 | 15.8 | 324 | 16.4 |
| ≥1500 to <3000 | 543 | 36.6 | 367 | 36.2 | 455 | 36.9 | 1122 | 46.0 | 482 | 45.2 | 919 | 46.4 |
| ≥3000 | 799 | 53.9 | 541 | 53.4 | 661 | 53.6 | 927 | 38.0 | 416 | 39.0 | 736 | 37.2 |
| Missing | 105 | 6.6 | 74 | 6.8 | 84 | 6.4 | 237 | 8.9 | 98 | 8.4 | 194 | 8.9 |
| **Marital status, n (%)** | | | | | | | | | | | | |
| Living alone | 215 | 14.2 | 139 | 13.4 | 184 | 14.6 | 635 | 25.1 | 271 | 24.5 | 524 | 25.5 |
| Living as couple | 1303 | 85.8 | 896 | 86.6 | 1079 | 85.4 | 1897 | 74.9 | 834 | 75.5 | 1529 | 74.5 |
| Missing | 69 | 4.3 | 52 | 4.8 | 54 | 4.1 | 143 | 5.3 | 60 | 5.2 | 120 | 5.5 |
| **Histology, n (%)** | | | | | | | | | | | | |
| Invasive carc., NST | 1273 | 80.3 | 898 | 82.8 | 1033 | 78.5 | 2037 | 76.2 | 927 | 79.6 | 1612 | 74.2 |
| Invasive lobular carc. | 170 | 10.7 | 95 | 8.8 | 167 | 12.7 | 396 | 14.8 | 132 | 11.3 | 374 | 17.2 |
| Mixed NST/lobular | 47 | 3.0 | 32 | 2.9 | 46 | 3.5 | 82 | 3.1 | 37 | 3.2 | 71 | 3.3 |
| Others | 95 | 6.0 | 60 | 5.5 | 70 | 5.3 | 159 | 5.9 | 68 | 5.8 | 116 | 5.3 |
| Missing | 2 | 0.1 | 2 | 0.2 | 1 | 0.1 | 1 | 0.0 | 1 | 0.1 | 0 | 0.0 |
| **TNM stage, n (%)** | | | | | | | | | | | | |
| I | 683 | 43.1 | 285 | 26.2 | 581 | 44.1 | 1509 | 56.4 | 355 | 30.5 | 1207 | 55.6 |
| II | 708 | 44.6 | 608 | 56.0 | 563 | 42.8 | 967 | 36.2 | 627 | 53.8 | 798 | 36.7 |
| III | 195 | 12.3 | 193 | 17.8 | 172 | 13.1 | 198 | 7.4 | 183 | 15.7 | 167 | 7.7 |
| Missing | 1 | 0.0 | 1 | 0.1 | 1 | 0.1 | 1 | 0.1 | 0 | 0.0 | 1 | 0.0 |
| **Histologic grade, n (%)** | | | | | | | | | | | | |
| 1 | 243 | 15.4 | 47 | 4.4 | 221 | 16.8 | 533 | 20.1 | 47 | 4.0 | 425 | 19.6 |
| 2 | 805 | 51.0 | 515 | 47.7 | 748 | 57.0 | 1449 | 54.7 | 540 | 46.5 | 1330 | 61.3 |
| 3 | 530 | 33.6 | 518 | 48.0 | 344 | 26.2 | 667 | 25.2 | 575 | 49.5 | 414 | 19.1 |
| Missing | 9 | 0.6 | 2 | 0.2 | 4 | 0.3 | 26 | 1.0 | 1 | 0.1 | 4 | 0.2 |
| **IHC-defined subtype of breast cancer, n (%)** | | | | | | | | | | | | |
| HR+/HER2- | 1169 | 73.7 | 690 | 63.5 | 198 | 15.0 | 2148 | 80.3 | 707 | 60.7 | 212 | 9.8 |
| HR+/HER2+ | 208 | 13.1 | 190 | 17.5 | 1119 | 85.0 | 227 | 8.5 | 183 | 15.7 | 1956 | 90.0 |
| HR-/HER2+ | 71 | 4.5 | 71 | 6.5 | 0 | 0.0 | 102 | 3.8 | 99 | 8.5 | 4 | 0.2 |
| HR-/HER2- | 139 | 8.8 | 136 | 12.5 | 0 | 0.0 | 198 | 7.4 | 176 | 15.1 | 1 | 0.0 |
| **Surgery type, n (%)** | | | | | | | | | | | | |
| BCS | 1060 | 66.8 | 634 | 58.3 | 880 | 66.8 | 2085 | 77.9 | 794 | 68.2 | 1695 | 78.0 |
| Mastectomy | 527 | 33.2 | 453 | 41.7 | 437 | 33.2 | 590 | 22.1 | 371 | 31.8 | 478 | 22.0 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Axillary management, n (%)** | | | | | | | | | | | | |
| Axillary dissection | 772 | 48.6 | 682 | 62.7 | 639 | 48.5 | 902 | 33.7 | 646 | 55.5 | 734 | 33.8 |
| Sentinel ganglia/none | 815 | 51.4 | 405 | 37.3 | 678 | 51.5 | 1772 | 66.3 | 518 | 44.5 | 1438 | 66.2 |
| **Radiotherapy, n (%)** | | | | | | | | | | | | |
| Yes | 1443 | 90.9 | 1007 | 92.6 | 1192 | 90.5 | 2438 | 91.1 | 1079 | 92.6 | 1990 | 91.6 |
| No | 144 | 9.1 | 80 | 7.4 | 125 | 9.5 | 237 | 8.9 | 86 | 7.4 | 183 | 8.4 |
| **(Neo)adjuvant CT type, n (%)** | | | | | | | | | | | | |
| Anthracyclines-taxanes | 987 | 62.3 | 987 | 91.1 | 766 | 58.3 | 944 | 35.3 | 944 | 81.2 | 689 | 31.8 |
| Anthracyclines-based | 35 | 2.2 | 35 | 3.2 | 32 | 2.4 | 61 | 2.3 | 61 | 5.2 | 48 | 2.2 |
| Taxanes-based | 62 | 3.9 | 62 | 5.7 | 51 | 3.9 | 156 | 5.8 | 156 | 13.4 | 120 | 5.5 |
| Other | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 0.0 | 1 | 0.1 | 1 | 0.0 |
| Missing regimen | 3 | 0.2 | 3 | 0.3 | 2 | 0.2 | 3 | 0.1 | 3 | 0.3 | 3 | 0.1 |
| No | 500 | 31.6 | 0 | 0.0 | 466 | 35.4 | 1510 | 56.5 | 0 | 0.0 | 1312 | 60.5 |
| **HER2-directed therapy, n (%)** | | | | | | | | | | | | |
| Yes | 236 | 14.9 | 235 | 21.6 | 158 | 12.0 | 241 | 9.0 | 240 | 20.6 | 142 | 6.5 |
| No | 1351 | 85.1 | 852 | 78.4 | 1159 | 88.0 | 2434 | 91.0 | 925 | 79.4 | 2031 | 93.5 |
| **Adjuvant endocrine therapy type, n (%)** | | | | | | | | | | | | |
| Tamoxifen ± LHRH | 1166 | 73.6 | 746 | 68.8 | 1166 | 88.7 | 168 | 6.3 | 51 | 4.4 | 168 | 7.7 |
| AI ± LHRH | 91 | 5.7 | 61 | 5.6 | 91 | 6.9 | 1906 | 71.4 | 770 | 66.2 | 1906 | 87.8 |
| LHRH | 9 | 0.6 | 6 | 0.6 | 9 | 0.7 | 1 | 0.0 | 1 | 0.1 | 1 | 0.0 |
| Tamoxifen → AI ± LHRH | 49 | 3.1 | 36 | 3.3 | 49 | 3.8 | 95 | 3.6 | 38 | 3.3 | 95 | 4.4 |
| Missing agent | 2 | 0.1 | 2 | 0.2 | 2 | 0.2 | 3 | 0.1 | 1 | 0.1 | 3 | 0.1 |
| No | 270 | 17.0 | 236 | 21.8 | 0 | 0.0 | 502 | 18.8 | 304 | 26.1 | 0 | 0.0 |
| **HADS-defined anxiety, n (%)** | 522 | 34.2 | 344 | 33.0 | 434 | 34.3 | 1091 | 42.6 | 448 | 40.0 | 892 | 42.8 |
| Normal | 387 | 25.3 | 266 | 25.5 | 327 | 25.8 | 680 | 26.5 | 314 | 28.0 | 554 | 26.6 |
| Borderline | 619 | 40.5 | 434 | 41.6 | 505 | 39.9 | 793 | 30.9 | 359 | 32.0 | 639 | 30.6 |
| Anxiety | 59 | 3.7 | 43 | 4.0 | 51 | 3.9 | 111 | 4.1 | 44 | 3.8 | 88 | 4.0 |
| Missing | | | | | | | | | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **HADS-defined depression, n (%)** | 1243 | 81.3 | 837 | 80.2 | 1031 | 81.4 | 2135 | 83.3 | 928 | 82.8 | 1732 | 83.1 |
| Normal | 174 | 11.4 | 121 | 11.6 | 141 | 11.1 | 268 | 10.5 | 121 | 10.8 | 221 | 10.6 |
| Borderline | 111 | 7.3 | 86 | 8.2 | 94 | 7.4 | 161 | 6.3 | 72 | 6.4 | 132 | 6.3 |
| Depression | 59 | 3.7 | 43 | 4.0 | 51 | 3.9 | 111 | 4.1 | 44 | 3.8 | 88 | 4.0 |
| Missing | | | | | | | | | | | | |
| **Menses at 2 years after visit, n (%)** | | | | | | | | | | | | |
| Absent | 869 | 67.6 | 665 | 76.4 | 746 | 69.1 | - | - | - | - | - | - |
| Present | 417 | 32.4 | 205 | 25.6 | 334 | 31.0 | | | | | | |
| Missing | 301 | 19.0 | 217 | 20.0 | 237 | 18.0 | | | | | | |

BCS – Breast conserving surgery; BMI – Body mass index; CT – Chemotherapy; HADS – Hospital Anxiety and Depression Scale; IQR – Interquartile range; n – number; Missing values do not add to the percentage count of non-missing categories.

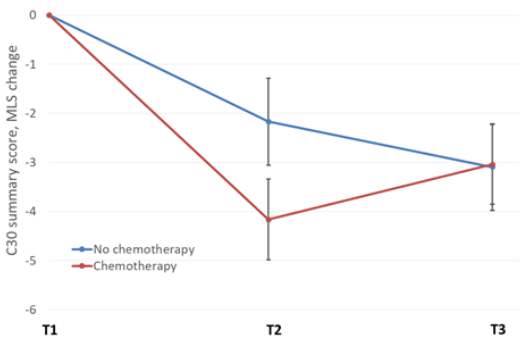
**PRO assessments**

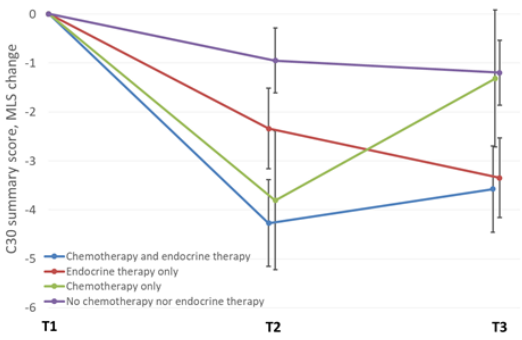
*PROs over time*

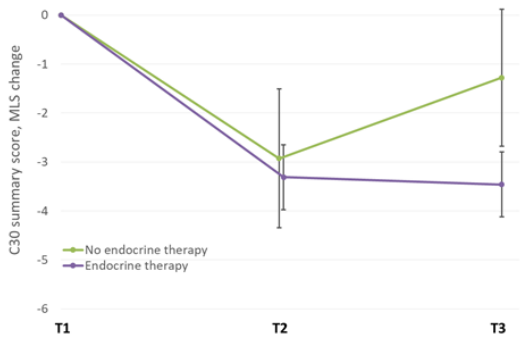
The overall QoL was negatively impacted 2-years after diagnosis in the general population (C30-SumSc, p<0.001). In addition, we observed a significant negative impact on role, cognitive and social functions, and also pain, dyspnea, fatigue, body image, systemic therapy side effects, constipation and breast and arm symptoms (all p<0.001) (M4 Figure 1, M4 Supplementary Figure 2 and M4 Supplementary Table 3). Considering all domains, no substantial recovery was noticed from the end of primary treatment to the 2-year after diagnosis time point, except for emotional function, future perspective and appetite loss, which slightly improved during this period (all p<0.001).

**M4 Figure 1 -** Mean least square change of EORTC QLQ C30 summary score from diagnosis (T1) to "end of primary treatment" (T2) and the "2-years after diagnosis visit" (T3) in patients treated and not treated with chemotherapy or endocrine therapy in the overall cohort (non-mutually exclusive groups) (1A), and in premenopausal (1B) and postmenopausal (1C) patients. Error bars refer to the 95% confidence interval of the estimate. Estimates and confidence intervals derived from multivariate generalized estimating equations models.

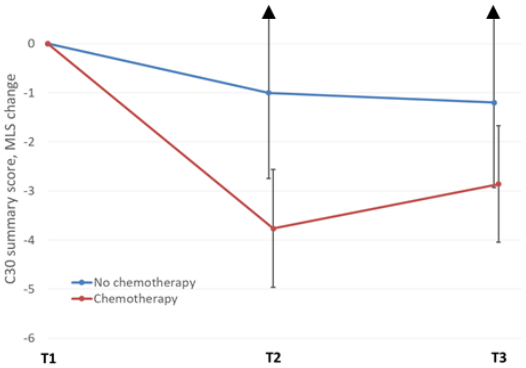
A

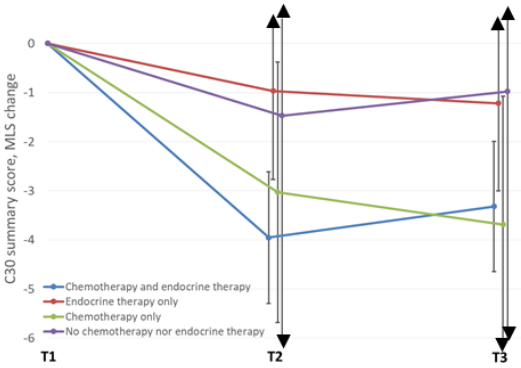


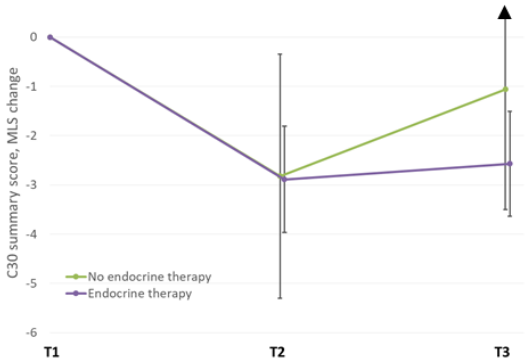



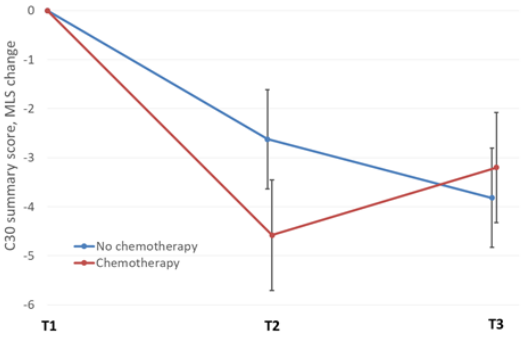
B

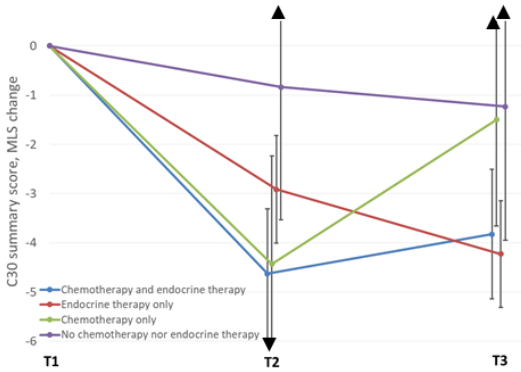

C30 summary score, MLS change
0
-1
-2
-3
-4
-5
-6
No chemotherapy
Chemotherapy
T1
T2
T3

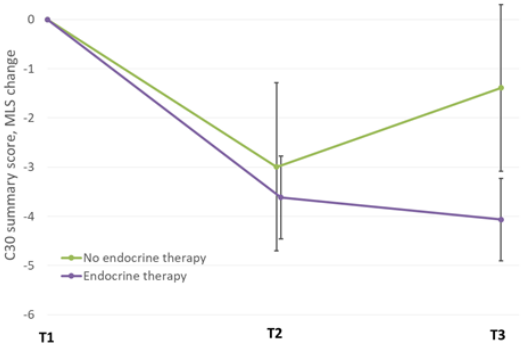

C30 summary score, MLS change
0
-1
-2
-3
-4
-5
-6
Chemotherapy and endocrine therapy
Endocrine therapy only
Chemotherapy only
No chemotherapy nor endocrine therapy
T1
T2
T3


C30 summary score, MLS change
0
-1
-2
-3
-4
-5
-6
No endocrine therapy
Endocrine therapy
T1
T2
T3

C

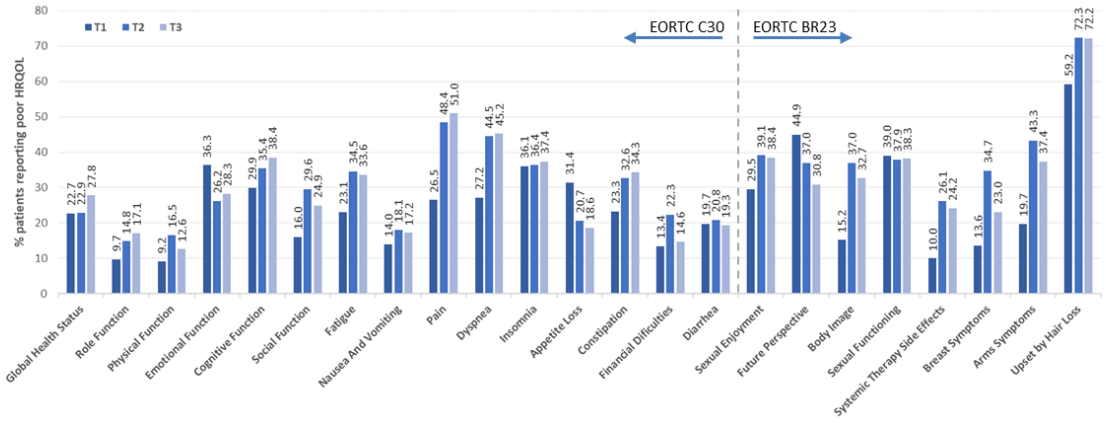

C30 summary score, MLS change
0
-1
-2
-3
-4
-5
-6
No chemotherapy
Chemotherapy
T1
T2
T3


C30 summary score, MLS change
0
-1
-2
-3
-4
-5
-6
Chemotherapy and endocrine therapy
Endocrine therapy only
Chemotherapy only
No chemotherapy nor endocrine therapy
T1
T2
T3

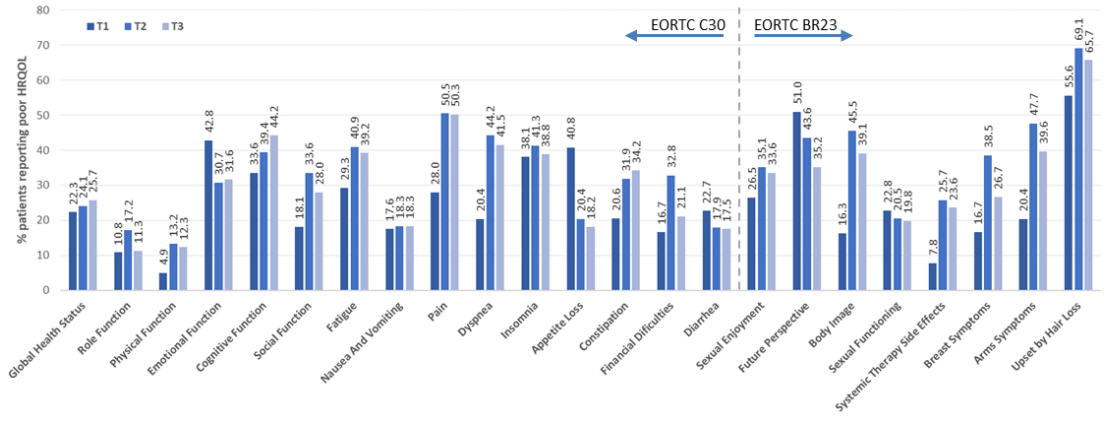

C30 summary score, MLS change
0
-1
-2
-3
-4
-5
-6
No endocrine therapy
Endocrine therapy
T1
T2
T3

**M4 Supplementary Figure 2 –** Prevalence (%) of patients reporting poor functions or severe symptoms in the EORTC QLQ C30 and BR23 domains at "diagnosis" (T1), "end of primary treatment visit" (T2) and at "2-years after diagnosis visit" (T3). Results in the overall cohort (S2A), and for premenopausal (S2B) and postmenopausal (S2C) patients.

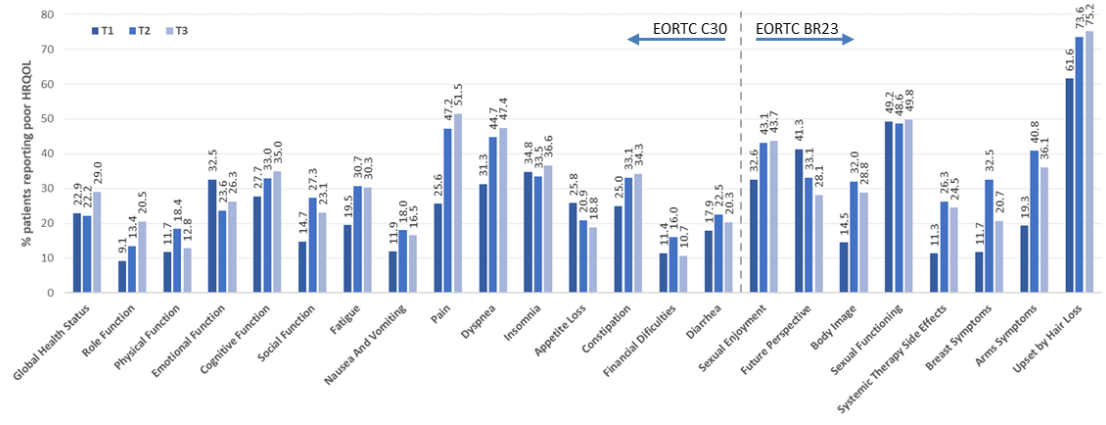
**S2A**




**S2B**




**S2C**



**M4 Supplementary table 3 –** Patient reported outcomes scores at “diagnosis” (T1), “end of primary treatment visit” (T2) and at “2 years after diagnosis visit” (T3), overall and according to menopausal status.

| | | T1 | | T2 | | T3 | |
|---|---|---|---|---|---|---|---|
| | | Completion rate (%) | mean (SD) | Completion rate (%) | mean (SD) | Completion rate (%) | mean (SD) |
| All | **EORTC QLQ-C30, summary score** | | | | | | |
| | Summary score | 92.7 | 83.47 (12.29) | 90.0 | 80.02 (14.44) | 93.0 | 80.08 (14.21) |
| | **EORTC QLQ-C30, functional scales** | | | | | | |
| | Global health status | 95.7 | 68.87 (18.70) | 92.2 | 68.73 (18.13) | 97.4 | 66.13 (17.94) |
| | Physical function | 96.8 | 90.49 (13.63) | 94.3 | 84.24 (15.27) | 98.9 | 84.05 (15.80) |
| | Role function | 96.7 | 86.86 (21.49) | 94.2 | 80.55 (23.84) | 98.4 | 82.50 (23.30) |
| | Emotional function | 96.6 | 65.73 (24.16) | 94.4 | 72.77 (23.84) | 98.7 | 70.71 (24.49) |
| | **EORTC QLQ-C30, symptoms scales** | | | | | | |
| | Cognitive function | 96.6 | 82.15 (21.31) | 94.5 | 78.85 (23.27) | 98.6 | 77.01 (24.31) |
| | Social functioning | 95.3 | 91.07 (17.74) | 93.9 | 83.61 (23.51) | 98.4 | 85.77 (22.28) |
| | Fatigue | 96.6 | 26.89 (23.70) | 94.1 | 34.90 (25.16) | 98.5 | 34.18 (24.32) |
| | Nausea/Vomiting | 96.6 | 3.60 (10.87) | 94.3 | 4.92 (12.70) | 98.6 | 4.92 (13.58) |
| | Pain | 96.8 | 15.57 (21.31) | 94.5 | 27.27 (25.78) | 98.4 | 28.61 (26.69) |
| | Dyspnea | 96.1 | 11.59 (21.39) | 93.4 | 19.45 (25.35) | 97.7 | 19.63 (25.24) |
| | Insomnia | 96.3 | 42.53 (33.31) | 93.8 | 40.95 (34.04) | 97.7 | 41.79 (34.43) |
| | Appetite | 96.5 | 13.87 (23.49) | 93.9 | 9.17 (20.20) | 98.0 | 8.32 (19.66) |
| | Constipation | 96.2 | 10.82 (22.41) | 93.8 | 15.73 (26.04) | 98.1 | 16.98 (27.40) |
| | Financial difficulties | 94.0 | 6.02 (17.19) | 93.1 | 11.42 (24.35) | 98.1 | 7.44 (20.49) |
| | Diarrhea | 95.8 | 8.15 (18.24) | 93.6 | 9.09 (19.88) | 97.0 | 8.57 (19.67) |
| | **EORTC BR23, functional scales** | | | | | | |
| | Sexual enjoyment | 45.6 | 64.02 (26.92) | 48.4 | 56.90 (26.81) | 48.1 | 57.53 (27.75) |
| | Future perspectives | 93.6 | 49.55 (31.32) | 93.5 | 56.07 (31.69) | 96.9 | 60.67 (31.38) |
| | Body image | 93.3 | 88.73 (19.45) | 93.6 | 74.20 (28.70) | 97.3 | 76.85 (27.50) |
| | Sexual function | 89.9 | 26.40 (26.32) | 89.5 | 25.75 (24.96) | 93.2 | 25.82 (25.18) |
| | **EORTC BR23, symptoms scales** | | | | | | |
| | Systemic therapy side effects | 95.2 | 11.01 (11.41) | 93.9 | 19.37 (15.96) | 97.8 | 18.27 (14.38) |
| | Breast symptoms | 84.0 | 12.86 (16.41) | 93.9 | 25.36 (19.96) | 97.5 | 19.26 (18.06) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Arm symptoms | 92.7 | 12.97 (21.07) | 94.0 | 25.97 (26.46) | 97.4 | 23.20 (25.76) |
| | Hair loss | 11.1 | 32.77 (34.24) | 21.0 | 48.77 (39.22) | 25.0 | 43.85 (36.50) |
| Premenopausal | **EORTC QLQ-C30, summary score** | | | | | | |
| | Summary score | 93.9 | 82.27 (12.34) | 92.0 | 78.92 (14.84) | 96.2 | 79.57 (14.64) |
| | **EORTC QLQ-C30, functional scales** | | | | | | |
| | Global health status | 96.0 | 68.70 (18.82) | 92.5 | 67.94 (17.75) | 98.0 | 67.13 (17.91) |
| | Physical function | 96.9 | 93.66 (10.86) | 93.9 | 85.98 (14.19) | 99.1 | 87.19 (13.93) |
| | Role function | 96.7 | 85.43 (22.05) | 94.0 | 78.22 (24.48) | 98.9 | 82.71 (22.89) |
| | Emotional function | 96.5 | 62.18 (24.47) | 94.1 | 70.06 (25.07) | 99.1 | 68.73 (25.21) |
| | **EORTC QLQ-C30, symptoms scales** | | | | | | |
| | Cognitive function | 96.5 | 80.31 (22.72) | 94.1 | 76.49 (24.87) | 99.0 | 74.12 (26.08) |
| | Social functioning | 95.5 | 89.73 (18.56) | 93.7 | 81.13 (25.13) | 98.9 | 84.29 (22.90) |
| | Fatigue | 96.7 | 30.93 (24.45) | 94.0 | 38.48 (26.02) | 98.9 | 37.19 (25.19) |
| | Nausea/Vomiting | 96.9 | 4.83 (12.96) | 94.0 | 5.13 (13.26) | 99.1 | 5.30 (14.23) |
| | Pain | 96.8 | 16.35 (20.83) | 94.0 | 28.77 (26.28) | 99.1 | 28.01 (26.14) |
| | Dyspnea | 96.5 | 8.62 (18.89) | 93.4 | 19.44 (25.34) | 98.8 | 17.94 (24.69) |
| | Insomnia | 96.6 | 44.81 (33.31) | 93.6 | 44.44 (35.33) | 98.5 | 42.93 (34.90) |
| | Appetite | 96.6 | 18.40 (26.04) | 93.8 | 9.25 (20.57) | 98.6 | 8.12 (19.45) |
| | Constipation | 96.2 | 8.95 (19.75) | 93.9 | 15.23 (25.71) | 98.6 | 16.85 (27.25) |
| | Financial difficulties | 94.0 | 7.44 (18.73) | 93.0 | 17.71 (29.45) | 98.6 | 11.17 (24.65) |
| | Diarrhea | 96.2 | 9.50 (19.63) | 93.9 | 7.99 (19.34) | 98.3 | 7.59 (18.54) |
| | **EORTC BR23, functional scales** | | | | | | |
| | Sexual enjoyment | 63.5 | 66.10 (26.68) | 66.0 | 59.69 (26.78) | 67.8 | 60.75 (28.45) |
| | Future perspectives | 94.8 | 45.17 (32.52) | 93.3 | 51.11 (32.08) | 97.5 | 57.21 (32.29) |
| | Body image | 94.6 | 87.88 (19.85) | 93.6 | 68.06 (30.51) | 97.7 | 72.77 (29.54) |
| | Sexual function | 92.8 | 35.26 (26.52) | 91.6 | 35.17 (25.14) | 95.7 | 36.26 (25.36) |
| | **EORTC BR23, symptoms scales** | | | | | | |
| | Systemic therapy side effects | 95.6 | 9.78 (10.83) | 93.8 | 19.65 (16.07) | 98.2 | 18.59 (14.29) |
| | Breast symptoms | 85.8 | 14.66 (17.39) | 93.5 | 27.31 (20.43) | 97.9 | 21.21 (19.07) |
| | Arm symptoms | 93.2 | 13.30 (21.14) | 93.6 | 28.91 (27.13) | 98.0 | 24.80 (26.31) |
| | Hair loss | 11.8 | 30.66 (34.20) | 16.3 | 50.06 (41.23) | 21.3 | 41.91 (38.20) |
| Postmenopausal | **EORTC QLQ-C30, summary score** | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Summary score | 92.0 | 84.19 (12.21) | 88.9 | 80.69 (14.14) | 91.1 | 80.40 (13.93) |
| | **EORTC QLQ-C30, functional scales** | | | | | | |
| | Global health status | 95.5 | 68.96 (18.63) | 92.1 | 69.20 (18.33) | 97.0 | 65.53 (17.94) |
| | Physical function | 96.7 | 88.61 (14.72) | 94.6 | 83.21 (15.79) | 98.8 | 82.18 (16.54) |
| | Role function | 96.6 | 87.70 (21.11) | 94.4 | 81.93 (23.35) | 98.1 | 82.38 (23.54) |
| | Emotional function | 96.7 | 67.84 (23.74) | 94.6 | 74.37 (22.95) | 98.5 | 71.89 (23.98) |
| | **EORTC QLQ-C30, symptoms scales** | | | | | | |
| | Cognitive function | 96.6 | 83.24 (20.36) | 94.7 | 80.25 (22.16) | 98.4 | 78.74 (23.02) |
| | Social functioning | 95.2 | 91.87 (17.19) | 94.0 | 85.08 (22.38) | 98.1 | 86.66 (21.86) |
| | Fatigue | 96.5 | 24.49 (22.91) | 94.2 | 32.78 (24.40) | 98.2 | 32.37 (23.60) |
| | Nausea/Vomiting | 96.5 | 2.86 (9.34) | 94.5 | 4.80 (12.35) | 98.4 | 4.69 (13.18) |
| | Pain | 96.8 | 15.10 (21.58) | 94.9 | 26.39 (25.44) | 97.9 | 28.97 (27.02) |
| | Dyspnea | 95.8 | 13.37 (22.56) | 93.4 | 19.46 (25.36) | 97.1 | 20.65 (25.52) |
| | Insomnia | 96.1 | 41.17 (33.24) | 93.9 | 38.89 (33.08) | 97.2 | 41.11 (34.13) |
| | Appetite | 96.4 | 11.18 (21.39) | 94.0 | 9.12 (19.99) | 97.7 | 8.44 (19.78) |
| | Constipation | 96.1 | 11.93 (23.79) | 93.7 | 16.02 (26.23) | 97.8 | 17.06 (27.50) |
| | Financial difficulties | 94.1 | 5.18 (16.15) | 93.2 | 7.70 (19.84) | 97.8 | 5.21 (17.16) |
| | Diarrhea | 95.6 | 7.34 (17.30) | 93.5 | 9.75 (20.17) | 96.3 | 9.16 (20.30) |
| | **EORTC BR23, functional scales** | | | | | | |
| | Sexual enjoyment | 35.0 | 61.78 (27.00) | 37.9 | 54.01 (26.55) | 36.4 | 53.97 (26.52) |
| | Future perspectives | 92.9 | 52.20 (30.27) | 93.6 | 59.01 (31.09) | 96.5 | 62.74 (30.64) |
| | Body image | 92.5 | 89.24 (19.19) | 93.6 | 77.84 (26.93) | 97.1 | 79.29 (25.90) |
| | Sexual function | 88.1 | 20.87 (24.64) | 88.3 | 19.95 (23.01) | 91.7 | 19.36 (22.79) |
| | **EORTC BR23, symptoms scales** | | | | | | |
| | Systemic therapy side effects | 95.0 | 11.74 (11.68) | 94.0 | 19.21 (15.90) | 97.6 | 18.07 (14.43) |
| | Breast symptoms | 82.9 | 11.76 (15.67) | 94.2 | 24.21 (19.60) | 97.3 | 18.10 (17.33) |
| | Arm symptoms | 92.4 | 12.77 (21.03) | 94.2 | 24.23 (25.91) | 97.0 | 22.23 (25.38) |
| | Hair loss | 10.6 | 34.15 (34.25) | 23.8 | 48.25 (38.39) | 27.2 | 44.75 (35.68) |

*ET and/or CT impact on general QoL*

Only ET was associated with deteriorated C30-SumSc 2-years after diagnosis ($p_{int}$=0.004) that persisted over time (M4 Figure 1, M4 Table 2 [see page 95] and M4 Supplementary Table 4 [see page 99]). In contrast, after a transient deterioration, there was no detrimental effect of CT on C30-SumSc at 2-years ($p_{int}$=0.924). Young age, comorbidities, smoking, low income, and anxiety/depression were also associated with QoL deterioration at 2-years (M4 Supplementary Table 4 shows multivariate models for C30-SumSc, remaining models not shown).

We then assessed the impact of treatment on general QoL (C30-SumSc) according to menopausal status. In premenopausal patients, neither ET ($p_{int}$=0.242) nor CT ($p_{int}$=0.100) were associated with a significant decrease of C30-SumSc after multivariate adjusting. In postmenopausal women, ET ($p_{int}$=0.004), but not CT ($p_{int}$=0.394), was associated with a substantial decrease in general QoL (MLS change at 2-years of -4.07 vs. -1.39 for ET vs. no ET). Prevalence of poor functions and severe symptoms and mean changes in QoL scores 2-years after diagnosis for patients treated or not with CT and/or ET are shown for the overall cohort and according to menopausal status in M4 Table 2, M4 Figure 2 [see page 101] and M4 Supplementary Figures 2-3 [see page 89 and 103].

*QLQ-C30*

*Patient-reported functional scales*

In the overall cohort, at 2-years, statistically significant worse QoL was observed among patients treated with ET (vs. no ET) for role functioning (p for interaction between treatment group-time [$p_{int}$]=0.005) and social functioning ($p_{int}$=0.032); CT (vs. no CT) impacted negatively physical functioning ($p_{int}$<0.001) and cognitive functioning ($p_{int}$<0.001) (M4 Table 2, M4 Figure 2a). In premenopausal patients, a statistically significant worse QoL was observed with CT (vs. no CT) for physical functioning ($p_{int}$<0.001) and cognitive functioning ($p_{int}$<0.001). ET did not impact any functional domain (M4 Table 2, M4 Figure 2b). In postmenopausal patients, statistically significant worse QoL was seen with ET for global health status ($p_{int}$=0.006), role functioning ($p_{int}$=0.001) and social functioning ($p_{int}$=0.012). CT did not impact any functional domain (M4 Table 2, M4 Figure 2c).

*Patient-reported symptom scales*

In the overall cohort, at 2-years, statistically significant worse QoL was observed with ET (vs. no ET) for pain ($p_{int}$=0.001). Insomnia improved among those not treated with ET (vs. ET) ($p_{int}$=0.014); CT (vs. no CT) impacted negatively dyspnea ($p_{int}$<0.001) and financial difficulties

($p_{int}$=0.015). Appetite loss improved among those treated with CT (vs. no CT) ($p_{int}$<0.001) (M4 Table 2, M4 Figure 2a). In premenopausal patients, statistically significant worse QoL was observed with CT (vs. no CT) for dyspnea ($p_{int}$=0.030), and financial difficulties ($p_{int}$=0.045). Appetite loss improved among those treated with CT (vs. no CT) ($p_{int}$<0.001). ET did not impact any symptom domain (M4 Table 2, M4 Figure 2b). In postmenopausal patients, statistically significant worse QoL was seen with ET for nausea ($p_{int}$=0.001) and pain ($p_{int}$=0.001) and CT (vs. no CT) impacted negatively dyspnea ($p_{int}$=0.011). Appetite loss improved among those treated with CT (vs. no CT) ($p_{int}$=0.009) (M4 Table 2, M4 Figure 2c).

*QLQ-BR23*

*Patient-reported functional scales*

In the overall cohort and by menopausal status, statistically significant worse QoL was observed with CT (vs. no CT) for body image ($p_{int}$<0.001) at 2-years. ET did not impact any functional domain (M4 Table 2, M4 Supplementary Figure 3a-c).

*Patient-reported symptom scales*

In the overall cohort, at 2-years, statistically significant worse QoL was observed with ET (vs. no ET) for systemic therapy side effects ($p_{int}$<0.001) and breast symptoms ($p_{int}$=0.024); CT (vs. no CT) impacted negatively breast symptoms ($p_{int}$<0.001) (M4 Table 2, M4 Supplementary Figure 3a). In premenopausal and postmenopausal patients, statistically significant worse QoL was observed with CT (vs. no CT) for breast symptoms ($p_{int}$<0.001 and 0.040, respectively) and ET impacted negatively systemic therapy side effects ($p_{int}$=0.030 and 0.004, respectively)(M4 Table 2, M4 Supplementary Figure 3b-c). Comparative analysis of sequential CT/ET, CT and ET-only and no systemic treatment groups were consistent with the above findings (M4 Supplementary Figure 4/5 [see page 105 and 107]). Independent of menopausal status, the sequential therapy with CT and ET have the highest impact on several QoL domains; however, global health status was mainly impacted by ET for the overall cohort and for postmenopausal women and by CT for premenopausal. Emotional function and future perspective recover was smaller among the groups treated with ET.

**M4 Table 2 –** Mean least square change of specific domain according to exposure to chemotherapy and/or to endocrine therapy. P-value highlights the p-value of the interaction test between receipt of chemotherapy or endocrine therapy and time.

| Symptom, dimension or scale | Treatment | Overall cohort | | | Premenopausal | | | Postmenopausal | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | MLS change | 95% CI | p-val. | MLS change | 95% CI | p-val. | MLS change | 95% CI | p-val. |
| **EORTC QLQ-C30, summary score** | | | | | | | | | | |
| **Summary score** | No chemotherapy | -3.094 | -3.937 - -2.252 | 0.924 | -1.184 | -2.840 - 0.473 | 0.100 | -3.825 | -4.787 - -2.863 | 0.394 |
| | Chemotherapy | -3.038 | -3.817 - -2.260 | | -2.863 | -3.995 - -1.731 | | -3.199 | -4.272 - -2.127 | |
| | No endocr. therapy | -1.294 | -2.634 - 0.045 | 0.004 | -1.05 | -3.385 - 1.285 | 0.242 | -1.417 | -3.032 - 0.198 | 0.004 |
| | Endocrine therapy | -3.458 | -4.090 - -2.826 | | -2.572 | -3.593 - -1.552 | | -4.066 | -4.864 - -3.268 | |
| **EORTC QLQ-C30, functional scales** | | | | | | | | | | |
| **Global health status** | No chemotherapy | -3.379 | -4.558 - -2.199 | 0.054 | -1.838 | -4.123 - 0.447 | 0.697 | -3.95 | -5.320 - -2.580 | 0.118 |
| | Chemotherapy | -1.799 | -2.895 - -0.703 | | -1.288 | -2.851 - 0.274 | | -2.309 | -3.842 - -0.776 | |
| | No endocr. therapy | -1.215 | -3.094 - 0.665 | 0.129 | -2.971 | -6.193 - 0.250 | 0.317 | -0.31 | -2.612 - 1.993 | 0.006 |
| | Endocrine therapy | -2.825 | -3.713 - -1.937 | | -1.176 | -2.583 - 0.232 | | -3.934 | -5.073 - -2.795 | |
| **Physical functioning** | No chemotherapy | -5.006 | -5.926 - -4.087 | <0.001 | -3.707 | -5.252 - -2.161 | <0.001 | -5.493 | -6.632 - -4.353 | 0.055 |
| | Chemotherapy | -7.403 | -8.260 - -6.547 | | -7.655 | -8.719 - -6.591 | | -7.164 | -8.439 - -5.889 | |
| | No endocr. therapy | -5.402 | -6.870 - -3.933 | 0.190 | -6.754 | -8.946 - -4.562 | 0.720 | -4.696 | -6.613 - -2.779 | 0.079 |
| | Endocrine therapy | -6.488 | -7.182 - -5.795 | | -6.316 | -7.275 - -5.356 | | -6.611 | -7.559 - -5.663 | |
| **Role functioning** | No chemotherapy | -4.76 | -6.241 - -3.279 | 0.393 | -0.271 | -3.146 - 2.603 | 0.104 | -6.43 | -8.141 - -4.719 | 0.158 |
| | Chemotherapy | -3.878 | -5.256 - -2.499 | | -3.164 | -5.139 - -1.189 | | -4.578 | -6.493 - -2.664 | |
| | No endocr. therapy | -1.21 | -3.570 - 1.150 | 0.005 | -1.107 | -5.164 - 2.951 | 0.551 | -1.266 | -4.141 - 1.610 | 0.001 |
| | Endocrine therapy | -4.975 | -6.090 - -3.859 | | -2.453 | -4.231 - -0.675 | | -6.669 | -8.091 - -5.247 | |
| **Emotional functioning** | No chemotherapy | 3.816 | 2.373 - 5.259 | 0.004 | 4.947 | 2.043 - 7.851 | 0.124 | 3.408 | 1.774 - 5.041 | 0.061 |
| | Chemotherapy | 6.727 | 5.381 - 8.072 | | 7.711 | 5.716 - 9.707 | | 5.757 | 3.926 - 7.588 | |
| | No endocr. therapy | 6.592 | 4.283 - 8.901 | 0.253 | 7.525 | 3.411 - 11.639 | 0.716 | 6.11 | 3.355 - 8.866 | 0.188 |
| | Endocrine therapy | 5.102 | 4.014 - 6.191 | | 6.691 | 4.896 - 8.487 | | 4.044 | 2.685 - 5.404 | |
| **Cognitive functioning** | No chemotherapy | -2.759 | -4.256 - -1.262 | <0.001 | -1.378 | -4.453 - 1.697 | 0.001 | -3.258 | -4.918 - -1.599 | 0.121 |
| | Chemotherapy | -6.503 | -7.897 - -5.108 | | -7.789 | -9.903 - -5.676 | | -5.232 | -7.090 - -3.373 | |
| | No endocr. therapy | -4.235 | -6.631 - -1.840 | 0.633 | -6.421 | -10.785 - -2.058 | 0.736 | -3.079 | -5.877 - -0.282 | 0.410 |
| | Endocrine therapy | -4.88 | -6.009 - -3.751 | | -5.601 | -7.506 - -3.697 | | -4.39 | -5.771 - -3.010 | |
| | No chemotherapy | -4.959 | -6.269 - -3.649 | 0.666 | -4.35 | -6.965 - -1.734 | 0.368 | -5.183 | -6.672 - -3.693 | 0.811 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Social functioning** | Chemotherapy | -5.353 | -6.575 - -4.131 | | -5.809 | -7.609 - -4.009 | | -4.91 | -6.582 - -3.238 | |
| | No endocr. therapy | -3.088 | -5.189 - -0.987 | 0.032 | -4.888 | -8.603 - -1.172 | 0.795 | -2.165 | -4.685 - 0.355 | 0.012 |
| | Endocrine therapy | -5.629 | -6.616 - -4.642 | | -5.426 | -7.043 - -3.808 | | -5.761 | -6.999 - -4.523 | |
| **EORTC QLQ-C30, symptoms scales** | | | | | | | | | | |
| **Fatigue** | No chemotherapy | 6.418 | 4.852 - 7.985 | 0.591 | 4.104 | 0.932 - 7.275 | 0.262 | 7.278 | 5.515 - 9.041 | 0.763 |
| | Chemotherapy | 7.005 | 5.546 - 8.464 | | 6.308 | 4.128 - 8.489 | | 7.684 | 5.711 - 9.658 | |
| | No endocr. therapy | 5.913 | 3.411 - 8.414 | 0.477 | 5.063 | 0.579 - 9.546 | 0.797 | 6.352 | 3.381 - 9.323 | 0.416 |
| | Endocrine therapy | 6.915 | 5.735 - 8.095 | | 5.704 | 3.742 - 7.665 | | 7.728 | 6.262 - 9.194 | |
| **Nausea** | No chemotherapy | 1.43 | 0.593 - 2.266 | 0.128 | -0.665 | -2.448 - 1.119 | 0.358 | 2.208 | 1.308 - 3.109 | 0.032 |
| | Chemotherapy | 0.542 | -0.236 - 1.321 | | 0.351 | -0.876 - 1.578 | | 0.73 | -0.278 - 1.738 | |
| | No endocr. therapy | -0.044 | -1.379 - 1.291 | 0.105 | 1.206 | -1.316 - 3.727 | 0.316 | -0.695 | -2.210 - 0.820 | 0.001 |
| | Endocrine therapy | 1.177 | 0.547 - 1.808 | | -0.201 | -1.305 - 0.902 | | 2.101 | 1.352 - 2.849 | |
| **Pain** | No chemotherapy | 12.842 | 11.224 - 14.460 | 0.874 | 10.294 | 7.259 - 13.329 | 0.424 | 13.79 | 11.882 - 15.698 | 0.775 |
| | Chemotherapy | 13.02 | 11.514 - 14.527 | | 11.796 | 9.710 - 13.881 | | 14.208 | 12.071 - 16.344 | |
| | No endocr. therapy | 9.078 | 6.500 - 11.656 | 0.001 | 8.732 | 4.448 - 13.016 | 0.197 | 9.259 | 6.051 - 12.466 | 0.001 |
| | Endocrine therapy | 13.799 | 12.581 - 15.018 | | 11.809 | 9.933 - 13.686 | | 15.128 | 13.543 - 16.714 | |
| **Dyspnea** | No chemotherapy | 5.712 | 4.143 - 7.280 | <0.001 | 6.23 | 3.337 - 9.123 | 0.03 | 5.516 | 3.644 - 7.389 | 0.011 |
| | Chemotherapy | 9.634 | 8.176 - 11.092 | | 10.125 | 8.138 - 12.113 | | 9.144 | 7.050 - 11.237 | |
| | No endocr. therapy | 7.219 | 4.717 - 9.721 | 0.605 | 7.695 | 3.612 - 11.778 | 0.536 | 6.963 | 3.809 - 10.117 | 0.909 |
| | Endocrine therapy | 7.949 | 6.767 - 9.131 | | 9.103 | 7.313 - 10.894 | | 7.169 | 5.611 - 8.727 | |
| **Insomnia** | No chemotherapy | -0.612 | -2.872 - 1.647 | 0.355 | -4.833 | -9.165 - -0.502 | 0.185 | 0.951 | -1.681 - 3.582 | 0.058 |
| | Chemotherapy | -2.067 | -4.165 - 0.031 | | -1.281 | -4.256 - 1.694 | | -2.856 | -5.793 - 0.080 | |
| | No endocr. therapy | -5.477 | -9.068 - -1.886 | 0.014 | -7.192 | -13.309 - -1.074 | 0.095 | -4.623 | -9.031 - -0.215 | 0.054 |
| | Endocrine therapy | -0.478 | -2.178 - 1.223 | | -1.506 | -4.183 - 1.170 | | 0.21 | -1.978 - 2.398 | |
| **Appetite Loss** | No chemotherapy | -4.044 | -5.475 - -2.614 | <0.001 | -9.294 | -12.200 - -6.388 | 0.175 | -2.102 | -3.696 - -0.509 | 0.009 |
| | Chemotherapy | -8.485 | -9.817 - -7.153 | | -11.736 | -13.734 - -9.738 | | -5.293 | -7.078 - -3.508 | |
| | No endocr. therapy | -7.356 | -9.639 - -5.073 | 0.375 | -11.378 | -15.482 - -7.274 | 0.824 | -5.248 | -7.929 - -2.567 | 0.158 |
| | Endocrine therapy | -6.214 | -7.294 - -5.134 | | -10.871 | -12.669 - -9.073 | | -3.094 | -4.421 - -1.767 | |
| **Constipation** | No chemotherapy | 5.637 | 3.915 - 7.359 | 0.468 | 7.676 | 4.531 - 10.821 | 0.959 | 4.888 | 2.817 - 6.959 | 0.81 |
| | Chemotherapy | 6.508 | 4.904 - 8.111 | | 7.776 | 5.618 - 9.935 | | 5.269 | 2.949 - 7.589 | |
| | No endocr. therapy | 4.299 | 1.543 - 7.055 | 0.156 | 3.694 | -0.756 - 8.144 | 0.052 | 4.621 | 1.122 - 8.119 | 0.785 |
| | Endocrine therapy | 6.503 | 5.206 - 7.800 | | 8.514 | 6.574 - 10.454 | | 5.162 | 3.440 - 6.884 | |
| **Financial difficulties** | No chemotherapy | 0.394 | -0.843 - 1.631 | 0.015 | 1.382 | -1.334 - 4.098 | 0.045 | 0.031 | -1.251 - 1.313 | 0.833 |
| | Chemotherapy | 2.493 | 1.335 - 3.650 | | 4.759 | 2.882 - 6.636 | | 0.239 | -1.203 - 1.680 | |
| | No endocr. therapy | 1.123 | -0.865 - 3.111 | 0.671 | 3.262 | -0.611 - 7.135 | 0.823 | 0.009 | -2.161 - 2.180 | 0.909 |
| | Endocrine therapy | 1.599 | 0.665 - 2.534 | | 3.744 | 2.059 - 5.430 | | 0.150 | -0.917 - 1.217 | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Diarrhea** | No chemotherapy | 0.951 | -0.342 - 2.245 | 0.091 | -1.619 | -4.076 - 0.837 | 0.555 | 1.895 | 0.376 - 3.414 | 0.629 |
| | Chemotherapy | -0.57 | -1.772 - 0.632 | | -2.517 | -4.204 - -0.831 | | 1.334 | -0.364 - 3.032 | |
| | No endocr. therapy | -1.136 | -3.202 - 0.931 | 0.183 | -2.972 | -6.449 - 0.505 | 0.648 | -0.187 | -2.747 - 2.373 | 0.118 |
| | Endocrine therapy | 0.417 | -0.557 - 1.390 | | -2.088 | -3.606 - -0.571 | | 2.091 | 0.829 - 3.352 | |
| **EORTC BR23, functional scales** | | | | | | | | | | |
| **Sexual enjoyment**[¥] | No chemotherapy | -4.575 | -7.354 - -1.796 | 0.124 | -1.717 | -6.113 - 2.679 | 0.055 | -6.605 | -10.138 - -3.072 | 0.527 |
| | Chemotherapy | -7.42 | -9.756 - -5.084 | | -6.943 | -9.961 - -3.924 | | -8.252 | -11.935 - -4.569 | |
| | No endocr. therapy | -5.994 | -10.038 - -1.950 | 0.893 | -3.855 | -9.950 - 2.241 | 0.618 | -7.956 | -13.291 - -2.622 | 0.814 |
| | Endocrine therapy | -6.303 | -8.298 - -4.308 | | -5.553 | -8.282 - -2.823 | | -7.228 | -10.132 - -4.324 | |
| **Future perspective** | No chemotherapy | 11.476 | 9.513 - 13.439 | 0.625 | 12.645 | 8.775 - 16.514 | 0.945 | 11.024 | 8.771 - 13.276 | 0.657 |
| | Chemotherapy | 12.144 | 10.324 - 13.965 | | 12.48 | 9.828 - 15.132 | | 11.789 | 9.278 - 14.300 | |
| | No endocr. therapy | 12.575 | 9.441 - 15.708 | 0.609 | 12.654 | 7.178 - 18.130 | 0.962 | 12.507 | 8.715 - 16.299 | 0.511 |
| | Endocrine therapy | 11.671 | 10.196 - 13.147 | | 12.51 | 10.124 - 14.896 | | 11.087 | 9.218 - 12.957 | |
| **Body image** | No chemotherapy | -8.173 | -9.712 - -6.635 | <0.001 | -9.087 | -12.240 - -5.934 | <0.001 | -7.833 | -9.545 - -6.122 | <0.001 |
| | Chemotherapy | -15.243 | -16.669 - -13.817 | | -17.813 | -19.975 - -15.652 | | -12.705 | -14.612 - -10.798 | |
| | No endocr. therapy | -11.645 | -14.108 - -9.183 | 0.771 | -13.649 | -18.126 - -9.173 | 0.511 | -10.565 | -13.451 - -7.678 | 0.673 |
| | Endocrine therapy | -12.049 | -13.209 - -10.889 | | -15.285 | -17.238 - -13.332 | | -9.872 | -11.295 - -8.449 | |
| **Sexual functioning** | No chemotherapy | 0.603 | -1.068 - 2.273 | 0.253 | 3.277 | -0.110 - 6.664 | 0.262 | -0.419 | -2.262 - 1.423 | 0.152 |
| | Chemotherapy | -0.725 | -2.269 - 0.819 | | 0.929 | -1.389 - 3.248 | | -2.438 | -4.495 - -0.381 | |
| | No endocr. therapy | 0.886 | -1.758 - 3.530 | 0.412 | 0.376 | -4.377 - 5.129 | 0.558 | 1.152 | -1.929 - 4.233 | 0.079 |
| | Endocrine therapy | -0.338 | -1.594 - 0.917 | | 1.93 | -0.160 - 4.020 | | -1.929 | -3.462 - -0.397 | |
| **EORTC BR23, symptoms scales** | | | | | | | | | | |
| **Systemic therapy side effects** | No chemotherapy | 6.57 | 5.710 - 7.430 | 0.157 | 8.195 | 6.571 - 9.818 | 0.474 | 5.973 | 4.962 - 6.984 | 0.977 |
| | Chemotherapy | 7.418 | 6.617 - 8.219 | | 8.915 | 7.800 - 10.029 | | 5.951 | 4.819 - 7.082 | |
| | No endocr. therapy | 4.617 | 3.245 - 5.988 | <0.001 | 6.356 | 4.063 - 8.650 | 0.03 | 3.713 | 2.013 - 5.412 | 0.004 |
| | Endocrine therapy | 7.561 | 6.913 - 8.209 | | 9.128 | 8.126 - 10.130 | | 6.513 | 5.673 - 7.353 | |
| **Breast symptoms** | No chemotherapy | 8.109 | 6.923 - 9.295 | <0.001 | 9.735 | 7.344 - 12.126 | 0.001 | 7.499 | 6.161 - 8.836 | 0.040 |
| | Chemotherapy | 5.128 | 4.027 - 6.229 | | 4.844 | 3.208 - 6.480 | | 5.399 | 3.902 - 6.896 | |
| | No endocr. therapy | 4.533 | 2.642 - 6.425 | 0.024 | 4.038 | 0.681 - 7.396 | 0.131 | 4.822 | 2.563 - 7.082 | 0.092 |
| | Endocrine therapy | 6.947 | 6.054 - 7.840 | | 6.862 | 5.384 - 8.340 | | 6.99 | 5.878 - 8.101 | |
| **Arm symptoms** | No chemotherapy | 9.144 | 7.552 - 10.737 | 0.065 | 8.742 | 5.638 - 11.846 | 0.069 | 9.302 | 7.465 - 11.139 | 0.550 |
| | Chemotherapy | 11.192 | 9.714 - 12.669 | | 12.229 | 10.106 - 14.351 | | 10.143 | 8.091 - 12.196 | |
| | No endocr. therapy | 8.544 | 6.008 - 11.081 | 0.146 | 10.398 | 6.022 - 14.774 | 0.725 | 7.505 | 4.417 - 10.594 | 0.124 |
| | Endocrine therapy | 10.624 | 9.426 - 11.822 | | 11.255 | 9.342 - 13.169 | | 10.207 | 8.680 - 11.733 | |
| **Upset by hair loss*** | No chemotherapy | 7.286 | 1.451 - 13.122 | 0.100 | 8.075 | -3.305 - 19.455 | 0.208 | 6.822 | 0.145 - 13.499 | 0.250 |
| | Chemotherapy | 14.377 | 8.236 - 20.519 | | 17.383 | 8.364 - 26.401 | | 13.207 | 4.598 - 21.816 | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No endocr. therapy | 9.924 | -0.275 - 20.123 | 0.878 | 7.839 | -10.677 - 26.354 | 0.495 | 11.017 | -1.114 - 23.148 | 0.747 |
| | Endocrine therapy | 10.801 | 6.139 - 15.462 | | 14.817 | 7.142 - 22.492 | | 8.796 | 2.919 - 14.672 | |

Asterisks denote that question was only to be answered (*) if patients stated to have been sexually active or (**) if patients stated they had experienced hair loss, resulting in fewer patients responding to these questions compared with other questions.

Models include as covariates: age, Charlson's comorbidity index, BMI, smoking, marital status, education level, income, disease staging center volume, type of surgery, axillary management, receipt of trastuzumab, receipt of radiotherapy, presence of anxiety and presence of depression, all of which collected at diagnosis.

CI – confidence interval; Endocr. – endocrine; EORTC QLQ - European Organisation for Research and Treatment of Cancer quality of life questionnaire; MLS – Mean least square; p-val. – p-value.

**M4 Supplementary table 4 –** Generalized estimating equations of C30 summary score from diagnosis to 2 years after diagnosis: A) models including the interaction between use of adjuvant endocrine therapy and time, and B) models including the interaction between use of adjuvant chemotherapy and time.

**A**

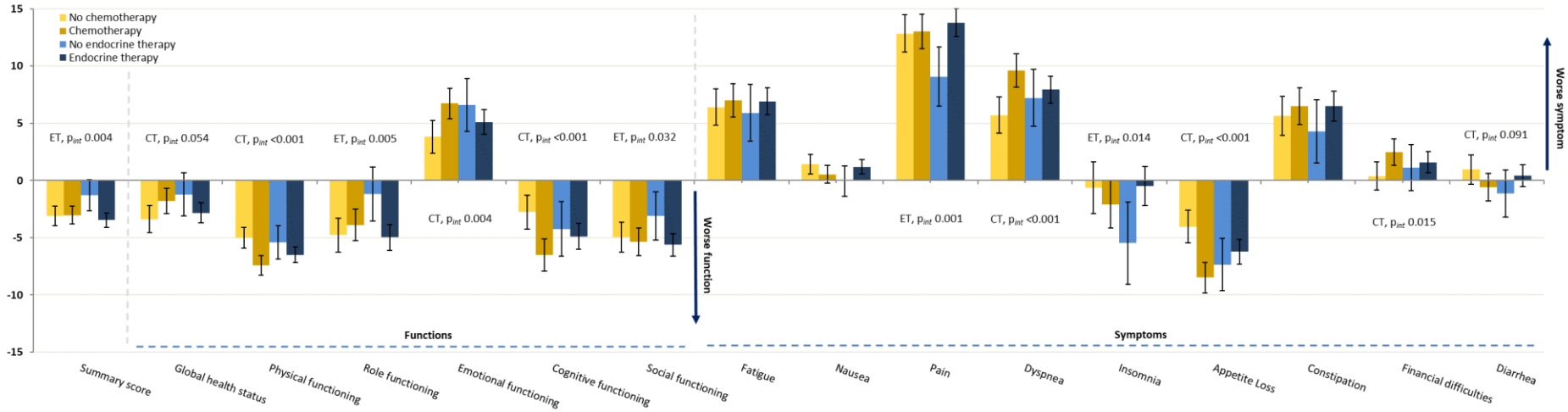
| | Overall cohort | | | Premenopausal | | | Postmenopausal | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Coefficient** | **95% CI** | **p-value** | **Coefficient** | **95% CI** | **p-value** | **Coefficient** | **95% CI** | **p-value** |
| Use of adjuvant ET | 0.375 | -0.685 - 1.435 | 0.488 | 0.617 | -1.221 - 2.455 | 0.511 | 0.434 | -0.856 - 1.723 | 0.510 |
| Time | -1.294 | -2.634 - 0.045 | 0.058 | -1.050 | -3.385 - 1.285 | 0.378 | -1.417 | -3.032 - 0.198 | 0.085 |
| Interaction adjuvant ET*time | -2.164 | -3.644 - -0.683 | 0.004 | -1.523 | -4.071 - 1.025 | 0.242 | -2.649 | -4.450 - -0.847 | 0.004 |
| Use of adjuvant CT | -0.086 | -0.825 - 0.654 | 0.820 | -1.416 | -2.669 - -0.164 | 0.027 | 0.662 | -0.256 - 1.580 | 0.157 |
| Age (categorical) | 0.341 | 0.060 - 0.621 | 0.017 | -0.131 | -0.796 - 0.535 | 0.701 | 0.738 | 0.222 - 1.253 | 0.005 |
| Charlson ≥ 1 | -1.886 | -2.631 - -1.140 | 0.000 | -0.125 | -1.587 - 1.337 | 0.867 | -2.499 | -3.357 - -1.642 | 0.000 |
| BMI (categorical) | -1.187 | -1.563 - -0.812 | 0.000 | -0.290 | -0.927 - 0.347 | 0.373 | -1.663 | -2.125 - -1.200 | 0.000 |
| Current smoker | -2.623 | -3.424 - -1.822 | 0.000 | -2.139 | -3.325 - -0.953 | 0.000 | -2.835 | -3.933 - -1.736 | 0.000 |
| Living as a couple | -0.383 | -1.209 - 0.444 | 0.364 | -1.040 | -2.594 - 0.514 | 0.190 | -0.016 | -0.980 - 0.948 | 0.974 |
| Education (categorical) | -0.250 | -0.489 - -0.012 | 0.040 | 0.027 | -0.396 - 0.450 | 0.900 | -0.414 | -0.702 - -0.127 | 0.005 |
| Income (categorical) | 0.622 | 0.462 - 0.781 | 0.000 | 0.780 | 0.492 - 1.067 | 0.000 | 0.552 | 0.361 - 0.743 | 0.000 |
| Center volume (> 100 patients) | 0.224 | -0.639 - 1.086 | 0.611 | 0.507 | -0.899 - 1.912 | 0.480 | -0.105 | -1.197 - 0.987 | 0.851 |
| TNM Stage (categorical) | -0.760 | -1.362 - -0.159 | 0.013 | -0.418 | -1.338 - 0.501 | 0.372 | -1.042 | -1.839 - -0.245 | 0.010 |
| Mastectomy (vs. BCS) | -0.344 | -1.178 - 0.491 | 0.420 | 0.305 | -0.949 - 1.559 | 0.633 | -0.858 | -1.982 - 0.267 | 0.135 |
| SLND or none (vs. ALND) | 0.595 | -0.196 - 1.386 | 0.140 | 0.735 | -0.488 - 1.957 | 0.239 | 0.538 | -0.501 - 1.577 | 0.310 |
| Use of adjuvant radiotherapy | -1.178 | -2.353 - -0.003 | 0.049 | 0.460 | -1.452 - 2.373 | 0.637 | -2.062 | -3.556 - -0.568 | 0.007 |
| Use of adjuvant trastuzumab | -0.567 | -1.522 - 0.387 | 0.244 | -0.509 | -1.881 - 0.864 | 0.468 | -0.737 | -2.078 - 0.603 | 0.281 |
| Anxiety (categorical) | -3.385 | -3.751 - -3.018 | 0.000 | -3.400 | -3.998 - -2.803 | 0.000 | -3.361 | -3.823 - -2.899 | 0.000 |
| Depression (categorical) | -6.635 | -7.202 - -6.069 | 0.000 | -6.015 | -6.928 - -5.102 | 0.000 | -7.001 | -7.720 - -6.282 | 0.000 |

**B**

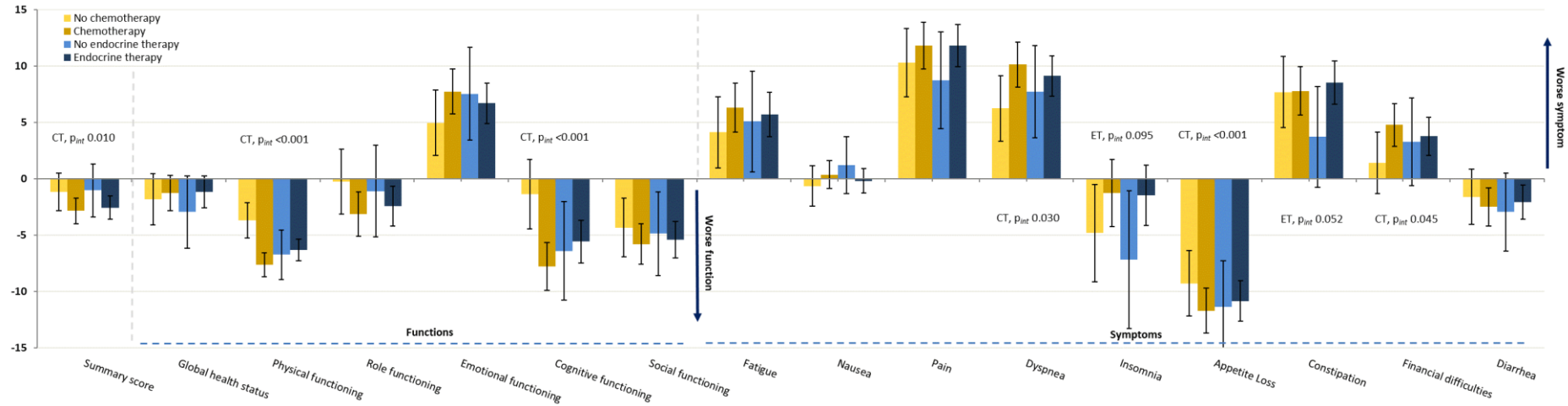
| | Overall cohort | | | Premenopausal | | | Postmenopausal | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Coefficient** | **95% CI** | **p-value** | **Coefficient** | **95% CI** | **p-value** | **Coefficient** | **95% CI** | **p-value** |
| Use of adjuvant ET | -0.113 | -1.043 - 0.817 | 0.811 | -0.580 | -2.183 - 1.023 | 0.478 | 0.357 | -0.799 - 1.512 | 0.545 |
| Time | -3.094 | -3.937 - -2.252 | 0.000 | -1.184 | -2.840 - 0.473 | 0.161 | -3.825 | -4.787 - -2.863 | 0.000 |
| Interaction adjuvant ET*time | 0.056 | -1.091 - 1.203 | 0.924 | -1.679 | -3.686 - 0.327 | 0.100 | 0.626 | -0.815 - 2.067 | 0.394 |
| Use of adjuvant CT | -0.703 | -1.465 - 0.058 | 0.070 | -0.148 | -1.465 - 1.169 | 0.826 | -0.876 | -1.808 - 0.055 | 0.065 |
| Age (categorical) | 0.339 | 0.058 - 0.620 | 0.018 | -0.133 | -0.798 - 0.533 | 0.696 | 0.733 | 0.217 - 1.249 | 0.005 |
| Charlson ≥ 1 | -1.889 | -2.634 - -1.143 | 0.000 | -0.135 | -1.597 - 1.326 | 0.856 | -2.502 | -3.360 - -1.644 | 0.000 |
| BMI (categorical) | -1.187 | -1.563 - -0.811 | 0.000 | -0.287 | -0.924 - 0.350 | 0.376 | -1.661 | -2.124 - -1.198 | 0.000 |
| Current smoker | -2.624 | -3.425 - -1.822 | 0.000 | -2.130 | -3.316 - -0.944 | 0.000 | -2.838 | -3.937 - -1.738 | 0.000 |
| Living as a couple | -0.383 | -1.210 - 0.444 | 0.364 | -1.031 | -2.584 - 0.523 | 0.194 | -0.014 | -0.979 - 0.952 | 0.978 |
| Education (categorical) | -0.253 | -0.492 - -0.015 | 0.038 | 0.028 | -0.395 - 0.451 | 0.898 | -0.420 | -0.707 - -0.132 | 0.004 |
| Income (categorical) | 0.621 | 0.461 - 0.781 | 0.000 | 0.778 | 0.490 - 1.065 | 0.000 | 0.551 | 0.360 - 0.742 | 0.000 |
| Center volume (> 100 patients) | 0.221 | -0.642 - 1.083 | 0.616 | 0.510 | -0.896 - 1.915 | 0.477 | -0.110 | -1.203 - 0.983 | 0.844 |
| TNM Stage (categorical) | -0.755 | -1.357 - -0.154 | 0.014 | -0.419 | -1.338 - 0.499 | 0.371 | -1.031 | -1.829 - -0.234 | 0.011 |
| Mastectomy (vs. BCS) | -0.341 | -1.177 - 0.494 | 0.423 | 0.309 | -0.945 - 1.563 | 0.629 | -0.854 | -1.979 - 0.272 | 0.137 |
| SLND or none (vs. ALND) | 0.600 | -0.191 - 1.392 | 0.137 | 0.737 | -0.485 - 1.959 | 0.237 | 0.550 | -0.490 - 1.590 | 0.300 |
| Use of adjuvant radiotherapy | -1.189 | -2.364 - -0.013 | 0.047 | 0.457 | -1.455 - 2.368 | 0.640 | -2.081 | -3.576 - -0.586 | 0.006 |
| Use of adjuvant trastuzumab | -0.563 | -1.519 - 0.392 | 0.248 | -0.511 | -1.884 - 0.861 | 0.465 | -0.726 | -2.068 - 0.616 | 0.289 |
| Anxiety (categorical) | -3.384 | -3.750 - -3.017 | 0.000 | -3.404 | -4.001 - -2.807 | 0.000 | -3.357 | -3.820 - -2.895 | 0.000 |
| Depression (categorical) | -6.638 | -7.205 - -6.071 | 0.000 | -6.012 | -6.925 - -5.099 | 0.000 | -7.009 | -7.729 - -6.289 | 0.000 |

**M4 Figure 2 –** Mean least square change of EORTC QLQ C30 PRO domains score from diagnosis to the "2-years after diagnosis visit" in patients treated and not treated with chemotherapy or endocrine therapy in the overall cohort (2A), and in premenopausal (2B) and postmenopausal (2C) patients. Error bars refer to the 95% confidence interval of the estimate. P-values refer to the interaction (pint) of the treatment with chemotherapy (CT) or endocrine therapy (ET) and time. Only p-values <0.1 are shown. Estimates and confidence intervals derived from multivariate generalized estimating equations models.

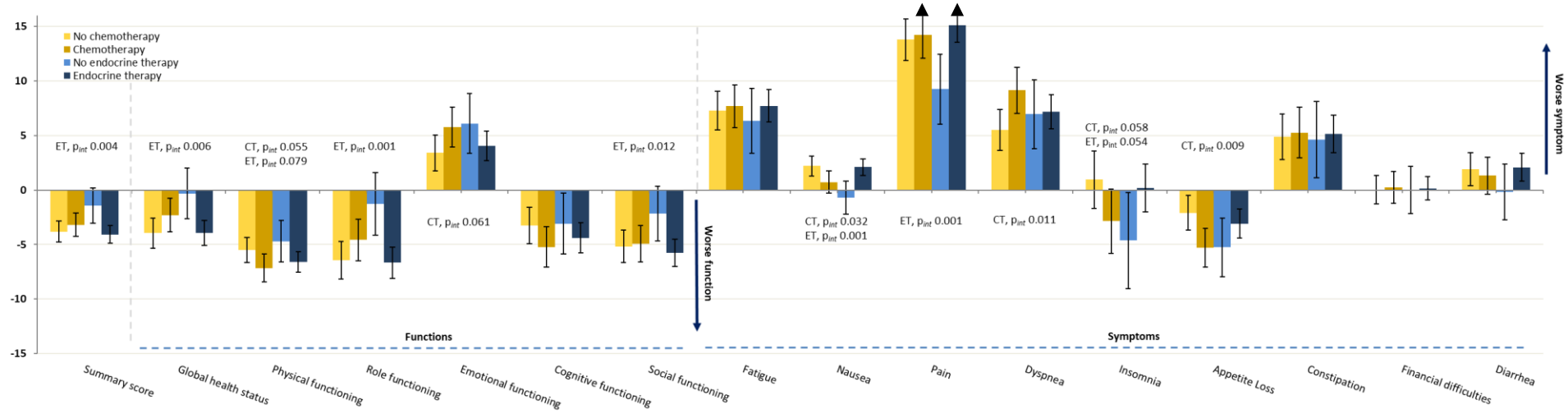
**A**



**B**

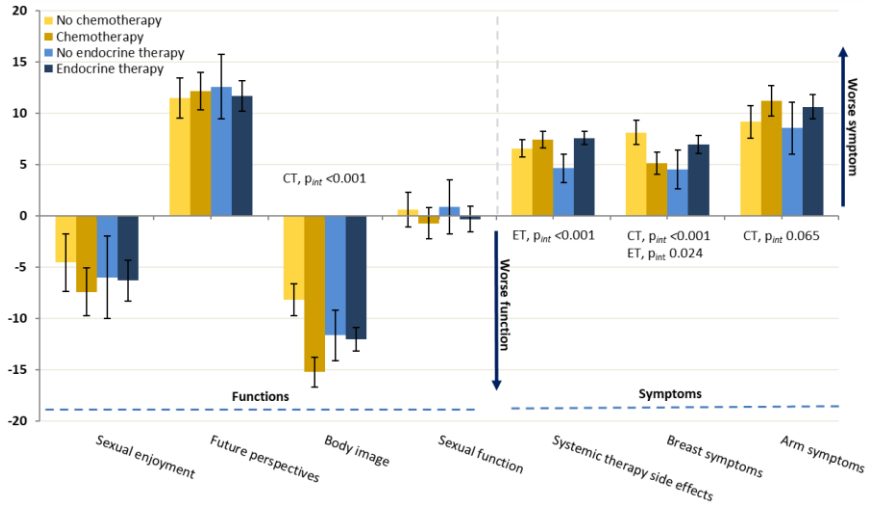

No chemotherapy
Chemotherapy
No endocrine therapy
Endocrine therapy
CT, $p_{int}$ 0.010
CT, $p_{int}$ <0.001
CT, $p_{int}$ <0.001
CT, $p_{int}$ 0.030
ET, $p_{int}$ 0.095
CT, $p_{int}$ <0.001
ET, $p_{int}$ 0.052
CT, $p_{int}$ 0.045
Worse function
Worse symptom
Functions
Symptoms
Summary score
Global health status
Physical functioning
Role functioning
Emotional functioning
Cognitive functioning
Social functioning
Fatigue
Nausea
Pain
Dyspnea
Insomnia
Appetite Loss
Constipation
Financial difficulties
Diarrhea

**C**

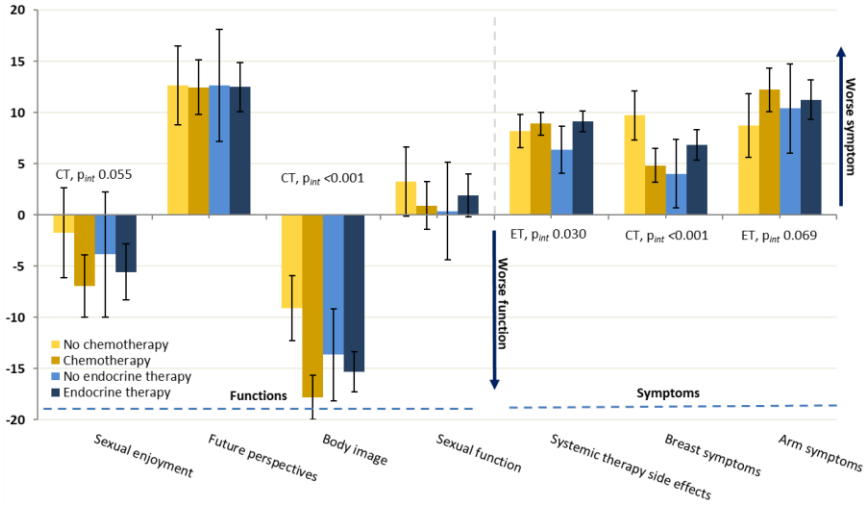

No chemotherapy
Chemotherapy
No endocrine therapy
Endocrine therapy
ET, $p_{int}$ 0.004
ET, $p_{int}$ 0.006
CT, $p_{int}$ 0.055
ET, $p_{int}$ 0.079
ET, $p_{int}$ 0.001
CT, $p_{int}$ 0.061
ET, $p_{int}$ 0.012
CT, $p_{int}$ 0.032
ET, $p_{int}$ 0.001
ET, $p_{int}$ 0.001
CT, $p_{int}$ 0.011
CT, $p_{int}$ 0.058
ET, $p_{int}$ 0.054
CT, $p_{int}$ 0.009
Worse function
Worse symptom
Functions
Symptoms
Summary score
Global health status
Physical functioning
Role functioning
Emotional functioning
Cognitive functioning
Social functioning
Fatigue
Nausea
Pain
Dyspnea
Insomnia
Appetite Loss
Constipation
Financial difficulties
Diarrhea

**M4 Supplementary Figure 3 –** Mean least square change of EORTC QLQ BR23 PRO domains score from diagnosis to the "2-years after diagnosis visit" in patients treated and not treated with chemotherapy or endocrine therapy in the overall cohort (S3A), and in premenopausal (S3B) and postmenopausal (S3C) patients. Hair loss is not shown due to high dispersion of the data. Error bars refer to the 95% confidence interval of the estimate. P-values refer to the interaction (pint) of the treatment with chemotherapy (CT) or endocrine therapy (ET) and time. Only p-values <0.1 are shown. Estimates and confidence intervals derived from multivariate generalized estimating equations models.

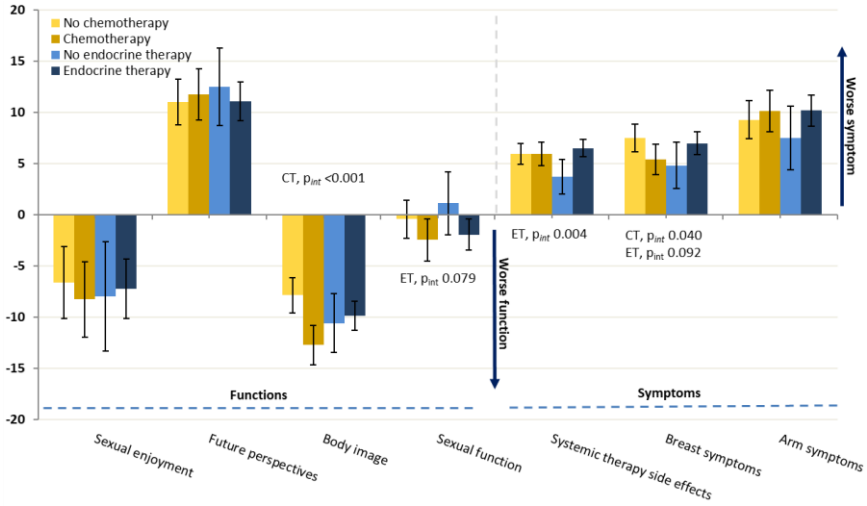
**A**



**B**

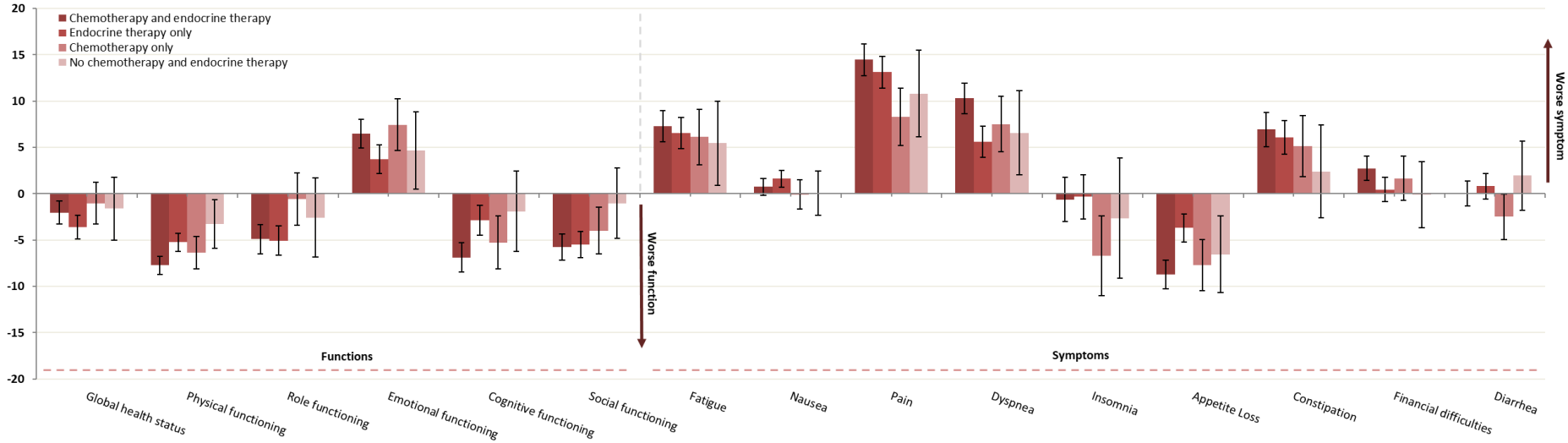

20
15
10
5
0
-5
-10
-15
-20
CT, $p_{int}$ 0.055
CT, $p_{int}$ <0.001
ET, $p_{int}$ 0.030
CT, $p_{int}$ <0.001
ET, $p_{int}$ 0.069
Worse symptom
Worse function
No chemotherapy
Chemotherapy
No endocrine therapy
Endocrine therapy
Functions
Symptoms
Sexual enjoyment
Future perspectives
Body image
Sexual function
Systemic therapy side effects
Breast symptoms
Arm symptoms

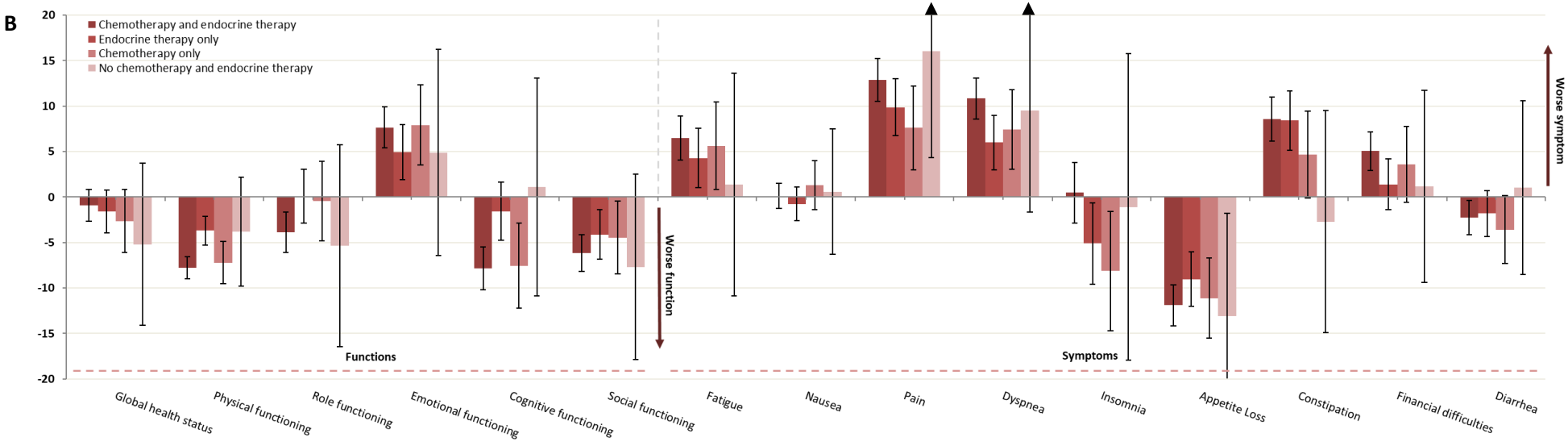
**C**

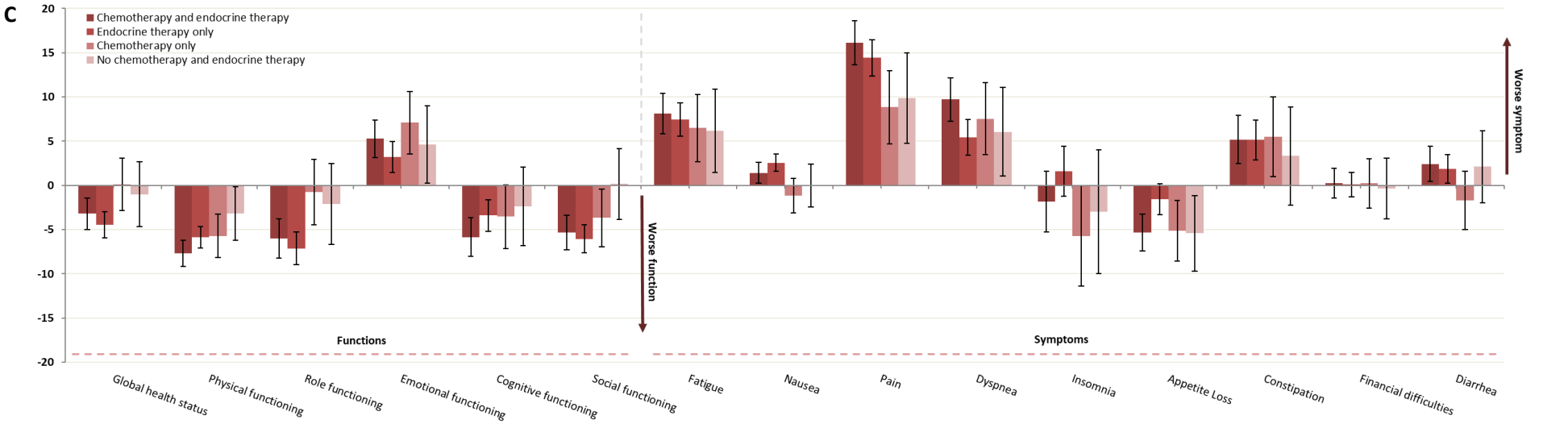

20
15
10
5
0
-5
-10
-15
-20
No chemotherapy
Chemotherapy
No endocrine therapy
Endocrine therapy
CT, $p_{int}$ <0.001
ET, $p_{int}$ 0.079
ET, $p_{int}$ 0.004
CT, $p_{int}$ 0.040
ET, $p_{int}$ 0.092
Worse symptom
Worse function
Functions
Symptoms
Sexual enjoyment
Future perspectives
Body image
Sexual function
Systemic therapy side effects
Breast symptoms
Arm symptoms

**M4 Supplementary Figure 4 –** Mean least square change of EORTC QLQ C30 PRO domains score from diagnosis to the "2-years after diagnosis visit" in patients treated and not treated with chemotherapy and/or endocrine therapy in the overall cohort (S4A), and in premenopausal (S4B) and postmenopausal (S4C) patients. Error bars refer to the 95% confidence interval of the estimate. Estimates and confidence intervals derived from multivariate generalized estimating equations models.

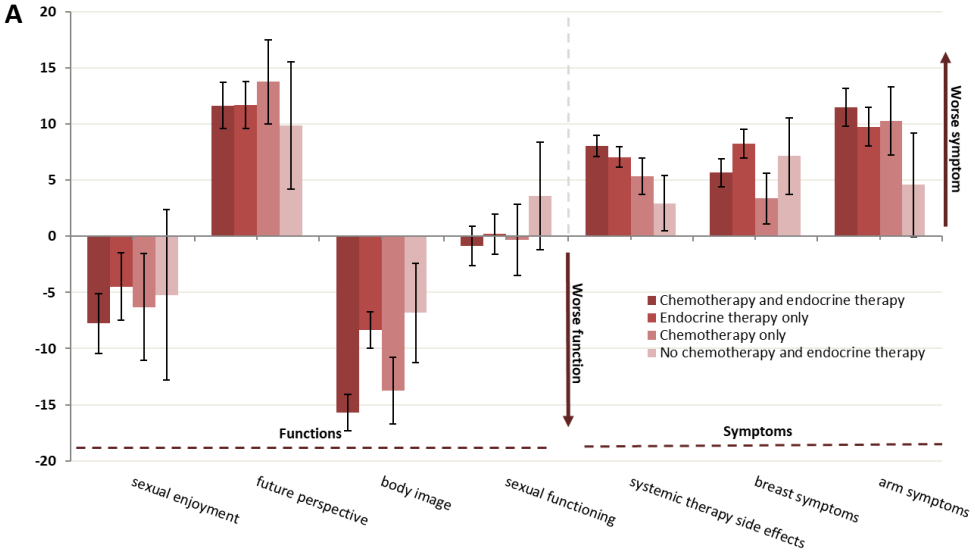
**A**

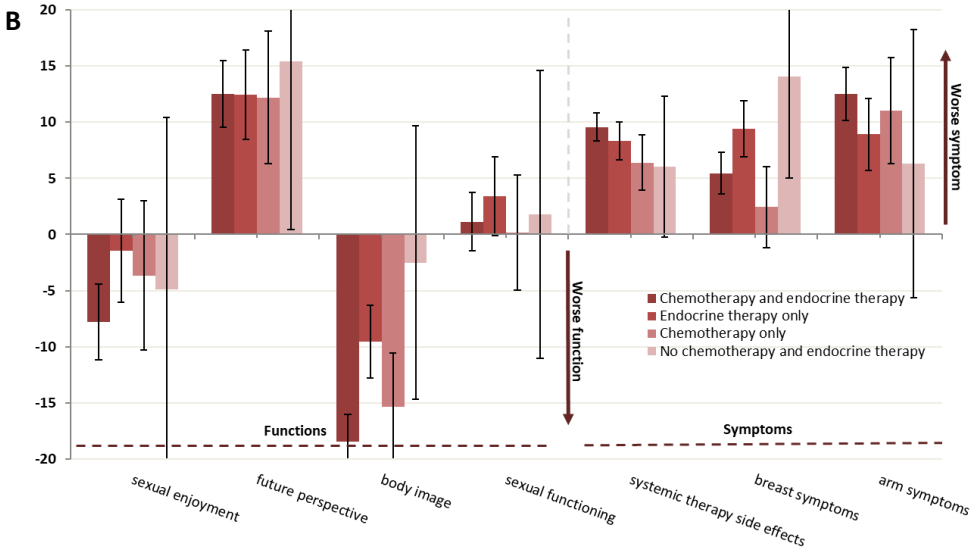


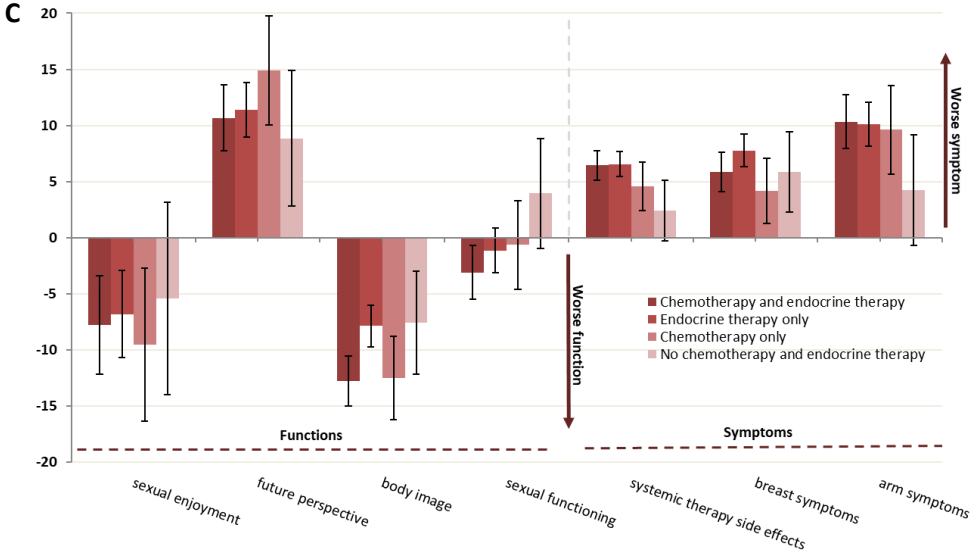

B
Chemotherapy and endocrine therapy
Endocrine therapy only
Chemotherapy only
No chemotherapy and endocrine therapy
20
15
10
5
0
-5
-10
-15
-20
Worse symptom
Worse function
Functions
Symptoms
Global health status
Physical functioning
Role functioning
Emotional functioning
Cognitive functioning
Social functioning
Fatigue
Nausea
Pain
Dyspnea
Insomnia
Appetite Loss
Constipation
Financial difficulties
Diarrhea


C
Chemotherapy and endocrine therapy
Endocrine therapy only
Chemotherapy only
No chemotherapy and endocrine therapy
20
15
10
5
0
-5
-10
-15
-20
Worse symptom
Worse function
Functions
Symptoms
Global health status
Physical functioning
Role functioning
Emotional functioning
Cognitive functioning
Social functioning
Fatigue
Nausea
Pain
Dyspnea
Insomnia
Appetite Loss
Constipation
Financial difficulties
Diarrhea

**M4 Supplementary Figure 5 –** Mean least square change of EORTC QLQ BR23 PRO domains score from diagnosis to the "2-years after diagnosis visit" in patients treated and not treated with chemotherapy and/or endocrine therapy in the overall cohort (S5A), and in premenopausal (S5B) and postmenopausal (S5C) patients. Hair loss is not shown due to high dispersion of the data. Error bars refer to the 95% confidence interval of the estimate. Estimates and confidence intervals derived from multivariate generalized estimating equations models.




B
20
15
10
5
0
-5
-10
-15
-20
Worse symptom
Worse function
Chemotherapy and endocrine therapy
Endocrine therapy only
Chemotherapy only
No chemotherapy and endocrine therapy
Functions
Symptoms
sexual enjoyment
future perspective
body image
sexual functioning
systemic therapy side effects
breast symptoms
arm symptoms


C
20
15
10
5
0
-5
-10
-15
-20
Worse symptom
Worse function
Chemotherapy and endocrine therapy
Endocrine therapy only
Chemotherapy only
No chemotherapy and endocrine therapy
Functions
Symptoms
sexual enjoyment
future perspective
body image
sexual functioning
systemic therapy side effects
breast symptoms
arm symptoms

### 5.4.7 Discussion

In this study, we investigated the variation in QoL from early BC diagnosis, thus before any intervention, to 2-years afterwards among 4262 patients enrolled in the prospective CANTO cohort, a large, real-world contemporary study of patients treated for BC across France. Using validated general and BC specific PROs, we found that patients report overall significantly deteriorated QoL 2-years after BC diagnosis that is impacted by both ET and CT independently. ET represented a considerable and persistent burden for some BC survivors' QoL, affecting the C30-SumSc and a substantial number of domains, while CT effect seems to have a more transient negative impact on QoL. Nevertheless, differential patterns of change in QoL were observed according to adjuvant treatment class and after stratification by menopausal status at diagnosis.

Corresponding with the improved BC survival, the need for patients and healthcare providers to understand the differential effect that distinct classes of adjuvant treatments may have on late QoL is emerging as a priority. Previous research suggested that most physical and psychosocial symptoms that commonly follow adjuvant BC treatment usually resolve in the first year after BC diagnosis and that most of BC survivors recover high functional levels of QoL[152–154] Nevertheless, it has been also reported that some patients may experience more persistent and distressing troubles that include longer-term physical, cognitive, and sexual disturbances.[144,145,155,156] In this study, we found that a substantial number of BC survivors report poor QoL (and deteriorated from diagnosis) 2-years after diagnosis, including a decrease in the C30-SumSc, but also 27.8% of patients reporting poor global health status, 38.4% severe cognitive dysfunction, 51% severe pain, 45.5% severe dyspnea and 33.6% severe fatigue.

Interestingly, when compared to diagnosis and thus before any intervention, our data seem to indicate that the receipt of distinct classes of adjuvant treatment was associated with differential patterns of QoL 2-years after. Prior studies have yielded inconsistent results in this regard. Some suggested that CT leads to cumulative, yet transient, QoL deterioration, which resolves shortly after treatment completion, whereas ET has a more prolonged negative effect on QoL, and other studies have suggested no major differences in QoL by treatment group.[144,145,155–157] For example, a pooled analysis of International BC Study Group trials showed a measurable impact of CT on QoL during active treatment, which was, however, transitory.[155] Nevertheless, persistence of QoL deterioration was associated with treatment strategy over time, with patients treated with chemoendocrine treatment scoring lower than patients treated only with tamoxifen. A previous cross-sectional study evaluating the QoL of BC survivors on average 3-years after BC diagnosis suggested no overall major differences in QoL between

adjuvant treatments groups.[145] This is consistent, with a recent analysis of the TAILORx trial that compared the impact of ET vs. ET+CT in the cognitive function, fatigue and endocrine symptoms.[157] Overall, although the addition of CT to ET led to greater cognitive impairment, fatigue and endocrine symptoms in the first 3-6 months, this change diluted between groups at a follow-up up to 36 months. Our study, making a comprehensive evaluation of with the use of a QoL summary score and several Qol domains, expands this knowledge. Patients were assessed at 2-years after diagnosis and both CT and ET seemed to impact QoL, particularly the C30-SumSc, each however playing a distinct role in different domains. ET had a persistently negative and clinically meaningful impact in C30-SumSc and in multiple functions and symptoms, including role and social function and pain, insomnia and systemic therapy side effects. In contrast, ET seems to attenuate the recovery in domains that typically improve overtime such as emotional function and future perspectives. In contrast, the impact of CT seemed to be transient and restricted to physical and cognitive function, financial difficulties, body image and breast symptoms, with no impact in the C30-SumSc at 2-years post-diagnosis. Our approach to evaluate the contributions of ET and CT after stratification by menopausal status adds further to the literature. In premenopausal patients, receipt of CT although fading overtime overall, it was associated with significant deterioration of several QoL domains. In addition, while CT seems to be the only driver of cognitive impairment in premenopausal women, both ET and CT contribute additively to cognitive deterioration in postmenopausal women. In postmenopausal patients, deterioration of QoL was associated substantially with ET. Treatment and treatment implications can greatly differ by menopausal status partially explaining these differences. Eighty-nine percent of premenopausal women in our cohort who received ET were treated with tamoxifen compared to 88% of postmenopausal women who received AIs, therefore it is possible that the use of AI might have driven our findings on the postmenopausal cohort. This is in line with recent longitudinal cohort data of 186 BC patients that suggested significantly reduced physical QoL for patients treated with AIs 1-year after initiation of ET compared with tamoxifen, but it contrasts with clinical trial data that have traditionally suggested only small differences in QoL by type of ET. If this is correct, the recent trend towards escalation of ET, either by extending the total duration of treatment or, in premenopausal women by intensifying treatment with the use of OFS with tamoxifen or AIs, might therefore substantially add to the burden of ET on QoL. In premenopausal women the impact of CT in QoL might indeed reflect transient or permanent ovarian function failure, suggesting that uptake of OFS in these patients may have a major impact on their QoL.

For this study, we used a large national French cohort that is representative of the overall BC population (77.8% HR+/HER2- BC, 51.5% stage I, 86.0% of CT treated patients received anthracyclines-taxanes) and that offered a unique opportunity to have a detailed and up-to-date perspective of the impact of CT and ET in QoL of BC patients. Nevertheless, we acknowledge some limitations. The proportion of patients with missing QoL questionnaire at 2-years after diagnosis was over 25%. While not optimal, this can be expected given the real-world research. Specific populations, as older and less educated/lower income patients might be underrepresented in this study thus deserving a focused approach in future research. Also, this study included patients who were diagnosed between 2012 and 2015, and treatment patterns have evolved since. The proportion of patients currently on adjuvant OFS plus AI or tamoxifen is higher than what was noted in the present study, which might underestimate the toxicity of ET in premenopausal women. Likewise, the most frequent adjuvant anthracycline-taxane combination regimen in CANTO was FEC-T (5-fluorouracil-epirubicin-cyclophosphamide followed by a taxane), while in current practice EC/AC-T (epirubicin/doxorubicin-cyclophosphamide followed by a taxane) is predominant. A minority of patients was treated with anthracyclines-sparing regimens which is, in some practices, an emerging regimen to treat early breast cancer. In addition, we did not explore the QoL impact by endocrine or CT regimen, since it is out of the scope of this paper. Moreover, there is not just one QoL metric, but many outcomes that have to be assessed to capture the overall impact of treatment on QoL, nevertheless we integrated a QoL summary score as primary outcome. Furthermore, we used EORTC QLQ BR23 module instead of the BR45 which was unavailable at CANTO study inception and is now in phase IV testing. Given that the QLQ BR45 might better capture specific BC treatment toxicities (e.g. joint pain and muscle ache), our results may be a conservative picture of the ET impact. In addition, due to the observational design and although we performed a comprehensive adjustment of our models, we cannot exclude unmeasured confounding, including factors such as treatment adherence. Lastly, no formal adjustment for multiplicity has been performed given the observational nature of the study.

In conclusion, QoL was deteriorated at 2-years after BC diagnosis in multiple functions and symptoms. QoL deterioration was associated with ET in postmenopausal women, and receipt of CT seemed to have a larger impact in premenopausal women. This differential effect of treatment classes by menopausal status on QoL should be considered when discussing optimal adjuvant therapy options and survivorship care as they may have implications for adherence and long-term health and psychosocial outcomes. While systemic treatment is a major driver in QoL, we recognize that the optimal support is a continuum that must consider,

among others, the psychological disruption of cancer diagnosis and the sequelae of local interventions. Our data challenge the common idea that ET is an innocent player in the QoL arena and highlight that appropriate selection of women for ET treatment escalation should be a research priority.

### 5.4.8 Funding

This work was supported by Agence Nationale De La Recherche [ANR-10-COHO-0004 to F. A.]; Susan G. Komen [CCR17483507 to I. V.]; Fondation ARC pour la recherche sur le cancer [*CANTO-WORK programme labillisé* to I. V.]; and Odyssea [NA to I. V.]. Funding sources did not interfere in any step of the study, namely design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; nor decision to submit the manuscript for publication.

## 5.5 Serum detection of non-adherence to adjuvant tamoxifen and breast cancer recurrence risk.

### 5.5.1 Introductory notes

This project quantifies adherence 1 year after adjuvant tamoxifen prescription by two methods to access adherence, specifically a self-reported questionnaire or by serum assessment. It further quantifies the impact of non-adherence on cancer outcomes. This project is under submission. Arlindo R. Ferreira participated in the study design, led data analysis, and further participated in results interpretation and manuscript writing.

### 5.5.2 Authors

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### 5.5.3 Abstract

Purpose: Non-adherence to long-term treatments is often under recognized by physicians, and a gold standard for its assessment does not exist. In breast cancer, non-adherence to medication constitutes a major obstacle for optimal outcomes. We sought to evaluate the rate of biochemical non-adherence to adjuvant tamoxifen one year after

treatment prescription using drug serum assessment and to examine its effects on short-term distant disease-free-survival (DDFS).

Patients and Methods: We studied 1177 premenopausal women enrolled on a large prospective national clinical study (CANTO/NCT01993498). Definition of biochemical non-adherence was based on a tamoxifen serum level <60 ng/ml. Patients were also requested to self-report adherence to tamoxifen during concomitant follow-up visits. Survival analyses were conducted using propensity score inverse probability treatment weighting and Cox proportional hazards models.

Results: Serum assessment of tamoxifen identified 16.0% of patients (n=188) below the set adherence threshold. Patient-reported rate of non-adherence was lower (12.3%). Of 188 biochemical non-adherent patients, 104 (55%) stated they had been regularly taking tamoxifen. After a median follow-up time of 24.2 months since tamoxifen serum assessment, biochemical non-adherent patients had significantly shorter DDFS (adjusted hazard ratio of distant recurrence or death 2.31 [1.05-5.06]; p=0.036) and an absolute 5.9% increase in the risk of DDFS at 3 years.

Conclusions: Therapeutic drug monitoring may be a useful method to promptly identify patients who do not take adjuvant tamoxifen as prescribed and are at risk of poorer outcomes. Targeted interventions facilitating patients' adherence are needed and have the potential to improve short-term breast cancer outcomes.

Trial registration: NCT01993498.

### 5.5.4 Introduction

Previous studies suggested that 30-50% of patients with chronic conditions in developed countries are non-adherent to prescribed medications.[158,159] Annually in the US, non-adherence to chronic medications is responsible for increased mortality, hospitalizations and health-care costs.[158,160] Non-adherence also impacts patient-physician relationships, possibly leading to breakdowns in trust and communication.[159,161] In addition, since health care systems are evolving into models where health care providers' payments are tied to outcomes, non-adherence can also impact health care providers reimbursement.[162] Therefore, optimizing adherence may lead to dramatic improvements in health outcomes, patient satisfaction and costs.

To be able to design effective programs supporting adherence it is first essential to better recognize when actual medication use differs from the prescribed regimen. There is no gold standard to identify non-adherence, with the prevalent use of indirect methods, commonly based on pharmacy prescription refills, patient-administered questionnaires, which although informative do not capture the actual medication intake. Particularly, it has been shown that patient self-report tend to overestimate adherence rates from two- to four-fold and pharmacy claims do not perfectly reflect the medication intake, especially if low out-of-pocket costs.[163] Direct methods, as measurement of the level of the drug or its metabolites in the blood or urine are less well studied and are not currently used in clinical practice.[158,161,164,165] Furthermore, non-adherence is a complex phenomenon with a multitude of factors associated, including patient, health care provider and disease-specific features making it hard to identify and intervene on causes of non-adherence.[158,160]

Eighty percent of breast cancer patients have hormone receptor-positive (HR+) disease and more than 90% of these patients present with stage I-III disease rendering them eligible for curative treatment.[10] For patients with HR+ breast cancer receiving adjuvant endocrine therapy, previous studies suggested that non-adherence is a prevalent issue.[166–168] Because 5-years of adjuvant endocrine therapy reduces recurrence rate by 50% throughout the first 10 years and mortality by a third throughout the first 15 years[10] and extending the duration of endocrine therapy beyond five years can also impact risk of recurrence by up to 40%, non-adherence constitutes a major obstacle for optimal disease and survival outcomes.[10,67] In premenopausal patients with HR+ breast cancer, especially those younger than 40, non-adherence to adjuvant endocrine therapy seems to be a major issue, and evidence suggested poorer survival outcomes in this population compared to older ones, partly due to higher non-adherence rates.[169]

CANcer TOxicities (CANTO) study (NCT01993498) has collected prospectively detailed tumor, treatment, toxicities, health-related patient reported outcomes (HRPROs) and biological data, on a cohort of 12,012 women with newly diagnosed early breast cancer. In this study we evaluated the hypothesis that therapeutic drug monitoring may promptly identify patients who are non-adherent to breast cancer adjuvant endocrine therapy and at risk of a worse outcome. To do this, we examined non-adherence by tamoxifen serum assessment among premenopausal patients of the CANTO cohort, in the first year after the start of adjuvant endocrine treatment and its impact on short-term breast cancer survival outcomes.

### 5.5.5 Patients and Methods

Study design and patient selection

*Data Source*

The CANTO cohort enrolled patients across the entire national French territory from 2012 to 2018. Eligibility criteria include patients 18 years of age or older, with a primary diagnosis of invasive stage cT0-cT3, cN0-3 breast cancer and no previous treatments for current breast cancer. Patients are assessed at diagnosis and shortly after primary treatment (primary surgery, chemotherapy or radiotherapy, whichever comes last), near to endocrine therapy prescription, if indicated, and then at Year 1, 3, and 5 after the initial post-primary treatment evaluation. Data collection at each time point includes clinical, treatment (including medication adherence assessed by trained clinical research nurse [CRN]), toxicity data, HRPROs and serum samples.[80] The protocol is available with the full text of this article.

*Study oversight*

CANTO is coordinated by UNICANCER (National French Cancer Centers Cooperative Group). The study was approved by the national regulatory authorities and ethics committee (ID-RCB: 2011-A01095-36; 11-039). All patients enrolled in the study provided written informed consent including consent for the biological data collection.

Variables assessment

*Assessment of non-adherence*

Non-adherence at Year 1 post-tamoxifen prescription was defined as non-persistence (early discontinuation) and/or suboptimal medication implementation (interruptions, skipped doses), in accordance with EMERGE guidelines at least one year after tamoxifen prescription.[170] We focused on women who potentially initiated tamoxifen and we excluded those who were prescribed tamoxifen but did not agree to initiate the treatment, as captured by CANTO clinical report form (CRF): “Endocrine therapy: yes/no”; “if no, state reason: patient’s refusal?; contraindication? Non-indication?”.

Non-adherence to tamoxifen at Year 1 post-tamoxifen prescription was determined using an objective and direct method, tamoxifen serum assessment (biochemical non-adherence) (primary outcome) and a subjective and indirect method, patient’s self-declaration (secondary outcome).

*Definition of primary outcome (biochemical non-adherence)*

Blood samples were immediately stored at -80° C after collection. Tamoxifen serum level was determined by liquid chromatography-tandem mass spectrometry on 200-400 µL of serum

in the multiple reaction monitoring mode of a 6460 triple quadrupole mass spectrometer (Agilent Technologies, Waldbronn, Germany).[171]

We used a predefined threshold of 60 ng/ml, for defining biochemical non-adherence to tamoxifen on the basis of pharmacological studies.[172–174] Supplementary appendices M5 S1.1 and M5 S1.2 detail tamoxifen metabolism and pharmacokinetic and cut-off definition for biochemical non-adherence.

*Definition of secondary outcome*

Patient's self-declarations on adherence to tamoxifen were collected by trained CRNs at the same time point of blood collection for tamoxifen serum-assessment by semi-structured interviews. M5 Supplementary Appendix S1.2 details the definition of patient's self-declaration.

*Assessment of Survival Outcomes*

For survival analyses, we focused primarily in distant disease-free survival (DDFS), given that the loco-regional recurrences are frequently amenable to definitive treatment thus limiting results interpretation in a cohort with a relatively short follow-up and limited number of recurrences. DDFS was defined as time from tamoxifen serum assessment to date of distant recurrence or death by any cause.[175] Secondarily, we examined breast cancer free interval (BCFI), which was defined time from tamoxifen serum assessment to date of contralateral breast cancer, local, regional or distant recurrence or death by breast cancer.[175] Since our focus was to assess the impact of non-adherence at Year 1 after tamoxifen prescription, a landmark analysis was performed and per consort diagram all patients with a distant disease event before this time point were upfront excluded from this study.

*Study covariates*

All study covariates were categorized as per M5 Table 1, including baseline socio-demographic, clinical, and behavioral factors, treatment toxicities and HRPROs shortly after treatment prescription.

Statistical analysis

Concordance between serum assessment and patient's self-report was tested by chi-square test and estimated using Cramer-V coefficient. Multivariate logistic regression modeled the association of relevant covariates with non-adherence at Year 1 post-tamoxifen prescription. Several methods examined the independent impact of biochemical non-adherence and patient reported non-adherence on DDFS. Time-to-event outcomes were estimated and plotted using

the Kaplan-Meier method. To deal with confounding, as a primary analyses we used propensity score (PS) inverse probability treatment weighting (IPTW) in a Cox model.[176] To assess robustness of results a multivariable Cox proportional hazards (CPH) model was also performed as a sensitivity analysis. Variables included both in the PS IPTW and CPH mode were known breast cancer prognostic factors and included: age at diagnosis, TNM staging, type of surgery, receipt of (neo) adjuvant chemotherapy and center size. In a second sensitivity analysis, to incorporate known social, psychological and behavioral confounders PS IPTW was also weighted by marital status, education, body mass index, smoking habits, anxiety, depression and symptomatology at treatment initiation. PS diagnostics were performed using user-written package pstest for Stata (by E. Leuven and B Sianesi). Variance estimation was optimized by using a bootstrapped PS and, to deal with instability that can ensue from large weights, a stabilized IPTW was implemented.[176,177] Since the Year 1 visit did not occur exactly at the same time from diagnosis for all patients, description of time between scheduled visits in adherent and non-adherent patients was also performed. All time-to-event analyses met proportional hazards assumption as assessed by the Schoenfeld residuals. Given the low DDFS event rate, median follow-up was the median of the observed follow-up times using all patients. There were low rates of missing variables, which were considered missing at random among adherent and non-adherent patients (M5 Table 1), given balanced distribution among groups. Therefore, no multiple imputation was performed. Secondary analyses focused on BCFI were performed. All tests were 2-sided and p-values of ≤ 0.05 were considered statistically significant. No formal adjustment for multiplicity has been performed given the observational nature of the study. The analyses were performed using Stata 15.1 (StataCorp LP).

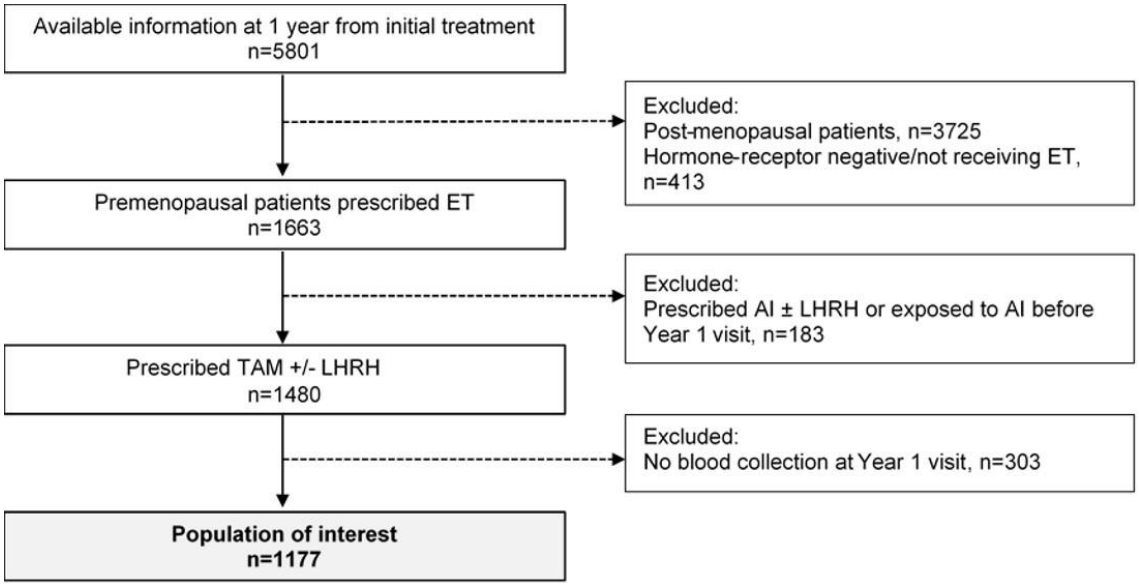
### 5.5.6 Results

Study Cohort

From the 5,801 women enrolled in CANTO with available data, we first excluded those who were post-menopausal at cancer diagnosis (n=3725), those with HRe-negative breast cancer or not receiving endocrine therapy (n=413) and those prescribed aromatase inhibitors before Year 1 visit (n=183). We then selected all women who were premenopausal at diagnosis and were prescribed and agreed to take adjuvant tamoxifen (n=1480). Finally, we selected women among whom tamoxifen serum assessment was performed at Year 1 post-tamoxifen prescription (n=1177) (M5 Figure 1). M5 Supplementary Table S1 describes characteristics of non-participant patients who were excluded due to absence of blood assessment (n=303/1480,

20.5%). Nonparticipant patients had lower likelihood to belong to a high-volume recruitment center; no other major differences emerged between groups.

**M5 Figure 1 –** Consort diagram of study participants. AI – aromatase inhibitor; ET – endocrine therapy; LHRH – luteinizing hormone releasing hormone; TAM – tamoxifen.




Among the analytic cohort, median time from tamoxifen prescription to measurement of non-adherence was 16.2 months (interquartile range [IQR] 15.1 - 17.8). Median age was 45 years (IQR 41 – 49). Patients' characteristics at baseline and treatment details are reported in M5 Table 1.

**M5 Table 1** – Demographic, social, clinical and pathological characteristics at baseline and treatment details

| | **Overall cohort** | | **Serum-defined adherence** | | | |
|---|---|---|---|---|---|---|
| | | | **Adherent** | | **Non-adherent** | |
| | Number | % | Number | % | Number | % |
| **Total number** | 1177 | 100 | 989 | 84.0 | 188 | 16.0 |
| **Tamoxifen serum concentration** | | | | | | |
| Median (IQR) | 110 (80 – 144) | | 119 (96 – 152) | | 6 (6 – 38) | |
| Min. – Max. | 6 – 298 | | 60 – 298 | | 6 – 60 | |
| **Age, years** | | | | | | |
| Median | 45.0 | | 45.0 | | 46.0 | |
| IQR | 41.0 – 49.0 | | 41.0 – 49.0 | | 42.0 – 50.0 | |
| **Age** | | | | | | |
| ≤35 | 91 | 7.7 | 73 | 7.4 | 18 | 9.6 |
| >35 to ≤40 | 164 | 13.9 | 143 | 14.5 | 21 | 11.2 |
| >40 to ≤50 | 762 | 64.7 | 641 | 64.8 | 121 | 64.4 |
| >50 | 160 | 13.6 | 132 | 13.3 | 28 | 14.9 |
| **Charlson's comorbidity score** | | | | | | |
| 0 | 975 | 88.2 | 830 | 89.4 | 145 | 81.9 |
| ≥1 | 130 | 11.8 | 98 | 10.6 | 32 | 18.1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Missing | 72 | 6.1 | 61 | 6.2 | 11 | 5.9 |
| **Body mass index** | | | | | | |
| Underweight | 47 | 4.0 | 39 | 3.9 | 8 | 4.3 |
| Normal | 728 | 62.0 | 615 | 62.2 | 113 | 60.4 |
| Overweight | 252 | 21.4 | 213 | 21.6 | 39 | 20.9 |
| Obese | 148 | 12.6 | 121 | 12.2 | 27 | 14.4 |
| Missing | 2 | 0.2 | 1 | 0.1 | 1 | 0.5 |
| **Smoking status** | | | | | | |
| No/previous smoker | 905 | 78.0 | 774 | 79.1 | 131 | 71.6 |
| Smoker | 256 | 22.0 | 204 | 20.9 | 52 | 28.4 |
| Missing | 16 | 1.4 | 11 | 1.1 | 5 | 2.7 |
| **Education** | | | | | | |
| Primary school | 50 | 4.5 | 39 | 4.1 | 11 | 6.3 |
| High school | 498 | 44.5 | 409 | 43.4 | 89 | 50.6 |
| College or higher | 570 | 51.0 | 494 | 52.4 | 76 | 43.2 |
| Missing | 59 | 5.0 | 47 | 4.8 | 12 | 6.4 |
| **Household income** | | | | | | |
| <1500 | 112 | 10.2 | 85 | 9.2 | 27 | 15.8 |
| ≥1500 to <3000 | 415 | 37.9 | 339 | 36.7 | 76 | 44.4 |
| ≥3000 | 568 | 51.9 | 500 | 54.1 | 68 | 39.8 |
| Missing | 82 | 7.0 | 65 | 6.6 | 17 | 9 |
| **Marital status** | | | | | | |
| Living as couple | 951 | 84.8 | 813 | 86.2 | 138 | 77.5 |
| Living alone | 170 | 15.2 | 130 | 13.8 | 40 | 22.5 |
| Missing | 56 | 4.8 | 46 | 4.7 | 10 | 5.3 |
| **Histology** | | | | | | |
| Invasive carcinoma, NST | 944 | 80.3 | 791 | 80.1 | 153 | 81.4 |
| Invasive lobular carcinoma | 132 | 11.2 | 112 | 11.3 | 20 | 10.6 |
| Mixed NST/lobular | 35 | 3 | 32 | 3.2 | 3 | 1.6 |
| Other | 65 | 5.5 | 53 | 5.4 | 12 | 6.4 |
| Missing | 1 | 0.1 | 1 | 0.1 | 0 | 0 |
| **Histologic grade** | | | | | | |
| 1 | 203 | 17.3 | 167 | 16.9 | 36 | 19.1 |
| 2 | 635 | 54.1 | 535 | 54.3 | 100 | 53.2 |
| 3 | 336 | 28.6 | 284 | 28.8 | 52 | 27.7 |
| Missing | 3 | 0.3 | 3 | 0.3 | 0 | 0 |
| **AJCC TNM stage** | | | | | | |
| I | 519 | 44.1 | 432 | 43.7 | 87 | 46.3 |
| II | 508 | 43.2 | 426 | 43.1 | 82 | 43.6 |
| III | 149 | 12.7 | 130 | 13.2 | 19 | 10.1 |
| Missing | 1 | 0.1 | 1 | 0.1 | 0 | 0 |
| **IHC-defined subtype** | | | | | | |
| HR+/HER2- | 995 | 84.5 | 834 | 84.3 | 161 | 85.6 |
| HR+/HER2+ | 182 | 15.5 | 155 | 15.7 | 27 | 14.4 |
| **Surgery type** | | | | | | |
| BCS | 788 | 66.9 | 659 | 66.6 | 129 | 68.6 |
| Mastectomy | 389 | 33.1 | 330 | 33.4 | 59 | 31.4 |
| **Axillary management** | | | | | | |
| Axillary dissection | 570 | 48.4 | 478 | 48.3 | 92 | 48.9 |
| Sentinel node/none | 607 | 51.6 | 511 | 51.7 | 96 | 51.1 |
| **Radiotherapy** | | | | | | |
| Yes | 1068 | 90.7 | 897 | 90.7 | 171 | 91 |
| No | 109 | 9.3 | 92 | 9.3 | 17 | 9 |
| **(Neo)adjuvant CT type** | | | | | | |
| Anthracyclines-taxanes | 691 | 58.7 | 590 | 59.7 | 101 | 53.7 |
| Anthracyclines-based | 24 | 2 | 18 | 1.8 | 6 | 3.2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Taxanes-based | 44 | 3.7 | 40 | 4 | 4 | 2.1 |
| Missing regimen | 1 | 0.1 | 1 | 0.1 | 0 | 0 |
| No | 417 | 35.4 | 340 | 34.4 | 77 | 41 |
| **HER2-directed therapy** | | | | | | |
| Yes | 146 | 12.4 | 129 | 13 | 17 | 9 |
| No | 1031 | 87.6 | 860 | 87 | 171 | 91 |
| **EORTC QLQ-C30 severe fatigue (>40) at tamoxifen prescription** | | | | | | |
| Yes | 440 | 40 | 350 | 37.6 | 90 | 53.3 |
| No | 660 | 60 | 581 | 62.4 | 79 | 46.7 |
| Missing | 77 | 6.5 | 58 | 5.9 | 19 | 10.1 |
| **EORTC QLQ-C30 severe insomnia (>40) at tamoxifen prescription** | | | | | | |
| Yes | 454 | 41.5 | 369 | 39.8 | 85 | 50.9 |
| No | 641 | 58.5 | 559 | 60.2 | 82 | 49.1 |
| Missing | 82 | 7.0 | 61 | 6.2 | 21 | 11.2 |
| **HADS anxiety at tamoxifen prescription** | | | | | | |
| Normal | 561 | 51 | 486 | 52.2 | 75 | 44.6 |
| Borderline | 291 | 26.5 | 241 | 25.9 | 50 | 29.8 |
| Anxiety | 247 | 22.5 | 204 | 21.9 | 43 | 25.6 |
| Missing | 78 | 6.6 | 58 | 5.9 | 20 | 10.6 |
| **HADS depression at tamoxifen prescription** | | | | | | |
| Normal | 902 | 82.1 | 775 | 83.2 | 127 | 75.6 |
| Borderline | 136 | 12.4 | 107 | 11.5 | 29 | 17.3 |
| Depression | 61 | 5.6 | 49 | 5.3 | 12 | 7.1 |
| Missing | 78 | 6.6 | 58 | 5.9 | 20 | 10.6 |
| **CTCAE v4 toxicities (any grade)** | | | | | | |
| Any gynecologic side effects | 584 | 50.8 | 483 | 49.8 | 101 | 55.8 |
| Hot-flashes | 863 | 75 | 727 | 75 | 136 | 75.1 |
| Musculoskeletal symptoms | 571 | 49.9 | 462 | 47.7 | 109 | 61.9 |
| Concentration impairment | 499 | 43.7 | 411 | 42.6 | 88 | 49.4 |
| Any neuropathy | 316 | 27.7 | 26.5 | 27.2 | 54 | 30.3 |
| **High recruitment center (>100 pts)** | | | | | | |
| Yes | 1152 | 97.9 | 968 | 97.9 | 184 | 97.9 |
| No | 25 | 2.1 | 21 | 2.1 | 4 | 2.1 |

AJCC – American Joint Committee on Cancer; BCS- Breast Conservative Surgery; CTCAE - Common Terminology Criteria for Adverse Events; EORTC QLQ - European Organisation for Research and Treatment of Cancer Quality of Life Questionnaires; HADS - Hospital Anxiety and Depression Scale, IHC – immunohistochemistry; IQR – interquartile range; NST – No Special Type; pts – patients.

Non-adherence at Year 1 post-tamoxifen prescription

Tamoxifen serum concentrations at Year 1 post-tamoxifen prescription ranged between <6 and 298 ng/ml, with a median of 110 ng/ml (M5 Table 1, M5 Figure S1 and M5 Table S2). Overall, 188 (16%) patients were below the set biochemical adherence threshold to tamoxifen at Year 1; 145 patients (12.3%) self-declared to be non-adherent: 89 (7.6%) reported tamoxifen discontinuation and 56 (4.7%) temporary interruptions. Among the 145 patients declaring to be non-adherent, only few (n=67) were able to provide a personal or medical reason for non-adherence. Among these, toxicity was mentioned by 57 patients. Of 188 biochemical non-

adherent patients, 104 (55.3%) stated they had been regularly taking tamoxifen over the last year. Conversely, 61 patients revealed a non-adherent behavior despite being adherent by serum assessment, of whom the majority (82%) reported transitory tamoxifen interruptions. Although biochemical and self-declaration non-adherence were significantly associated (p<.0001) only moderate concordance between the two methods was found (concordance: 86% [95% CI 84 to 88%]; Cramer V = 0.429) (M5 Table 2).

**M5 Table 2 –** Concordance of serum and self-declaration methods to assess adherence.

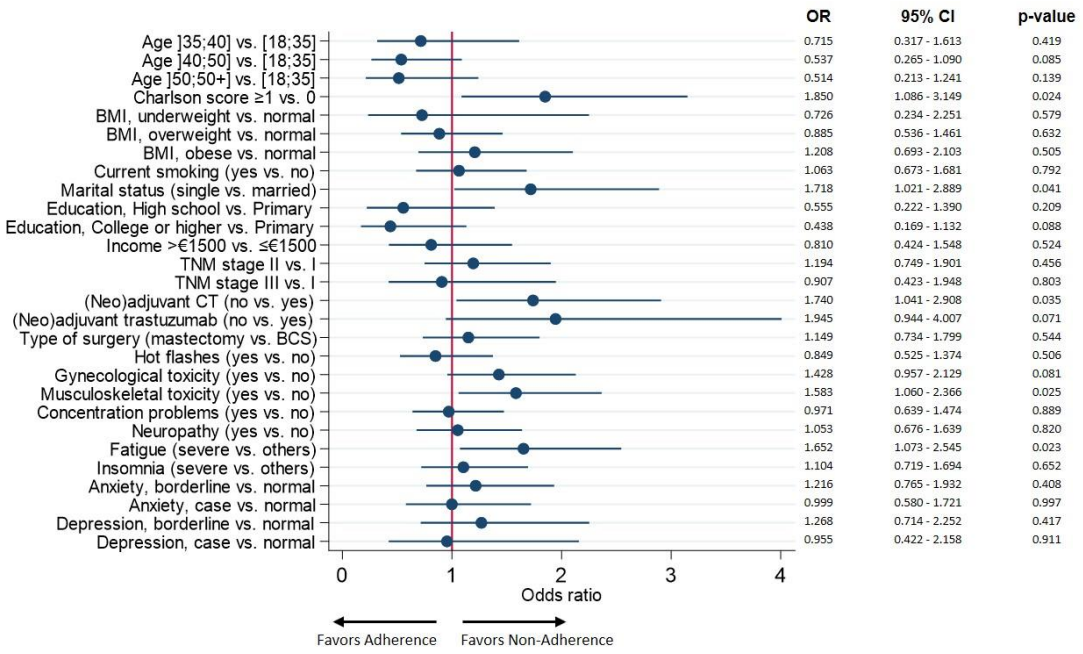
| | | Serum assessment | | |
|---|---|---|---|---|
| | | **Adherent, n (%)** | **Non-adherent, n (%)** | **Total** |
| **Self-declaration** | **Adherent, n (%)** | 928 (93.8) | 104 (55.3) | 1032 (87.7) |
| | **Non-adherent, n (%)** | 61 (6.2)* | 84 (44.6) | 145 (12.3) |
| | **Total** | 989 (84.0) | 188 (16.0) | 1177 |

Concordance: 86% (95% CI 84 to 88%)
Chi-square p-value <.0001
Cramer V = 0.4293

*61 patients were adherent by serum assessment but declared to be non-adherent: a) 50 due to treatment interruptions, b) 8 due to switch to AI for toxicity, c) 3 due to treatment discontinuation

Biochemical non-adherence was associated with multiple factors. Patients not living with a partner as a couple (vs. with a partner) (adjusted odds ratio [aOR]=1.72 [1.02-2.89]), those with more comorbidities (Charlson's comorbidity score ≥1 vs. 0) (aOR=1.85 [1.09-3.15]) and patients who did not receive treatment with (neo) adjuvant chemotherapy (vs. those who received chemotherapy) (aOR=1.74 [1.04-2.91]) had higher odds of biochemical non-adherence. In addition, symptoms after tamoxifen prescription (median time from prescription to assessment = 3.9 [3.0-5.1] months), including musculoskeletal symptoms (aOR=1.58 [1.06-2.37]) and severe fatigue (aOR=1.65 [1.07-2.5]) increased the risk of biochemical non-adherence (M5 Figure 2). Factors associated to patient reported non-adherence are shown in M5 Figure S2.

**M5 Figure 2 –** Multivariate estimates of variables associated with serum-defined adherence. BMI – Body mass index; CT – Chemotherapy. Severe Fatigue and insomnia defined as the respective subscale EORTC-C30 score> 40.[178] Anxiety and Depression defined using Hospital Anxiety and Depression Scale.[179]



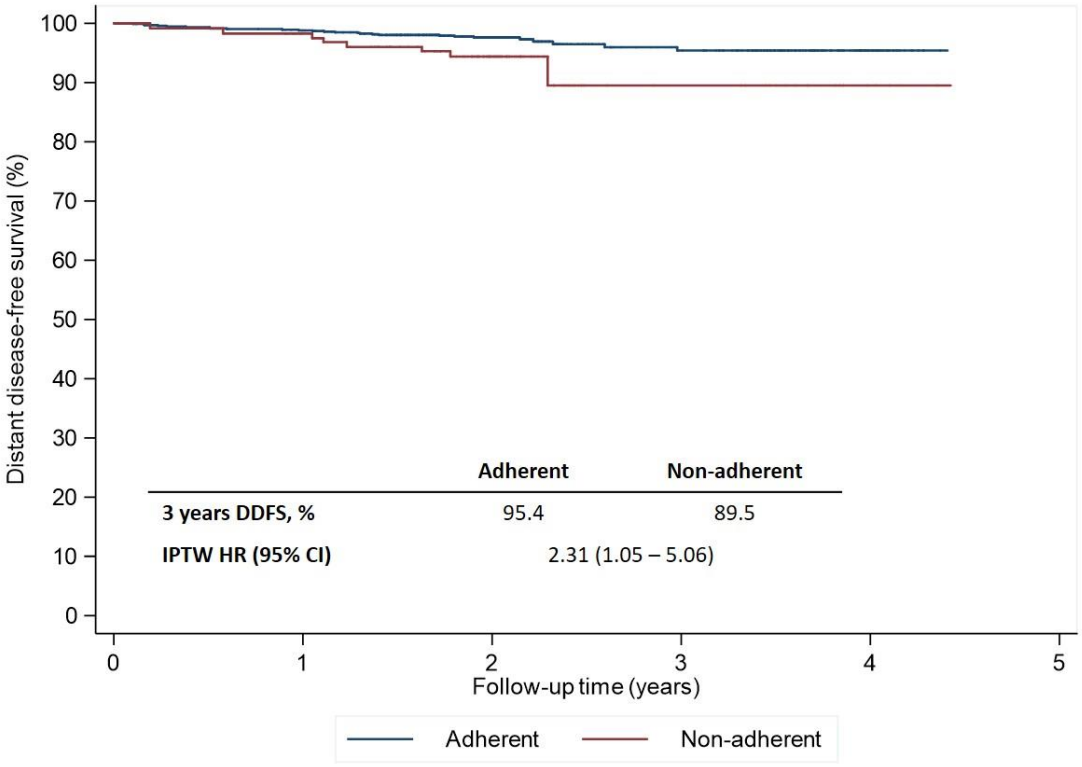

Impact of non-adherence at Year 1 post-tamoxifen prescription on survival outcomes

After a median follow-up of 24.2 months from tamoxifen prescription (IQR 22.8-27.0), 38 events were registered (M5 Table S3 details distribution of events). The median DDFS follow-up is balanced between adherence/non-adherence groups defined by serum assessment (median 24.3 [IQR 22.8 – 27.5] vs. 24.1 [IQR 21.3 – 25.8] for non-adherence).

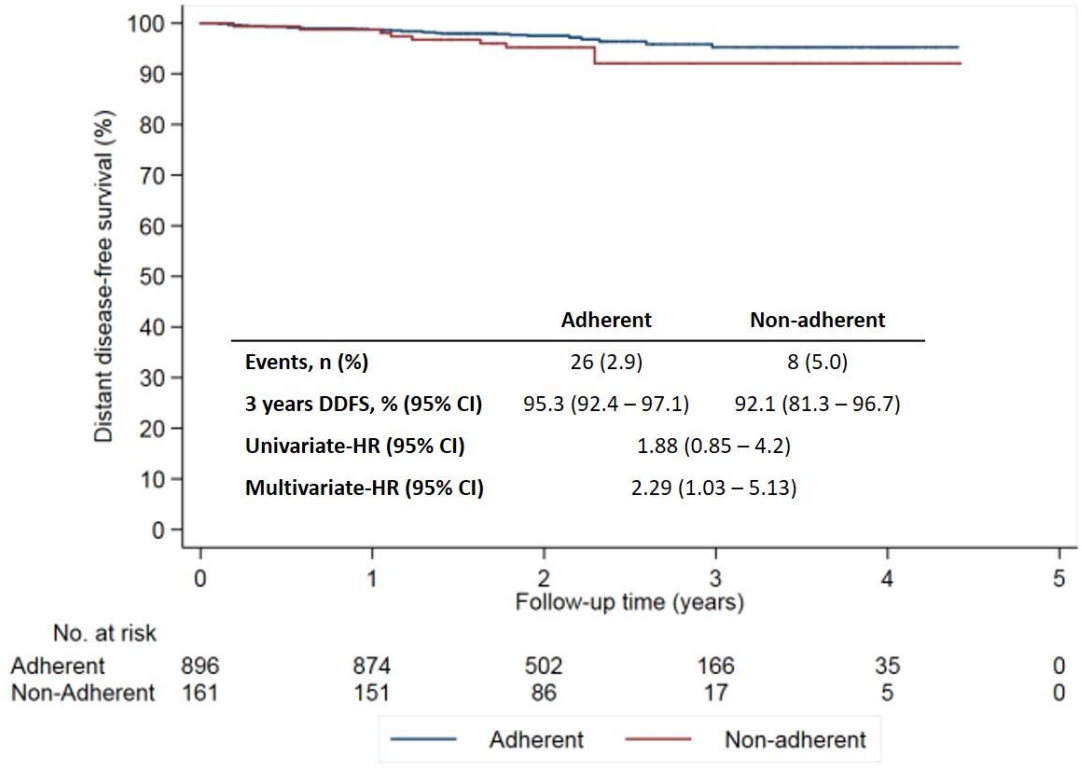
In the PS IPTW, the proportion of patients alive at three years was 95.4% in the adherent cohort and 89.5% in the non-adherent cohort (M5 Figure 3-A). In the multivariate IPTW model, non-adherent patients had a 131% increase in the risk of death or disease recurrence (Hazard ratio [HR] = 2.31 [1.05-5.06]) with a 5.9% absolute difference in the risk of a DDFS event at three years. The number needed to avoid non-adherence to impact 1 DDFS event at three years was 17. Diagnoses of the models performance are fully presented in M5 Supplementary Appendix S2. Sensitivity and secondary analyses demonstrated consistent results (M5 Figure 3-B and M5 Figure S3). M5 Table S4-S7 present full univariable and multivariable models. No difference in DDFS or BCFI outcomes was found between self-reported adherence and non-adherence (M5 Figure S4).

**M5 Figure 3 –** DDFS (distant recurrences and death) according to serum-defined adherence status in the propensity score weighted cohort (IPTW; A) and in the non-IPTW cohort (B). Time 0 defines time of the post-tamoxifen prescription visit and date of serum assessment of tamoxifen

**A**




**B**



### 5.5.7 Discussion

Non-adherence to adjuvant endocrine therapy for early breast cancer is often under-recognized partly due to the unavailability of a gold standard method for its detection and challenges in incorporating assessments of adherence into routine clinical practice.

Our study emphasizes that the real-life prevalence of non-adherence to medications is still not well-quantified: health-care providers tend to overestimate to what extent patients take their prescribed long-term oral treatments, whereas patients tend to underreport treatment discontinuations or interruptions.[180] Studies that tried to quantify the prevalence of non-adherence have yielded heterogeneous results; mostly reporting on indirect estimations obtained using patient self-report and prescription refills.[158,159,165] In breast cancer, previous studies, based on indirect methods, suggested that non-adherence to adjuvant endocrine therapy over 5 years ranges from 25% to more than 40-50%, with this proportion rising over time.[166–168,181] Only one study measured adherence to endocrine therapy by using an objective method based on drug serum assessment, although it did not provide correlations with breast cancer outcomes.[165] In our study serum assessment was able to identify a worryingly high proportion of patients, one in six, who were non-adherent to therapy at only one year after treatment prescription. Patient self-reports underestimated rates of non-adherence. Notably, 55% of patients who were non-adherent by serum assessment would not overtly acknowledge non-adherence.

Furthermore, non-adherence by serum assessment measured as early as Year 1 after treatment prescription emerged as marker of poorer outcomes regardless of other main prognostic factors, suggesting that risk of recurrence increases as soon as the patients start to be non-adherent. Although it is very unusual to see a significant impact on outcomes with such short-term follow-up among patients with HR+ breast cancer, prior researches are consistent with our findings. Controversial results were reported across different studies, suggesting the possibility that inadequate exposure to tamoxifen due to non-adherence may lead to a suboptimal concentration of its active metabolites.[174,182] Prior retrospective analyses based on pharmacy claims data also suggested a negative impact of non-adherence on breast cancer outcomes, but used an arbitrary cut-off of 80% medication possession ratio to define adequate adherence.[183–185] However, pharmacy claims typically cannot be obtained in real-time on an individual patient-level, and thus cannot be used to tailor treatment in the clinic.[158,166,167]

This study provides important insights on the complexity of non-adherence and on the multitude of its contributors. We found that sicker, non-partnered patients and those with

higher symptomatology burden, including more severe fatigue and musculoskeletal symptoms, had a higher likelihood of being non-adherent to therapy. Most of these associations have also been observed in other chronic diseases such as HIV, cardiac diseases and diabetes and are explained by several differences in social and clinical characteristics across patients. In addition to these previously known barriers, patients not having received adjuvant chemotherapy also were more likely not to be adherent to tamoxifen in our analysis. We hypothesize that patients who did not receive adjuvant chemotherapy are less aware of the health risks related to their disease and misconceive the beneficial impact of adjuvant endocrine therapy on breast cancer outcomes.

CANTO offered an unparalleled opportunity to test the performance of therapeutic drug monitoring in adjuvant treatment of breast cancer. Nevertheless, we acknowledge some limitations. First, we used non-previously validated thresholds of tamoxifen concentration to define biochemical non-adherence. However, we employed a conservative approach based on previous pharmacological studies[171–174] focused on the 3-month steady-state tamoxifen concentration, which all our patients should have achieved. In addition, we acknowledge that we did not assess the most active tamoxifen metabolites, but as mentioned the data on the impact of these metabolites on the outcome are still inconclusive. Second, the self-reported assessment of adherence and respective reasons were not based on validated scales, but still reflects what is currently done in clinical practice. Although CRNs systematically asked and collected the reasons for treatment interruption or discontinuation, a small number of patients disclosed this information limiting our ability to capture the complexity of factors affecting medication-taking behavior. Third, due to the low number of events and the lack of validation cohort, we cannot draw definitive conclusions on the generalizability of the negative impact of non-adherence on breast cancer outcomes. Nevertheless, our results are clinically plausible and the wide inclusion and exclusion criteria in CANTO call into the external validity of results. Fourth, we are aware that it is hard to isolate the true impact of non-adherence to tamoxifen on outcomes, because it is part of a multitude of health-related behaviors impacting prognosis.[186] Indeed, due to the observational design, we cannot exclude unmeasured confounding impacting our survival analyses. Nevertheless, we employed a PS weighting to relevant known prognostic factors aiming to a comprehensive adjustment in our analyses.[176,177] Fifth, we cannot exclude the impact of awareness of being observed on adherence (Hawthorne effect). Nevertheless, in our study the long-term observation, assessment of multiple clinical and biological data and evaluation of adherence using indirect and direct methods may minimize this effect.[187] Finally,

our results may not be generalizable to other populations as we restricted our analysis to the French pre-menopausal population with breast cancer.

This study adds to the understanding of the multifaceted and complex issue of 'non-adherence' to chronic medications, suggesting that therapeutic drug monitoring may serve as an important tool to identify non-adherence. Our results suggest that the introduction in clinical practice of an inexpensive blood test enables to identify non-adherent patients who are at risk of a distant relapse event very early in their adjuvant treatment trajectory. We could potentially avoid one distant relapse event if we helped 17 patients to take medications as prescribed. The impact of interventions to optimize adherence on a population level thus could be very large. Targeted interventions managing adherence to adjuvant endocrine therapy are needed and have the potential to improve breast cancer outcomes.

### 5.5.8 Acknowledgment of research support

This research was supported by an Institut National Cancer-France grant (SHS-E-SP 18-129) to Barbara Pistilli, a Career Catalyst Research grant from Susan G. Komen (CCR17483507) to Ines Vaz-Luis, a Fondation ARC pour la recherche sur le cancer grant (CANTO-WORK-programme labillisé) to Ines Vaz-Luis and Gwenn Menvielle and the Philanthropic Odyssea Gustave Roussy Program. The CANTO study is supported by the French Government under the "Investment for the Future" program managed by the National Research Agency (ANR), grant n° ANR-10-COHO-0004.

### 5.5.9 Supplementary material

**M5 SUPPLEMENTARY APPENDIX S1**

*M5 S1.1 Tamoxifen metabolism and pharmacokinetics considerations*

Tamoxifen has a steady-state concentration that is reached in ≈ 4 weeks and an elimination half-life of 7 days.[188] The long half-life of tamoxifen makes it detectable for up to 6 weeks after treatment discontinuation.[189–191] Serum concentration of tamoxifen is constant over its steady-state phase, and therefore tamoxifen concentration evaluation is not required to be performed at specific time points from tamoxifen intake.[192] In addition, tamoxifen has a different molecular mass from tamoxifen metabolites, thus its co-elution with other metabolites is not possible;[193] furthermore, standard chromatographic separation allows avoiding co-elution of tamoxifen with any other compounds.

Tamoxifen serum concentration does not vary by CYP2D6 polymorphisms unlike its main metabolite, endoxifen.[193–195] Drug-drug interactions with CYP2D6 inhibitors (e.g. paroxetine or

fluoxetine) do not influence tamoxifen levels. There are very few drugs, such as rifampicin, aminogluthetimide, curcumin and piperine, which may decrease tamoxifen serum levels.[196–198] CANTO collects extensive data on concomitant medications. In our study, none of women with tamoxifen serum levels <60 ng/ml was exposed to any of the drugs mentioned above that may interfere with tamoxifen serum levels. Finally, although some studies showed that tamoxifen serum levels may increase with increasing weight, in our cohort we did not find a significant correlation between tamoxifen serum levels and patients' weight.[172,199]

*M5 S1.2 Definition of biochemical non-adherence*

The cut-off to define non-adherence was pre-specified on the basis of previous pharmacological data that reported an average three-month steady-state tamoxifen concentration around 120 ng/ml, ranging from 70 to 180 ng/ml, in patients receiving 20 mg of tamoxifen per day.[172–174] Since all our patients were prescribed 20 mg of tamoxifen for more than three months (median time from tamoxifen prescription to measurement of non-adherence was 16.2 months, IQR 15.1 - 17.8), we used a putative threshold of 60 ng/ml for defining biochemical non-adherence to tamoxifen. Moreover, a prior study evaluating pharmacokinetics and pharmacogenetics of tamoxifen in a large cohort of premenopausal patients receiving adjuvant tamoxifen used ≤150 nM (corresponding to ≤60 ng/ml) as cut-off for identifying poorly adherent patients.[174]

We also investigated a possible linear relationship between tamoxifen serum concentration and survival outcomes. To do this, we applied several methods. First, we modeled tamoxifen concentration using spline functions. We fitted the spline modeling function including 3 knots and 4 degrees of freedom (natural splines as per Harrell et al, 2001). Although the graphical representation of hazard ratio value according to tamoxifen concentration may suggest a linear relationship between tamoxifen concentration and survival outcomes, the wide confidence intervals around these estimates do not allow us to confirm that such a relationship exists. In addition, we modeled the association between tamoxifen serum levels as a continuous variable and its association with cancer outcomes, assuming a linear relation between the two variables, which was not statistically significant. Thus, even if a linear relationship cannot be excluded, the available data do not allow to definitely conclude on this question. Full results are provided in the supplementary material.

In summary, the current data do not allow to exclude or confirm a linear relationship between tamoxifen serum concentration and survival outcomes. In contrast, the pre-defined cut-off of 60 ng/ml provides a clinically actionable threshold of non-adherence, identifying those at risk of poorer outcomes.

**M5 S1.1 A -** Multivariate Cox proportional hazards model for distant disease-free survival (A) and breast cancer free interval (B) using as explanatory variable a restricted cubic spline of tamoxifen serum levels (3 knots, at values corresponding to 60 ng/ml and the P50 and P75 of the data)

**A**

Cox regression -- Breslow method for ties

| | | | |
|---|---|---|---|
| No. of subjects = | 1,056 | Number of obs = | 1,056 |
| No. of failures = | 34 | | |
| Time at risk = | 28161.16963 | | |
| | | LR chi2(8) = | 59.71 |
| Log likelihood = | -196.39029 | Prob > chi2 = | 0.0000 |

| _t | Haz. Ratio | Std. Err. | z | P>\|z\| | [95% Conf. | Interval] |
|---|---|---|---|---|---|---|
| tam_level_rcs1 | .9922906 | .0060834 | -1.26 | 0.207 | .9804388 | 1.004286 |
| tam_level_rcs2 | 1.008232 | .0053608 | 1.54 | 0.123 | .99778 | 1.018794 |
| age1 | .9305044 | .0223773 | -3.00 | 0.003 | .8876632 | .9754132 |
| n_stade_tnm | | | | | | |
| STADE II | 1.655869 | 1.06062 | 0.79 | 0.431 | .4718601 | 5.810839 |
| STADE III | 9.398298 | 6.014756 | 3.50 | 0.000 | 2.680984 | 32.94611 |
| n_surgery | | | | | | |
| Mastectomie | 1.352698 | .5341309 | 0.77 | 0.444 | .6238677 | 2.932981 |
| 2.n_chemo_tp1 | .4008416 | .3355396 | -1.09 | 0.275 | .0777038 | 2.067774 |
| 1.n_pts_volume_2 | .9900578 | 1.01787 | -0.01 | 0.992 | .1319919 | 7.426324 |

**B**

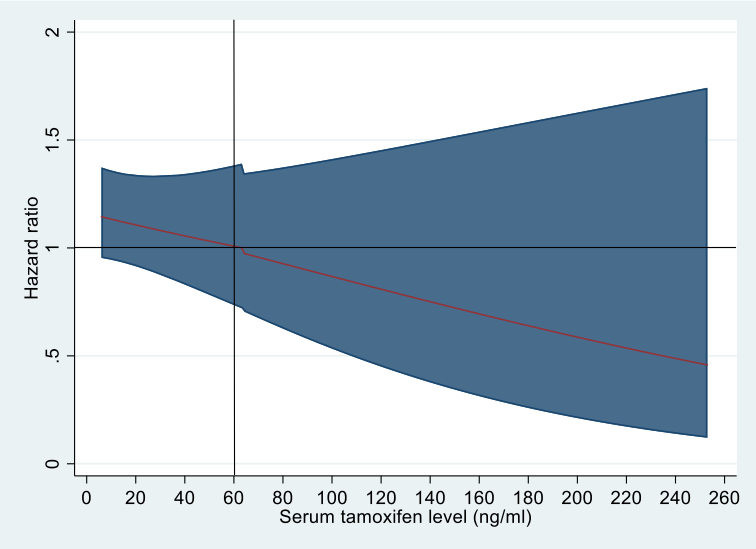
Cox regression -- Breslow method for ties

| | | | |
|---|---|---|---|
| No. of subjects = | 1,056 | Number of obs = | 1,056 |
| No. of failures = | 49 | | |
| Time at risk = | 28149.50615 | | |
| | | LR chi2(8) = | 56.17 |
| Log likelihood = | -294.66801 | Prob > chi2 = | 0.0000 |

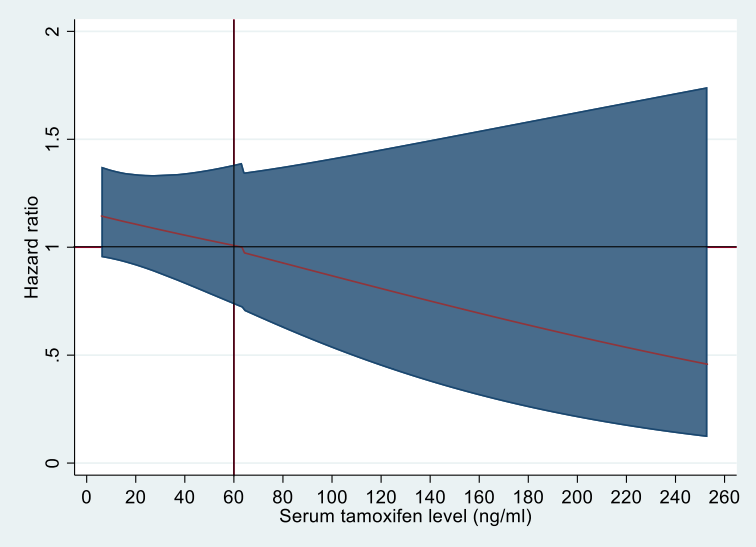
| _t | Haz. Ratio | Std. Err. | z | P>\|z\| | [95% Conf. | Interval] |
|---|---|---|---|---|---|---|
| tam_level_r~1 | 1.328378 | .2694972 | 1.40 | 0.162 | .8925499 | 1.977019 |
| tam_level_r~2 | .8471397 | .0997095 | -1.41 | 0.159 | .6726164 | 1.066946 |
| age1 | .9343054 | .0193198 | -3.29 | 0.001 | .8971964 | .9729493 |
| n_stade_tnm | | | | | | |
| STADE II | 2.239248 | 1.076472 | 1.68 | 0.094 | .8727799 | 5.745126 |
| STADE III | 9.833952 | 5.069485 | 4.43 | 0.000 | 3.580359 | 27.01031 |
| n_surgery | | | | | | |
| Mastectomie | 1.004011 | .3191401 | 0.01 | 0.990 | .5384817 | 1.872 |
| 2.n_chemo_tp1 | .9133665 | .4674599 | -0.18 | 0.859 | .3349663 | 2.490514 |
| 1.n_pts_vol~2 | 1.289398 | 1.31199 | 0.25 | 0.803 | .1754978 | 9.473326 |

**M5 S1.2 A -** Hazard ratio derived from multivariate Cox proportional hazards model for distant disease-free survival (A) and breast cancer free interval (B) as a function of the continuous serum tamoxifen level

**A**




**B**



**M5 S1.1 B -** Multivariate Cox proportional hazards model for distant disease-free survival using as explanatory variable continuous levels of serum tamoxifen (A) Multivariate Cox proportional hazards model for breast cancer free interval using as explanatory variable continuous levels of serum tamoxifen (B)

**A**

```
Cox regression -- Breslow method for ties

No. of subjects =        1,056                 Number of obs    =       1,056
No. of failures =           34
Time at risk    =  28161.16963
                                               LR chi2(7)       =       57.52
Log likelihood  =   -197.48189                 Prob > chi2      =      0.0000
```

| _t | Haz. Ratio | Std. Err. | z | P>\|z\| | [95% Conf. | Interval] |
|---|---|---|---|---|---|---|
| tamoxifene_~_ | 1.000716 | .0032964 | 0.22 | 0.828 | .9942757 | 1.007197 |
| age1 | .9314806 | .0225922 | -2.93 | 0.003 | .8882367 | .9768299 |
| n_stade_tnm | | | | | | |
| STADE II | 1.720393 | 1.09744 | 0.85 | 0.395 | .4927711 | 6.00634 |
| STADE III | 9.804075 | 6.264828 | 3.57 | 0.000 | 2.802121 | 34.30255 |
| n_surgery | | | | | | |
| Mastectomie | 1.308295 | .518693 | 0.68 | 0.498 | .6014981 | 2.845622 |
| 2.n_chemo_tp1 | .4002277 | .3332548 | -1.10 | 0.271 | .0782606 | 2.04678 |
| 1.n_pts_vol~2 | 1.051837 | 1.079841 | 0.05 | 0.961 | .1406321 | 7.867058 |

**B**

```
Cox regression -- Breslow method for ties

No. of subjects =        1,056                 Number of obs    =       1,056
No. of failures =           49
Time at risk    =  28149.50615
                                               LR chi2(7)       =       54.72
Log likelihood  =   -295.39307                 Prob > chi2      =      0.0000
```

| _t | Haz. Ratio | Std. Err. | z | P>\|z\| | [95% Conf. | Interval] |
|---|---|---|---|---|---|---|
| tamoxifene_~_ | .9973065 | .0027304 | -0.99 | 0.325 | .9919695 | 1.002672 |
| age1 | .9344741 | .0193068 | -3.28 | 0.001 | .8973894 | .9730913 |
| n_stade_tnm | | | | | | |
| STADE II | 2.238047 | 1.07326 | 1.68 | 0.093 | .8743266 | 5.728813 |
| STADE III | 9.867039 | 5.0877 | 4.44 | 0.000 | 3.591579 | 27.10743 |
| n_surgery | | | | | | |
| Mastectomie | .9942492 | .3176898 | -0.02 | 0.986 | .531512 | 1.859848 |
| 2.n_chemo_tp1 | .9043867 | .460753 | -0.20 | 0.844 | .333194 | 2.454772 |
| 1.n_pts_vol~2 | 1.318079 | 1.340945 | 0.27 | 0.786 | .1794624 | 9.680761 |

*M5 S1.3 Self-declaration of non-adherence*

Patient's self-declarations on adherence to tamoxifen were collected by *ad hoc* clinical research nurses using semi-structured interviews including the following questions (translated from the French here): a*. did you take your endocrine therapy regularly? ; b. did you ever stop to taking it ?; c. If yes, for how many days? When? Why?* The main variable of interest was patient's reported adherence and non-adherence as a binary variable. Patient's statements were *collected as per study* CRFs, as follows: a. ongoing hormone therapy yes/no; b. treatment interruption: yes/no; duration of treatment interruption (days); c. treatment discontinuation date; reasons of treatment discontinuation: toxicity/patient's refusal/physician's choice/end of treatment/other. A patient would be considered as having self-declared non-adherence if any of the following was reported: no ongoing hormone therapy *or* treatment interruption yes *or* any duration of treatment interruption reported *or* treatment discontinuation date preceding the Year 1 post-tamoxifen prescription assessment *or* patient's refusal/toxicity indicated as treatment discontinuation reasons.

A patient would be considered as adherent by self-declaration if all of the followings were reported: ongoing hormone therapy yes *and* treatment interruption no *and* duration of treatment interruption not reported *and* treatment discontinuation date not indicated or following Year 1 post-tamoxifen assessment *and* reasons for treatment discontinuation not indicated or physician's choice/end of treatment/other.

In addition, type of self-declared non-Adherence was also defined including a. tamoxifen suboptimal implementation (any voluntary or involuntary missed doses or treatment pauses followed by restarts) and b. tamoxifen early discontinuation (tamoxifen cessation or switch to aromatase-inhibitors because of tamoxifen-related side effects or patient's decision).

*M5 S1.4 Impact of concomitant medications on serum-assessed and self-declared non-adherence*

Tamoxifen serum concentration does not vary by CYP2D6 polymorphisms unlike its main metabolite, endoxifen.[193–195] Drug-drug interactions with CYP2D6 inhibitors (e.g. paroxetine or fluoxetine) do not influence tamoxifen levels. Particularly in our study, we did not find any statistical significant correlation between exposition to Selective Serotonin Reuptake Inhibitors (SSRIs)/Serotonin–norepinephrine reuptake inhibitors (SNRIs) and serum-assessed non-adherence ($p = 0.3401$). Among the 167 patients who declared to be taking SSRIs/SNRIs at the same time as tamoxifen, serum concentrations of tamoxifen at Year 1 had a median of 102 ng/ml (interquartile range: 72-132 ng/ml). In contrast, for the patients not taking SSRIs/SNRIs, serum concentrations of tamoxifen at Year 1 had a median of 113 ng/ml (interquartile range: 82-147

ng/ml). Despite minimal differences between groups, the median values in both groups were above the pre-specified biochemical non adherent cut-off used in this study (60 ng/ml). There are very few drugs, such as rifampicin, aminogluthetimide, curcumin and piperine, which may decrease tamoxifen serum levels.[196–198] CANTO collects extensive data on concomitant medications. In our study, none of women with tamoxifen serum levels <60 ng/ml was exposed to any of the drugs mentioned above that may interfere with tamoxifen serum levels. Of note four patients with tamoxifen serum levels ≥60 ng/ml were exposed to curcumin (range of tamoxifen serum dose among these patients: 74 – 217 ng/ml).

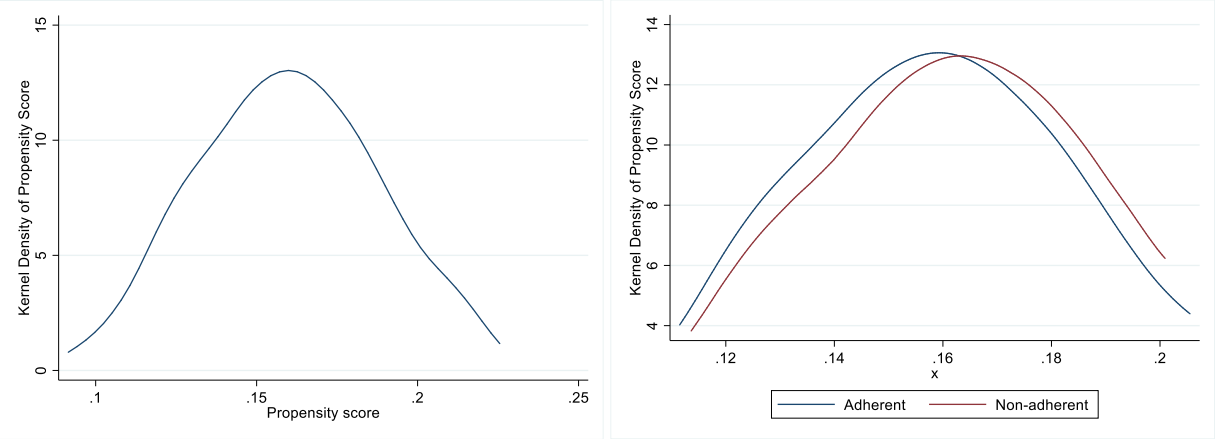
**M5 SUPPLEMENTARY APPENDIX S2**

*M5 S2.1 Propensity score IPTW diagnostics*

*Propensity score IPTW diagnostics for serum-defined adherence (A) and self-reported adherence (B)*

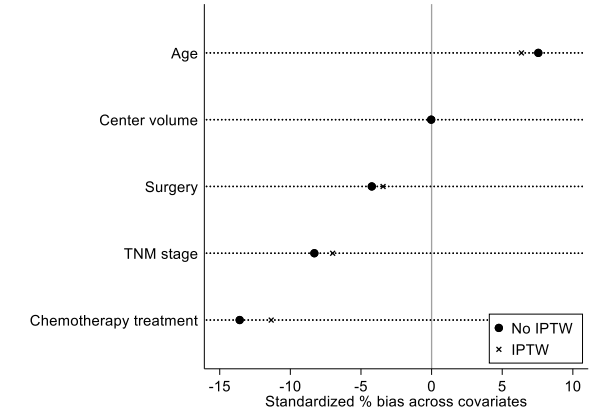
**M5 S2.1 A -** PS distribution, overall (A) and in adherent and non-adherent patients (B)

**A** **B**



**M5 S2.1 B -** Descriptive statistics on balance between adherent and non-adherent patients for variables included in the propensity score: A) in graphical format and B) in table format. "Unmatched" refers to non-adjusted cohort and "matched" to IPTW cohort.

**A**




**B**

| Variable | Unmatched / Matched | Mean Treated | Mean Control | %reduct \|bias\| | t-test t | t-test p>\|t\| | V(T)/ V(C) |
|---|---|---|---|---|---|---|---|
| age1 | U | 44.979 | 44.523 | 7.6 | 0.98 | 0.330 | 1.17 |
| | M | 44.979 | 44.595 | 6.4 | 15.9 | 0.82 | 0.411 | 1.17 |
| n_stade_tnm | U | 1.6383 | 1.6943 | -8.3 | | -1.03 | 0.304 | 0.91 |
| | M | 1.6383 | 1.6856 | -7.0 | 15.6 | -0.87 | 0.383 | 0.93 |
| n_chemo_tp | U | 1.5904 | 1.6562 | -13.6 | | -1.73 | 0.084 | 1.08 |
| | M | 1.5904 | 1.6454 | -11.4 | 16.4 | -1.44 | 0.151 | 1.06 |
| n_surgery | U | 1.3138 | 1.3337 | -4.2 | | -0.53 | 0.596 | 0.97 |
| | M | 1.3138 | 1.3299 | -3.4 | 18.8 | -0.43 | 0.666 | 0.98 |
| n_pts_volume_2 | U | .97872 | .97877 | -0.0 | | -0.00 | 0.997 | . |
| | M | .97872 | .97869 | 0.0 | 15.8 | 0.00 | 0.997 | . |

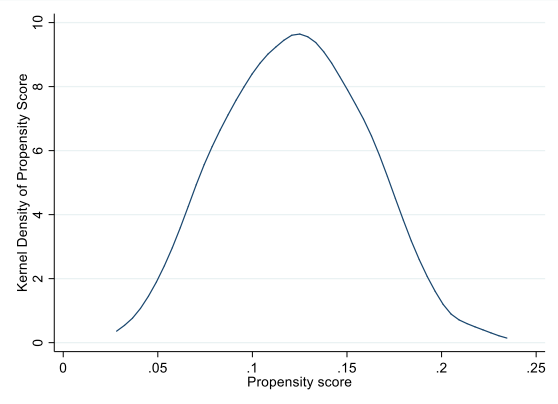
* if variance ratio outside [0.75; 1.33] for U and [0.75; 1.33] for M

| Sample | Ps R2 | LR chi2 | p>chi2 | MeanBias | MedBias | B | R | %Var |
|---|---|---|---|---|---|---|---|---|
| Unmatched | 0.003 | 3.13 | 0.679 | 6.7 | 7.6 | 13.9 | 1.14 | 0 |
| Matched | 0.002 | 2.21 | 0.819 | 5.6 | 6.4 | 11.7 | 1.13 | 0 |

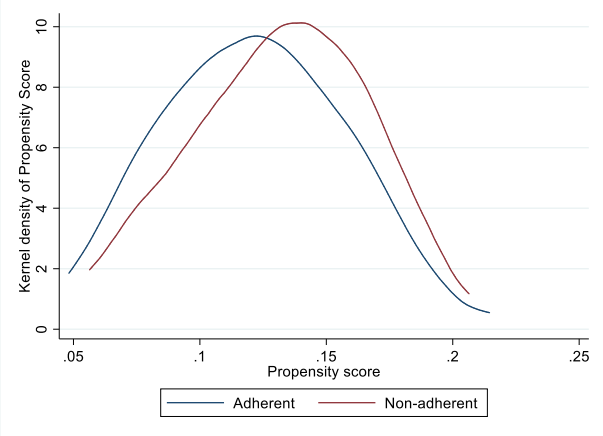
* if B>25%, R outside [0.5; 2]

**M5 S2.1 C -** PS distribution, overall (A) and in adherent and non-adherent patients (B)

**A**

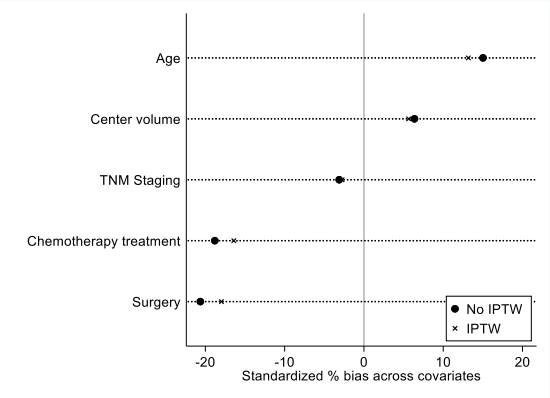



**B**



**M5 S2.1 D -** Descriptive statistics on balance between adherent and non-adherent patients for variables included in the propensity score: A) in graphical format and B) in table format. "Unmatched" refers to non-adjusted cohort and "matched" to IPTW cohort.

**A**




**B**

| Variable | Unmatched / Matched | Mean Treated | Mean Control | %reduct \|bias\| | | t-test t | t-test p>\|t\| | V(T)/ V(C) |
|---|---|---|---|---|---|---|---|---|
| age1 | U | 45.372 | 44.486 | 15.0 | | 1.70 | 0.089 | 1.03 |
| | M | 45.372 | 44.596 | 13.2 | 12.4 | 1.50 | 0.135 | 1.04 |
| n_stade_tnm | U | 1.6667 | 1.688 | -3.1 | | -0.35 | 0.727 | 0.98 |
| | M | 1.6667 | 1.6855 | -2.8 | 11.6 | -0.31 | 0.758 | 0.98 |
| n_chemo_tp | U | 1.5655 | 1.657 | -18.8 | | -2.16 | 0.031 | 1.10 |
| | M | 1.5655 | 1.6454 | -16.4 | 12.7 | -1.87 | 0.062 | 1.08 |
| n_surgery | U | 1.2483 | 1.3421 | -20.6 | | -2.25 | 0.025 | 0.83 |
| | M | 1.2483 | 1.33 | -18.0 | 12.9 | -1.97 | 0.049 | 0.85 |
| n_pts_volume_2 | U | .98621 | .97771 | 6.4 | | 0.66 | 0.507 | . |
| | M | .98621 | .9787 | 5.6 | 11.6 | 0.60 | 0.550 | . |

* if variance ratio outside [0.72; 1.39] for U and [0.72; 1.39] for M

| Sample | Ps R2 | LR chi2 | p>chi2 | MeanBias | MedBias | B | R | %Var |
|---|---|---|---|---|---|---|---|---|
| Unmatched | 0.014 | 11.98 | 0.035 | 12.8 | 15.0 | 31.0* | 0.91 | 0 |
| Matched | 0.011 | 9.23 | 0.100 | 11.2 | 13.2 | 27.3* | 0.91 | 0 |

* if B>25%, R outside [0.5; 2]

*Model fitness diagnostics*

**M5 S2.2 A -** Model fitness for distant disease-free survival when considering multivariable models with clinical variables (A), clinical variables plus adherence (B). Frame (C) further details likelihood ration test between models.

**A**

```
No. of subjects =        1,056                 Number of obs    =       1,056
No. of failures =           34
Time at risk    =  28161.16963
                                               LR chi2(6)       =       58.06
Log likelihood  =    -197.2149                 Prob > chi2      =      0.0000
```

| _t | Haz. Ratio | Std. Err. | z | P>\|z\| | [95% Conf. | Interval] |
|---|---|---|---|---|---|---|
| age1 | .9342952 | .022378 | -2.84 | 0.005 | .8914487 | .9792011 |
| n_stade_tnm | | | | | | |
| STADE II | 1.718308 | 1.097648 | 0.85 | 0.397 | .4913112 | 6.009596 |
| STADE III | 9.893208 | 6.326458 | 3.58 | 0.000 | 2.824979 | 34.64648 |
| n_chemo_tp | 2.529734 | 2.106609 | 1.11 | 0.265 | .4945907 | 12.9391 |
| n_surgery | 1.303793 | .5165844 | 0.67 | 0.503 | .5997196 | 2.83445 |
| n_pts_volume_2 | 1.306706 | .4558496 | 0.77 | 0.443 | .6595272 | 2.588946 |

**B**

```
No. of subjects =        1,056                 Number of obs    =       1,056
No. of failures =           34
Time at risk    =  28161.16963
                                               LR chi2(7)       =       61.58
Log likelihood  =   -195.45482                 Prob > chi2      =      0.0000
```

| _t | Haz. Ratio | Std. Err. | z | P>\|z\| | [95% Conf. | Interval] |
|---|---|---|---|---|---|---|
| n_tamoxifen_adhe~m | 2.294149 | .9420511 | 2.02 | 0.043 | 1.025866 | 5.130412 |
| age1 | .9381248 | .0221169 | -2.71 | 0.007 | .8957628 | .9824902 |
| n_stade_tnm | | | | | | |
| STADE II | 1.677311 | 1.075913 | 0.81 | 0.420 | .4771 | 5.896821 |
| STADE III | 9.863814 | 6.307697 | 3.58 | 0.000 | 2.816565 | 34.54379 |
| n_chemo_tp | 2.627443 | 2.197425 | 1.16 | 0.248 | .5100857 | 13.53392 |
| n_surgery | 1.356864 | .5342893 | 0.78 | 0.438 | .6271345 | 2.935703 |
| n_pts_volume_2 | 1.25469 | .4413376 | 0.65 | 0.519 | .6296889 | 2.500039 |

**C**

```
Likelihood-ratio test                                 LR chi2(1)  =      3.52
(Assumption: A nested in B)                           Prob > chi2 =    0.0606
```

**M5 S2.2 B -** Model fitness for breast cancer free interval when considering multivariable models with clinical variables (A), clinical variables plus adherence (B). Frame (C) further details likelihood ration test between models

**A**

```
No. of subjects =        1,056                 Number of obs    =       1,056
No. of failures =           49
Time at risk    =  28149.50615
                                               LR chi2(6)       =       53.74
Log likelihood  =   -295.87847                 Prob > chi2      =      0.0000
```

| _t | Haz. Ratio | Std. Err. | z | P>\|z\| | [95% Conf. | Interval] |
|---|---|---|---|---|---|---|
| age1 | .9321385 | .0192868 | -3.40 | 0.001 | .8950932 | .970717 |
| n_stade_tnm | | | | | | |
| STADE II | 2.244595 | 1.074074 | 1.69 | 0.091 | .8786677 | 5.733914 |
| STADE III | 9.845698 | 5.079033 | 4.43 | 0.000 | 3.582143 | 27.06139 |
| n_surgery | | | | | | |
| Mastectomie | .9836253 | .3158226 | -0.05 | 0.959 | .5242347 | 1.845583 |
| 2.n_chemo_tp1 | .9217388 | .4681993 | -0.16 | 0.873 | .3405949 | 2.494466 |
| 1.n_pts_vol~2 | 1.344159 | 1.368046 | 0.29 | 0.771 | .1828615 | 9.880501 |

**B**

```
No. of subjects =        1,056                 Number of obs     =       1,056
No. of failures =           49
Time at risk    =  28149.50615
                                               LR chi2(7)        =       59.39
Log likelihood  =   -293.05813                 Prob > chi2       =      0.0000
```

| _t | Haz. Ratio | Std. Err. | z | P>\|z\| | [95% Conf. | Interval] |
|---|---|---|---|---|---|---|
| n_tamoxifen~m | 2.36386 | .7929667 | 2.56 | 0.010 | 1.224849 | 4.562059 |
| age1 | .9346217 | .0188453 | -3.35 | 0.001 | .898406 | .9722973 |
| n_stade_tnm | | | | | | |
| STADE II | 2.208698 | 1.06318 | 1.65 | 0.100 | .8598087 | 5.67376 |
| STADE III | 9.902269 | 5.121913 | 4.43 | 0.000 | 3.592971 | 27.29076 |
| n_surgery | | | | | | |
| Mastectomie | 1.011401 | .3224111 | 0.04 | 0.972 | .5414768 | 1.88915 |
| 2.n_chemo_tp1 | .8930007 | .4566803 | -0.22 | 0.825 | .3277537 | 2.433078 |
| 1.n_pts_vol~2 | 1.202608 | 1.224043 | 0.18 | 0.856 | .1635879 | 8.840911 |

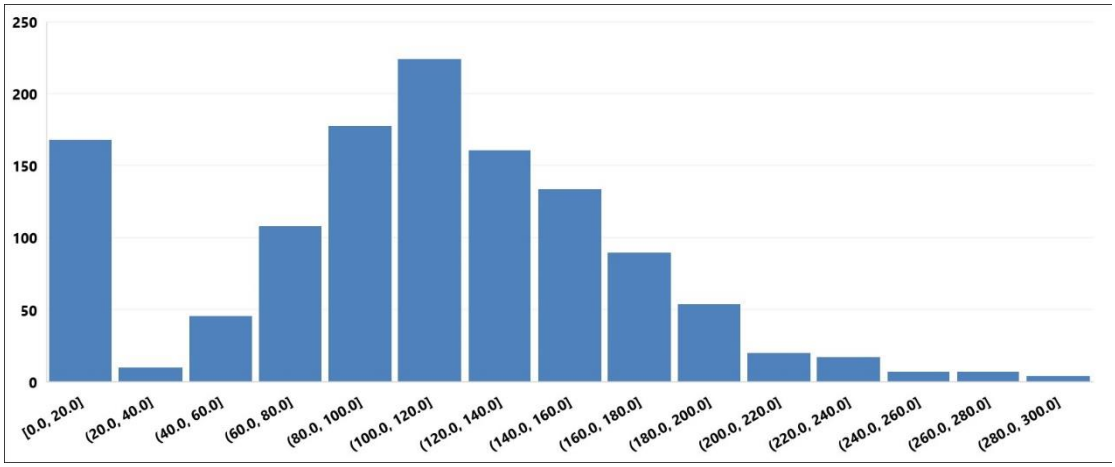
**C**

```
Likelihood-ratio test                          LR chi2(1)  =      5.64
(Assumption: A nested in B)                    Prob > chi2 =    0.0175
```

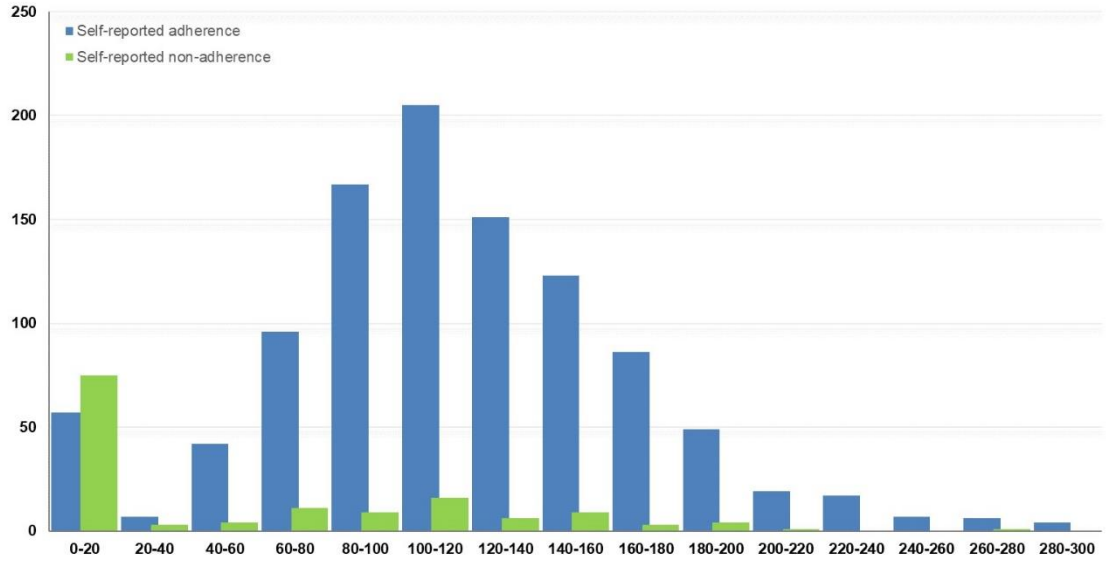
## M5 SUPPLEMENTARY FIGURES

**M5 Figure S1** – Distribution of tamoxifen concentration in the overall cohort (A) and according to self-reported adherence status (B)

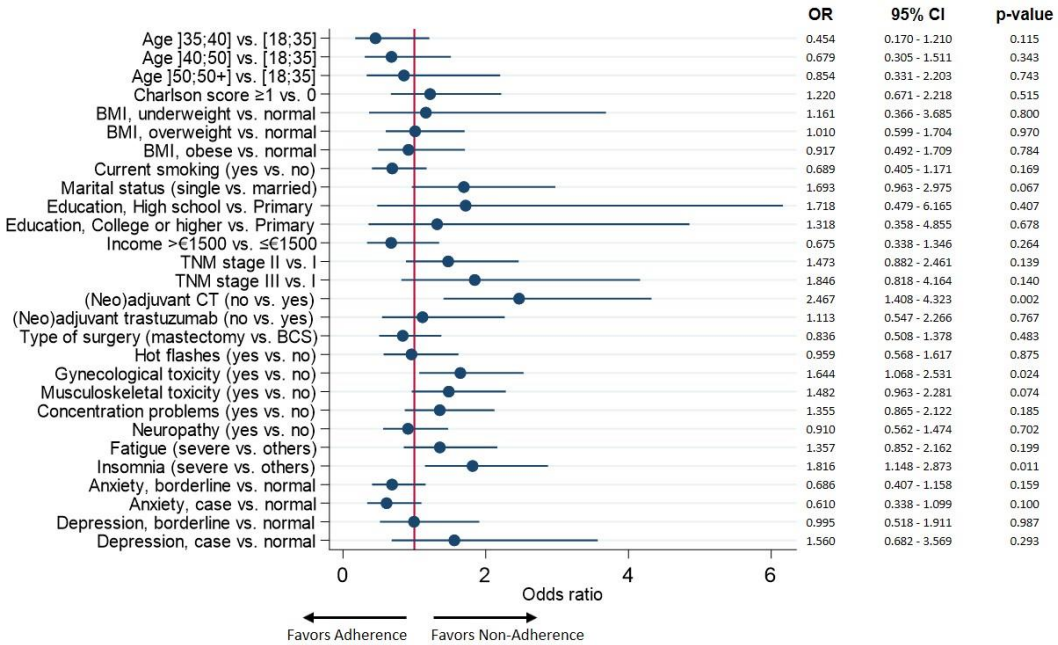
**A**



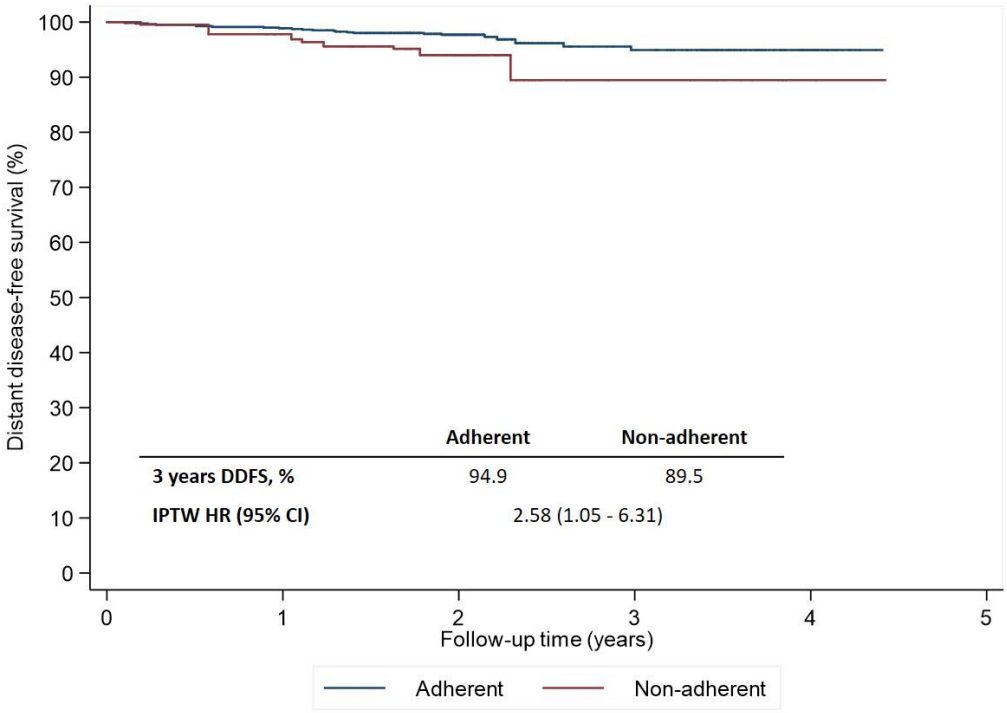
**B**




**M5 Figure S2** – Multivariate estimates of variables associated with self-reported adherence. *BMI – Body mass index; CT – Chemotherapy. Severe Fatigue and insomnia defined as the respective subscale EORTC-C30 score > 40.*[178] *Anxiety and Depression defined using Hospital Anxiety and Depression Scale.*[179]

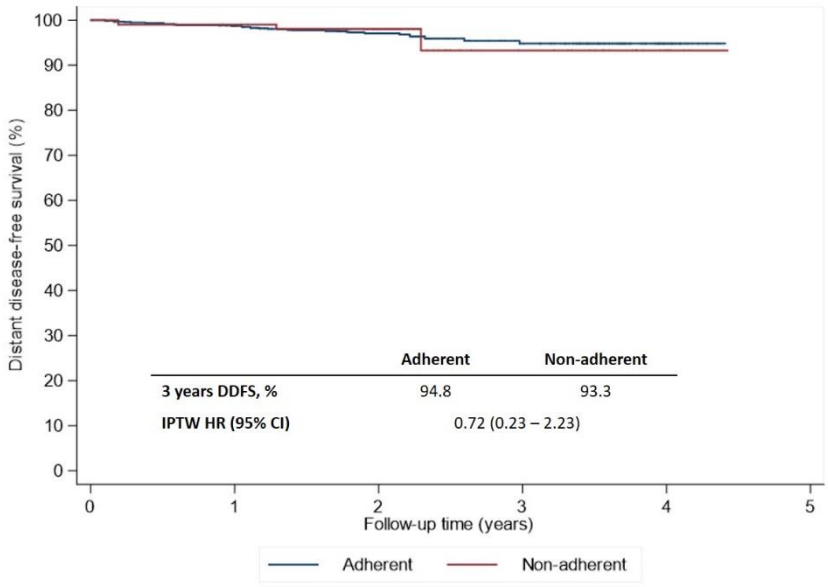


**M5 Figure S3 –** Sensitivity analysis of DDFS (distant recurrences and death) according to serum-defined adherence status using an extended list of variables in the propensity score weighting cohort (IPTW). *Time 0 defines time of the post-tamoxifen prescription visit and date of serum assessment of tamoxifen.*

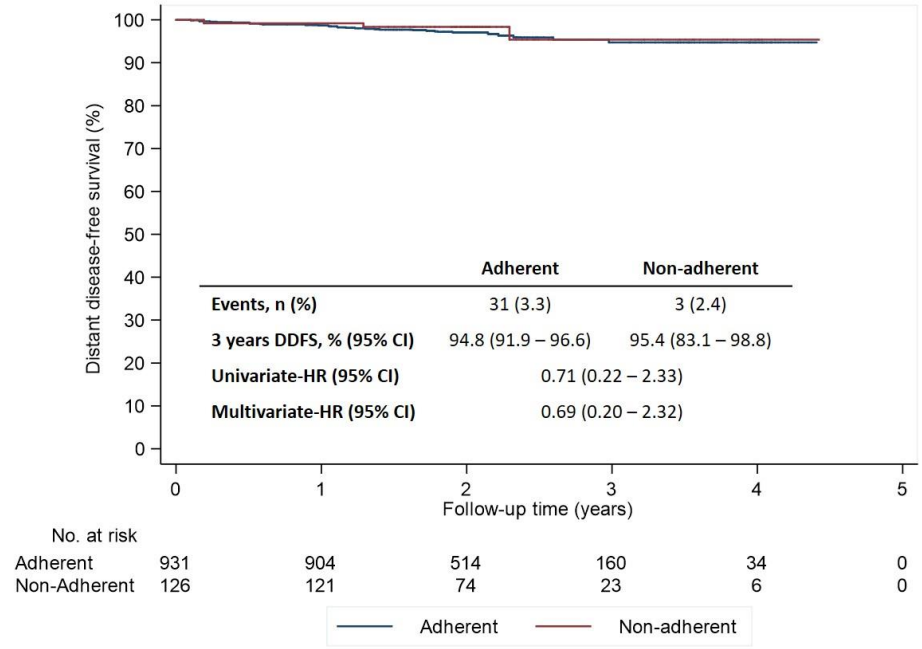


**M5 Figure S4–** DDFS (distant recurrences and death) according to self-reported adherence status in the propensity score weighted cohort (IPTW; A) and in the non-IPTW cohort (B). *Time 0 defines time of the post-tamoxifen prescription visit and date of serum assessment of tamoxifen.*

**A**




**B**



**M5 SUPPLEMENTARY TABLES**

**M5 Table S1 –** Demographic, social, clinical and pathological characteristics at baseline and treatment details within participants and premenopausal non-participants (patients without serum samples available).

| | Participants | | Non-participants | | p-value |
|---|---|---|---|---|---|
| | Number | % | Number | % | |
| **Total number** | 1177 | 79.5 | 303 | 20.5 | NA |
| **Age** | | | | | |
| ≤35 | 91 | 7.7 | 21 | 6.9 | 0.670 |
| >35 to ≤40 | 164 | 13.9 | 40 | 13.2 | |
| >40 to ≤50 | 762 | 64.7 | 207 | 68.3 | |
| >50 | 160 | 13.6 | 35 | 11.6 | |
| **Charlson's comorbidity score** | | | | | |
| 0 | 975 | 88.2 | 239 | 87.5 | 0.750 |
| ≥1 | 130 | 11.8 | 34 | 12.5 | |
| Missing | 72 | 6.1 | 30 | 9.9 | |
| **Body mass index** | | | | | |
| Underweight | 47 | 4.0 | 12 | 4 | 0.910 |
| Normal | 728 | 62.0 | 191 | 64.1 | |
| Overweight | 252 | 21.4 | 61 | 20.5 | |
| Obese | 148 | 12.6 | 34 | 11.4 | |
| Missing | 2 | 0.2 | 5 | 1.7 | |
| **Smoking status** | | | | | |
| No/previous smoker | 905 | 78.0 | 213 | 71 | 0.082 |
| Smoker | 256 | 22.0 | 87 | 29 | |
| Missing | 16 | 1.4 | 3 | 1 | |
| **Education** | | | | | |
| Primary school | 50 | 4.5 | 9 | 3.3 | 0.300 |
| High school | 498 | 44.5 | 133 | 49.4 | |
| College or higher | 570 | 51.0 | 127 | 47.2 | |
| Missing | 59 | 5.0 | 34 | 11.2 | |
| **Household income** | | | | | |
| <1500 | 112 | 10.2 | 38 | 14.4 | 0.150 |
| ≥1500 to <3000 | 415 | 37.9 | 96 | 36.4 | |
| ≥3000 | 568 | 51.9 | 130 | 49.2 | |
| Missing | 82 | 7.0 | 39 | 12.9 | |
| **Marital status** | | | | | |
| Living as couple | 951 | 84.8 | 228 | 83.8 | 0.680 |
| Living alone | 170 | 15.2 | 44 | 16.2 | |
| Missing | 56 | 4.8 | 31 | 10.2 | |
| **Histology** | | | | | |
| Invasive carcinoma, NST | 944 | 80.3 | 238 | 78.8 | 0.820 |
| Invasive lobular carcinoma | 132 | 11.2 | 38 | 12.6 | |
| Mixed NST/lobular | 35 | 3 | 11 | 3.6 | |
| Other | 65 | 5.5 | 15 | 5 | |
| Missing | 1 | 0.1 | 1 | 0.3 | |
| **Histologic grade** | | | | | |
| 1 | 203 | 17.3 | 54 | 17.9 | 0.140 |
| 2 | 635 | 54.1 | 178 | 59.1 | |
| 3 | 336 | 28.6 | 69 | 22.9 | |
| Missing | 3 | 0.3 | 2 | 0.7 | |
| **TNM stage** | | | | | |
| I | 519 | 44.1 | 117 | 39.3 | 0.170 |
| II | 508 | 43.2 | 133 | 44.6 | |

| | | | | | |
|---|---|---|---|---|---|
| III | 149 | 12.7 | 48 | 16.1 | |
| Missing | 1 | 0.1 | 5 | 1.7 | |
| **IHC-defined subtype** | | | | | |
| HR+/HER2- | 995 | 84.5 | 255 | 84.2 | 0.870 |
| HR+/HER2+ | 182 | 15.5 | 48 | 15.8 | |
| **Surgery type** | | | | | |
| BCS | 788 | 66.9 | 197 | 65.4 | 0.620 |
| Mastectomy | 389 | 33.1 | 104 | 34.6 | |
| Missing | 0 | 0 | 2 | 0.7 | |
| **Axillary management** | | | | | |
| Axillary dissection | 570 | 48.4 | 147 | 48.8 | 0.900 |
| Sentinel node/none | 607 | 51.6 | 154 | 51.2 | |
| Missing | 0 | 0 | 2 | 0.7 | |
| **Radiotherapy** | | | | | |
| Yes | 1068 | 90.7 | 277 | 91.4 | 0.710 |
| No | 109 | 9.3 | 26 | 8.6 | |
| **(Neo)adjuvant Chemotherapy type** | | | | | |
| Anthracyclines-taxanes | | | | | 0.062 |
| Anthracyclines-based | 691 | 58.7 | 171 | 56.4 | |
| Taxanes-based | 24 | 2 | 14 | 4.6 | |
| Missing regimen | 44 | 3.7 | 8 | 2.6 | |
| No | 1 | 0.1 | 4 | 1.3 | |
| | 417 | 35.4 | 106 | 35 | |
| **HER2-directed therapy** | | | | | |
| Yes | 146 | 12.4 | 36 | 11.9 | 0.800 |
| No | 1031 | 87.6 | 267 | 88.1 | |
| **High recruitment center (>100 pts)** | | | | | |
| Yes | 1152 | 97.9 | 31 | 10.2 | <0.001 |
| No | 25 | 2.1 | 272 | 89.8 | |

**M5 Table S2 –** Description of tamoxifen serum concentration according to method to assess adherence. **lower tamoxifen quantification limit*

| **Serum concentration of tamoxifen (ng/ml)** | **Serum defined** | | **Self-reported defined** | |
|---|---|---|---|---|
| | **Adherent** | **Non-adherent** | **Adherent** | **Non-adherent** |
| Median (IQR)<br>Min. – Max. | 119 (96 – 152)<br>60 – 298 | 6 (6 – 38)<br>6 – 60 | 115 (88 - 148)<br>6 – 298 | 10 (6 - 104)<br>6 - 272 |
| < 60, n (%)<br>< 6*, n (%)<br>6-60, n (%)<br>≥ 60, n (%) | 188 (16.0)<br>118 (10.1)<br>70 (5.9)<br>989 (84.0) | | 104 (10.1)<br>45 (4.4)<br>59 (5.7)<br>928 (89.9) | 84 (57.9)<br>73 (50.3)<br>11 (7.6)<br>61 (42.1) |

**M5 Table S3 –** Descriptive evaluation of survival data (with censor date on 31-05-2018)

| | Overall cohort | | IPTW weighted cohort | |
|---|---|---|---|---|
| | **Adherent** | **Non-adherent** | **Adherent** | **Non-adherent** |
| **Serum-defined adherence** | | | | |
| Observations, n (%) | 896 (84.8) | 161 (15.2) | | |
| Events, n (%)[1] | | | | |
| DDFS | 26 (2.9) | 8 (5.0) | | |
| BCFI | 37 (4.1) | 12(7.5) | | |
| Local recurrence | 5 | 0 | | |
| Regional recurrence | 4 | 1 | | |
| Distant recurrence | 26 | 8 | | |
| Contralateral recurrence | 4 | 3 | | |
| Death | 2 | 0 | | |
| Year of enrollment | | | | |
| Median (IQR) | 2013 (2013 - 2014) | 2013 (2013 - 2014) | | |
| Min. – Max. | 2012 - 2015 | 2012 - 2014 | | |
| DDFS follow-up, from Year 1 visit | | | | |
| Median (IQR) | 24.3 (22.8 – 27.5) | 24.1 (21.4 – 25.8) | | |
| 95% CI | 24.1 – 24.4 | 23.9 – 24.4 | | |
| Min. – Max. | 0.2 – 52.9 | 1.1 – 53.1 | | |
| DDFS follow-up, from baseline | | | | |
| Median (IQR) | 47.6 (44.0 – 51.7) | 46.1 (41.0 – 49.0) | | |
| 95% CI | 47.3 – 48.0 | 45.1 – 47.1 | | |
| Min. – Max. | 16.8 – 72.5 | 15.7 – 73.4 | | |
| DDFS, median (IQR) | NR (NR - NR) | NR (NR - NR) | NR (NR - NR) | NR (NR - NR) |
| DDFS point estimates, % (95% CI) | | | | |
| 1 year post Year 1 visit | 98.8 (97.8 – 99.3) | 98.7 (95.1 – 99.7) | 98.8 | 98.3 |
| 2 years post Year 1 visit | 97.6 (96.3 – 98.4) | 95.2 (90.2 – 97.7) | 97.6 | 94.4 |
| 3 years post Year 1 visit | 95.3 (92.4 – 97.1) | 92.1 (81.3 – 96.7) | 95.4 | 89.5 |
| 4 years post Year 1 visit | 95.3 (92.4 – 97.1) | 92.1 (81.3 – 96.7) | 95.4 | 89.5 |
| 5 years post Year 1 visit | NR | NR | NR | NR |
| BCFI, median (IQR) | NR (NR - NR) | NR (NR - NR) | NR (NR - NR) | NR (NR - NR) |
| BCFI point estimates, % (95% CI) | | | | |
| 1 year post Year 1 visit | 98.7 (97.6 – 99.2) | 97.5 (93.4 – 99.1) | 98.7 | 97.3 |
| 2 years post Year 1 visit | 96.7 (95.3 – 97.7) | 93.3 (87.8 – 96.3) | 96.8 | 92.4 |
| 3 years post Year 1 visit | 93.2 (89.9 – 95.5) | 87.3 (75.2 – 93.8) | 93.4 | 84.7 |
| 4 years post Year 1 visit | 92.7 (89.1 – 95.0) | 87.3 (75.2 – 93.8) | 92.8 | 84.7 |
| 5 years post Year 1 visit | NR | NR | NR | NR |
| **Self-reported adherence** | | | | |
| Observations, n (%) | 931 (88.1) | 126 (11.9) | | |
| Events, n (%)[1] | | | | |
| DDFS | 31 (3.3) | 3 (2.4) | | |
| BCFI | 44 (4.7) | 5 (4.0) | | |
| Local recurrence | 5 | 0 | | |
| Regional recurrence | 5 | 0 | | |
| Distant recurrence | 31 | 3 | | |
| Contralateral recurrence | 5 | 2 | | |
| Death | 2 | 0 | | |
| Year of enrollment | | | | |
| Median (IQR) | 2013 (2013 - 2014) | 2013 (2013 - 2014) | | |
| Min. – Max. | 2012 - 2015 | 2012 - 2014 | | |
| DDFS follow-up, from Year 1 visit | | | | |
| Median (IQR) | 24.2 (22.7 – 26.9) | 24.4 (23.0 – 28.1) | | |
| 95% CI | 24.1 – 24.4 | 24.0 – 24.9 | | |
| Min. – Max. | 0.2 – 52.9 | 1.1 – 53.1 | | |

| | | | | |
|---|---|---|---|---|
| DDFS, median (IQR) | NR (NR - NR) | NR (NR - NR) | NR (NR - NR) | NR (NR - NR) |
| DDFS point estimates, % (95% CI) | | | | |
| 1 year post Year 1 visit | 98.7 (97.7 – 99.3) | 99.2 (94.4 – 99.9) | 98.7 | 99.0 |
| 2 years post Year 1 visit | 97.1 (95.7 – 98.0) | 98.4 (93.6 – 99.6) | 97.1 | 98.1 |
| 3 years post Year 1 visit | 94.8 (91.9 – 96.6) | 95.4 (83.1 – 98.8) | 94.8 | 93.3 |
| 4 years post Year 1 visit | 94.8 (91.9 – 96.6) | 95.4 (83.1 – 98.8) | 94.8 | 93.3 |
| 5 years post Year 1 visit | NR | NR | NR | NR |
| BCFI, median (IQR) | NR (NR - NR) | NR (NR - NR) | NR (NR - NR) | NR (NR - NR) |
| BCFI point estimates, % (95% CI) | | | | |
| 1 year post Year 1 visit | 98.5 (97.5 – 99.1) | 98.4 (93.8 – 99.6) | 98.5 | 98.3 |
| 2 years post Year 1 visit | 96.2 (94.7 – 97.3) | 96.7 (91.5 – 98.8) | 96.1 | 96.6 |
| 3 years post Year 1 visit | 92.2 (88.7 – 94.6) | 93.8 (83.0 – 97.8) | 92.1 | 92.5 |
| 4 years post Year 1 visit | 91.6 (87.9 – 94.2) | 93.8 (83.0 – 97.8) | 91.6 | 92.5 |
| 5 years post Year 1 visit | NR | NR | NR | NR |

[1]*Numbers add to more than 100% because patients can have more than one type of recurrence.*

CI – confidence interval; DDFS – distant disease-free survival; IPTW – inverse-probability treatment weighting; IQR – interquartile range; NR – not reached.

**M5 Table S4 –** Univariate and multivariate association with DDFS (Cox proportional hazards model). Variable selection for multivariate analysis was based on prior information of variables associated with cancer survival outcomes. Number of variables used in the multivariate model was limited by the number of DDFS events.

| | Univariate association | | Multivariate association, serum-defined adherence | | Multivariate association, self-reported adherence | |
|---|---|---|---|---|---|---|
| | **HR (95% CI)** | **p-value** | **HR (95% CI)** | **p-value** | **HR (95% CI)** | **p-value** |
| **Age at diagnosis, for every 1-year increase** | 0.91 (0.87 – 0.95) | <0.001 | 0.94 (0.89 – 0.97) | 0.005 | 0.93 (0.88 – 0.98) | 0.003 |
| **Age at diagnosis** | | | | | | |
| ≤35 | (reference) | | | | | |
| >35 to ≤40 | 0.19 (0.05 – 0.70) | 0.013 | | | | |
| >40 to ≤50 | 0.25 (0.11 – 0.55) | 0.001 | | | | |
| >50 | 0.11 (0.02 – 0.51) | 0.005 | | | | |
| **Charlson's comorbidity score** | | | | | | |
| 0 | (reference) | | | | | |
| ≥1 | 2.12 (0.92 – 4.90) | 0.077 | | | | |
| **Body mass index** | | | | | | |
| Underweight | 0.92 (0.12 – 6.92) | 0.934 | | | | |
| Normal | (reference) | | | | | |
| Overweight | 1.45 (0.62 – 3.39) | 0.392 | | | | |
| Obese | 2.79 (1.23 – 6.31) | 0.014 | | | | |
| **Histology** | | | | | | |
| Invasive carcinoma, NST | (reference) | | | | | |
| Invasive lobular carcinoma | 0.46 (0.11 – 1.94) | 0.294 | | | | |
| Mixed NST/lobular | 2.28 (0.54 – 9.59) | 0.261 | | | | |
| Other | 1.59 (0.48 – 5.26) | 0.443 | | | | |
| **Histologic grade** | | | | | | |
| 1 | (reference) | | | | | |
| 2 | Unstable model | NR | | | | |
| 3 | Unstable model | NR | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **AJCC TNM stage** | | | | | | |
| I | (reference) | | (reference) | | (reference) | |
| II | 2.90 (0.91 – 9.28) | 0.072 | 1.68 (0.48 – 5.89) | 0.419 | 1.73 (0.50 – 6.03) | 0.390 |
| III | 19.6 (6.70 – 57.49) | <0.001 | 9.82 (2.81 – 34.35) | <0.001 | 10.05 (2.87 – 35.14) | <0.001 |
| **IHC-defined subtype** | | | | | | |
| HR+/HER2- | 0.78 (0.32 – 1.88) | 0.579 | | | | |
| HR+/HER2+ | (reference) | | | | | |
| **Surgery type** | | | | | | |
| Conserving surgery | (reference) | | (reference) | | (reference) | |
| Mastectomy | 3.70 (1.83 – 7.47) | <0.001 | 1.36 (0.63 – 2.95) | 0.431 | 1.30 (0.60 – 2.82) | 0.510 |
| **Axillary management** | | | | | | |
| Axillary dissection | (reference) | | | | | |
| Sentinel node/none | 0.12 (0.04 – 0.34) | <0.001 | | | | |
| **Radiotherapy** | | | | | | |
| Yes | 3.99 (0.54 – 29.18) | 0.173 | | | | |
| No | (reference) | | | | | |
| **(Neo)adjuvant CT type** | | | | | | |
| Yes | (reference) | | (reference) | | (reference) | |
| No | 0.10 (0.02 – 0.42) | 0.002 | 0.38 (0.07 – 1.97) | 0.250 | 0.41 (0.08 – 2.09) | 0.282 |
| **HER2-directed therapy** | | | | | | |
| Yes | 1.29 (0.50 – 3.35) | 0.595 | | | | |
| No | (reference) | | | | | |
| **High recruitment center (>100 pts)** | | | | | | |
| Yes | 0.68 (0.09 – 4.94) | 0.699 | 0.93 (0.12 – 6.94) | 0.923 | 1.11 (0.15 – 8.33) | 0.923 |
| No | (reference) | | (reference) | | (reference) | |
| **Tamoxifen adherence, serum defined** | 1.88 (0.85 – 4.15) | 0.120 | 2.34 (1.05 – 5.23) | 0.038 | | |
| **Tamoxifen adherence, self-reported** | 0.71 (0.21 – 2.33) | 0.575 | | | 0.69 (0.20 – 2.32) | 0.547 |

**M5 Table S5 –** Multivariate association with DDFS (Cox proportional hazards model, PS weighted cohort). Variable selection for multivariate analysis was based on prior information of variables associated with cancer survival outcomes. Number of variables used in the multivariate model was limited by the number of DDFS events.

| | Multivariate association, serum-defined adherence | | Multivariate association, self-reported adherence | |
|---|---|---|---|---|
| | **HR (95% CI)** | **p-value** | **HR (95% CI)** | **p-value** |
| **Age at diagnosis, for every 1-year increase** | 0.94 (0.90 – 0.98) | 0.003 | 0.93 (0.89 – 0.98) | 0.006 |
| **AJCC TNM stage** | | | | |
| I | (reference) | | (reference) | |
| II | 1.67 (0.44 – 6.41) | 0.454 | 1.80 (0.47 – 6.92) | 0.392 |
| III | 9.62 (2.58 – 35.85) | 0.001 | 11.18 (2.99 – 41.76) | <0.001 |
| **Surgery type** | | | | |
| Conserving surgery | (reference) | | (reference) | |
| Mastectomy | 1.41 (0.66 – 3.02) | 0.373 | 1.31 (0.61 – 2.81) | 0.481 |
| **(Neo)adjuvant CT type** | | | | |
| Yes | 2.52 (0.48 – 13.31) | 0.275 | 2.32 (0.45 – 12.05) | 0.313 |
| No | (reference) | | (reference) | |
| **High recruitment center (>100 pts)** | | | | |
| Yes | 1.38 (0.65 – 2.95) | 0.273 | 1.09 (0.24 – 4.94) | 0.906 |
| No | (reference) | | (reference) | |
| **Tamoxifen adherence, serum defined** | 2.31 (1.06 – 5.06) | 0.036 | | |
| **Tamoxifen adherence, self-reported** | | | 0.72 (0.23 – 2.23) | 0.573 |

**M5 Table S6 –** Univariate and multivariate association with breast cancer free interval (Cox proportional hazards model). Variable selection for multivariate analysis was based on prior information of variables associated with cancer survival outcomes. Number of variables used in the multivariate model was limited by the number of DFS excluding second primaries events.

| | Univariate association | | Multivariate association, serum-defined adherence | | Multivariate association, self-reported adherence | |
|---|---|---|---|---|---|---|
| | **HR (95% CI)** | **p-value** | **HR (95% CI)** | **p-value** | **HR (95% CI)** | **p-value** |
| **Age at diagnosis, for every 1-year increase** | 0.92 (0.88 – 0.96) | <0.001 | 0.93 (0.90 – 0.97) | 0.001 | 0.93 (0.89 – 0.97) | 0.001 |
| **Age at diagnosis** | | | | | | |
| ≤35 | (reference) | | | | | |
| >35 to ≤40 | 0.29 (0.11 – 0.76) | 0.012 | | | | |
| >40 to ≤50 | 0.25 (0.13 – 0.50) | <0.001 | | | | |
| >50 | 0.16 (0.05 – 0.50) | 0.002 | | | | |
| **Charlson's comorbidity score** | | | | | | |
| 0 | (reference) | | | | | |
| ≥1 | 1.59 (0.74 – 3.40) | 0.231 | | | | |
| **Body mass index** | | | | | | |
| Underweight | 1.75 (0.53 – 5.79) | 0.362 | | | | |
| Normal | (reference) | | | | | |
| Overweight | 1.16 (0.56 – 2.42) | 0.692 | | | | |
| Obese | 2.20 (1.08 – 4.46) | 0.030 | | | | |
| **Histology** | | | | | | |
| Invasive carcinoma, NST | (reference) | | | | | |
| Invasive lobular carcinoma | 0.31 (0.08 – 1.30) | 0.111 | | | | |
| Mixed NST/lobular | 1.61 (0.39 – 6.69) | 0.509 | | | | |
| Other | 2.23 (0.94 – 5.25) | 0.068 | | | | |
| **Histologic grade** | | | | | | |
| 1 | (reference) | | | | | |
| 2 | 1.97 (0.58 – 6.69) | 0.277 | | | | |
| 3 | 6.11 (1.85 – 20.16) | 0.003 | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **AJCC TNM stage** | | | | | | |
| I | (reference) | | (reference) | | (reference) | |
| II | 2.70 (1.17 – 6.22) | 0.020 | 2.21 (0.86 – 5.67) | 0.100 | 2.25 (0.88 – 5.74) | 0.090 |
| III | 11.59 (5.18 – 25.93) | <0.001 | 9.90 (3.59 – 27.29) | <0.001 | 9.94 (3.62 – 27.31) | <0.001 |
| **IHC-defined subtype** | | | | | | |
| HR+/HER2- | 0.73 (0.35 – 1.50) | 0.388 | | | | |
| HR+/HER2+ | (reference) | | | | | |
| **Surgery type** | | | | | | |
| Conserving surgery | (reference) | | (reference) | | (reference) | 0.945 |
| Mastectomy | 2.29 (1.31 – 4.02) | 0.004 | 1.01 (0.54 – 1.89) | 0.972 | 0.98 (0.52 – 1.83) | |
| **Axillary management** | | | | | | |
| Axillary dissection | (reference) | | | | | |
| Sentinel node/none | 0.29 (0.15 – 0.55) | <0.001 | | | | |
| **Radiotherapy** | | | | | | |
| Yes | 1.90 (0.59 – 6.13) | 0.281 | | | | |
| No | (reference) | | | | | |
| **(Neo)adjuvant CT type** | | | | | | |
| Yes | (reference) | 0.001 | (reference) | 0.825 | (reference) | |
| No | 0.26 (0.11 – 0.57) | | 0.89 (0.33 – 2.43) | | 0.93 (0.34 – 2.52) | 0.886 |
| **HER2-directed therapy** | | | | | | |
| Yes | 1.07 (0.45 – 2.51) | 0.881 | | | | |
| No | (reference) | | | | | |
| **High recruitment center (>100 pts)** | | | | | | |
| Yes | 0.96 (0.13 – 6.94) | 0.966 | 1.20 (0.16 – 8.84) | 0.856 | 1.38 (0.19 – 10.18) | 0.753 |
| No | (reference) | | (reference) | | (reference) | |
| **Tamoxifen adherence, serum defined** | 2.02 (1.05 – 3.89) | 0.034 | 2.36 (1.22 – 4.56) | 0.010 | | |
| **Tamoxifen adherence, self-reported** | 0.83 (0.33 – 2.10) | 0.696 | | | 0.82 (0.32 – 2.10) | 0.686 |

**M5 Table S7 –** Multivariate association with breast cancer free interval (Cox proportional hazards model, PS weighted cohort). Variable selection for multivariate analysis was based on prior information of variables associated with cancer survival outcomes. Number of variables used in the multivariate model was limited by the number of DFS excluding second primaries events.

| | Multivariate association, serum-defined adherence | | Multivariate association, self-reported adherence | |
|---|---|---|---|---|
| | **HR (95% CI)** | **p-value** | **HR (95% CI)** | **p-value** |
| **Age at diagnosis, for every 1-year increase** | 0.94 (0.90 – 0.97) | 0.001 | 0.93 (0.89 – 0.97) | 0.001 |
| **AJCC TNM stage** | | | | |
| I | (reference) | | (reference) | |
| II | 2.17 (0.79 – 5.96) | 0.132 | 2.19 (0.81 – 5.97) | 0.123 |
| III | 9.49 (3.29 – 27.36) | <0.001 | 9.84 (3.45 – 28.06) | <0.001 |
| **Surgery type** | | | | |
| Conserving surgery | (reference) | | (reference) | 0.985 |
| Mastectomy | 1.04 (0.56 – 1.94) | 0.899 | 0.99 (0.53 – 1.87) | |
| **(Neo)adjuvant CT type** | | | | |
| Yes | 1.14 (0.41 – 3.16) | 0.802 | 1.13 (0.42 – 3.04) | 0.814 |
| No | (reference) | | (reference) | |
| **High recruitment center (>100 pts)** | | | | |
| Yes | 1.22 (0.24 – 6.22) | 0.899 | 1.45 (0.30 – 7.02) | 0.647 |
| No | (reference) | | (reference) | |
| **Tamoxifen adherence, serum defined** | 2.38 (1.27 – 4.47) | 0.007 | | |
| **Tamoxifen adherence, self-reported** | | | 0.81 (0.33 – 1.97) | 0.639 |

## 6. Discussion and conclusions

Throughout the body of work of this thesis and using data derived from multiple sources, we found that adjuvant endocrine therapy in the form of AI for postmenopausal women and OFS for pre-menopausal women were successfully introduced in clinical practice and improved the overall survival of patients with hormone-receptor positive early breast cancer. We also showed that adjuvant systemic treatments for breast cancer modulate QoL, with endocrine therapy being a relevant driver of deteriorated overall QoL and of specific QoL sub-domains, especially in postmenopausal women. Finally, we uncover that as shortly as 1 year after endocrine therapy initiation, 1 in 6 women are not taking adjuvant tamoxifen. Taking our work as an all, we provide new pieces of information to support an informed conversation between patients and physicians, as well as we uncover QoL dimensions that are specifically harmed by adjuvant systemic treatment and that can benefit from tailored interventions to improve QoL, adherence to treatment and ultimately cancer outcomes.

### 6.1 Real-world evidence shows the uptake in clinical practice and supports the effectiveness of AI and ovarian function suppression for the adjuvant treatment of breast cancer

Over the last 2 decades, the landscape of adjuvant endocrine treatment of hormone-receptor positive breast cancer evolved from tamoxifen to a wider set of options. For postmenopausal women, starting in 2005, international guidelines included AI as an alternative to tamoxifen, either replacing tamoxifen or to be used in sequence.[85] For premenopausal women, starting in 2015, international guidelines recommended the use of ovarian suppression for patients at higher risk of recurrence.[84] These two examples were an opportunity to analyze both the uptake of new evidence into clinical practice and the adherence to international guidelines in Portuguese centers. In two of our studies, we summarized the introduction of AI and OFS into clinical practice in a group medium and large size hospitals in Lisbon region, Portugal.[200,201] In the first study of postmenopausal women, we recorded a fast uptake of adjuvant AI with approximately 40% of patients receiving this treatment in 2006 of which in approximately 60% of the cases as a switch strategy (tamoxifen followed by an AI or an AI followed by tamoxifen).[200] This pattern remained mostly stable through the period of analysis ranging from 2006 and 2008. However, at the center level, we recorded a more dynamic pattern, with centers quickly introducing AIs, e.g. center A that in 2006 was using AI in more than 60% of patients and in 2008 in close to 80%, and centers slowly introducing this treatment, e.g. center

D that in 2006 and 2007 only prescribed tamoxifen, but in 2008 prescribed AIs to more than 65% of patients thus becoming in that year the second center with the highest proportion of patients receiving AIs. While patient preferences and disease characteristics might partially explain these differences, center and physician factors might also contribute to these differences. In line with this observation, center of care was independently associated with the use of AIs in a multivariate model. In the second study of premenopausal women, we identified the use of adjuvant OFS at least since 2006 and an acceleration in the uptake of this drug after 2014 (from every 1 in 7 to every 1 in 4 patients).[201] This was an interesting finding, given the lack of consistent evidence to support the use of OFS before 2014, but to a certain extent aligned with the equipoise on the topic that prompted the pursuit of the seminal clinical trials in the field, as the SOFT, TEXT and ASTTRA trials. We could not dissect the use of OFS by center in this study. Taken together, while overall practice patterns seem to be aligned with the available evidence and international recommendations, we identified different center-specific patterns that are at least in part explained by local preferences. In addition, there is, at times, some clinical practices that occur despite the unavailability of definitive evidence to support its use, as highlighted by the use of adjuvant OFS in premenopausal women before 2014. Translation to practice of evidence derived from clinical trials and from clinical guidelines is known to be frequently sub-optimal.[91] While in some cases there might exist barriers to the introduction of new practices, there are others in which treatments are used in the absence of robust evidence supporting its use. As clinicians we need to be vigilant and seek a balanced use of treatment options and thus avoid under and overtreatment as well as the use of unproven interventions. The identification of cultural and administrative barriers to optimal care (both towards under and overtreatment) and the design of strategies to overcome such barriers should be an institutional priority in each center. Moreover, academic centers should monitor treatment heterogeneities and use them as opportunities to run definitive clinical trials when such differences are driven by treatment efficacy equipoise.

Beyond the introduction of new treatment practices, the most appropriate duration of treatment is also a contemporaneous theme in the adjuvant endocrine therapy arena.[202] Although extended adjuvant tamoxifen for up to 10 years (compared to 5 years) has shown to improve long-term overall survival, AI for up to approximately 7 or 10 years has not yet shown such robust improvement in outcomes.[67] Most studies evaluating adjuvant OFS administered this treatment for 2 years, but more recent studies administered OFS for 5 years (partially due to the objective of testing the efficacy of AIs in premenopausal women).[52,121] Our analyses showed that most patients received adjuvant tamoxifen and AIs for 5 years and adjuvant OFS

for 2 years. These practices reflect the available evidence at the time of treatment prescription. Updated analysis of the real-world treatment patters might help dissect the use and effectiveness of extended adjuvant endocrine treatment.

In the highlighted studies, both adjuvant AIs (when compared to tamoxifen) and OFS (when compared to no OFS) improved cancer outcomes as measured by overall survival. In both cases, to our knowledge, this is the first real-world evidence supporting the use of AI (vs. tamoxifen) and OFS (vs. no OFS) in routine clinical practice. In addition, while previous studies point to an interaction between histologic type and efficacy of AI/tamoxifen, in specific with a relative resistance of invasive lobular carcinomas to tamoxifen and thus a preferred sensitivity to AIs[103], we did not identify this signal when looking to specific subtypes of lobular breast carcinomas, as pure and mixed lobular carcinomas.[203] While a growing body of evidence is identifying relevant biologic differences between lobular and breast carcinomas of no special type, it may be that what separates the lobular family of tumors either was not enough to produce a difference in efficacy that our study was able to discern (power limitation) or may alternatively support a lack of efficacy difference inside the lobular family of tumors. In both studies we found a small absolute impact of AIs (over tamoxifen) and OFS (over no OFS) in the overall cohort: at 5 years, 5.4% for the comparison between AI-tamoxifen and 2.1% for the comparison OFS-no OFS. This absolute difference is however in line with the magnitude of effect detected in clinical trials and in other advances in the endocrine therapy arena. At the same time, this piece of evidence is reassuring of the positive impact of both AIs (for postmenopausal women) and OFS (for premenopausal women) in terms of saving lives of patients.

Nevertheless, tamoxifen, AI and ovarian function suppression are associated with specific tolerability and safety issues. On the one hand tamoxifen increases the risk for thromboembolic events and endometrial cancer (the later in postmenopausal women), while on the other hand AIs increase the risk for osteoporosis and bone fractures, but also arthralgias and other musculoskeletal disorders.[58] In the case of OFS, it is associated with more frequent hot flashes, loss of sexual interest and sleep disturbance, but also vaginal dryness.[66] Considering the profile of adverse events and the overall absolute small improvements in disease recurrence and overall survival, current guidelines recommend the use of AIs in postmenopausal women with higher risk of recurrence, as with positive lymph nodes, higher histologic grade or high ki67 and after discussing the tolerability issues of each drug with patients.[14] Another strategy is to expose patients to AIs during a period of the overall treatment plan following a switch approach. In fact, a remarkable conclusion from the EBCTCG metanalysis comparing tamoxifen to AIs (in monotherapy or sequence) is that the overall survival impact of an AI-only strategy vs. a

tamoxifen – AI switch strategy is very similar (RR 0.96, 95% CI 0.86 – 1.07; 7 years rate of death from any cause 14.5 vs. 13.6% for switch vs. AI-only treatment).[39] Likewise, guidelines also recommend the use of OFS not in all but in a subset of premenopausal women, in specific those ≤35 years old and those who classically would warrant treatment with chemotherapy (node positive and/or high grade) that remained premenopausal.[14] To a certain extent this approach challenges the observation that both adjuvant AIs and OFS are effective (relative benefit) in patients with hormone receptor-positive tumors irrespective of e.g. histologic grade, stage or HER2 status (in SOFT trial a significant interaction favoring OFS + tamoxifen vs. tamoxifen in terms of DFS was found, but there were very small numbers of HER2 positive tumors and such an interaction was not found for the OFS + AI vs. tamoxifen).[52,204] However, in both cases the exercise is to select patients at higher absolute risk of recurrence for treatment with AIs (in monotherapy or in sequence) or OFS and thus obtain the larger absolute gains. In our analyses we found that physicians are indeed selecting some subgroups of patients for AIs, as defined by higher disease stage, higher tumor histologic grade or tumor HER2 positivity. Similarly, physicians are selecting for OFS patients with higher tumor histologic grade, HER2 positivity and treatment with (neo)adjuvant chemotherapy. This is reasonable in the continuum of the risk of recurrence, as the larger absolute reductions in the risk of recurrence and death will be in felt in those patients with larger absolute risks at baseline. To accommodate the absolute risk of detrimental cancer outcomes, as well as known QoL differences between treatments and patients' preferences is thus a reasonable route with a considerable room for tailored approaches incorporating in postmenopausal women tamoxifen, AI or their sequence for 5 or for extended periods of up to 10 years, and in premenopausal women tamoxifen or OFS with either tamoxifen or AI, also for periods ranging from 5 to up to 10 years of total adjuvant endocrine therapy.

Despite the consistent improvement of outcomes derived from adjuvant endocrine treatment, the risk of recurrence in patients with hormone receptor-positive tumors persists, with more than 50% of recurrences occurring after 5 years of diagnosis and some of them occurring even 20 years after diagnosis.[41] In the metastatic setting new avenues were opened with the use of CDK4/6 inhibitors and by targeting the PIK3CA/AKT/mTOR pathway. Future improvements in the realm of adjuvant treatment of hormone-receptor positive breast cancers might occur by moving these agents to the adjuvant setting. Based on these observations, several studies are already testing the role of e.g. CDK4/6 inhibitors and PI3K inhibitors in the early disease setting.[205,206] Yet, the challenge of avoiding late relapses is still not being addressed with these innovations, as most of these interventions are focusing on the intensification of

treatment in the first year of adjuvant endocrine therapy. The investment in changing health behaviors, as exercise and alcohol consumption, and risk factors, as excess weight, could be independent sources of incremental gains coming from non-pharmacological interventions.[207–210] In addition, the evolving field of genomic signatures is showing promising new tools that with further validation might be helpful in identifying those patients that might be eligible for extended adjuvant endocrine therapy, as is the example of the gene signature breast cancer index.[211,212]

### 6.2 Adjuvant endocrine therapy is a relevant driver of QoL deterioration two years after diagnosis, a fact that should be considered when planning the optimal care and research priorities in the field of adjuvant systemic treatments for breast cancer

Over the last decades, the optimization of local and systemic treatments allowed for most patients with breast cancer to expect long-term survivals. A part of these gains derived from incremental improvements of adjuvant systemic treatments, as endocrine therapy in patients with hormone receptor-positive tumors, HER2-directed therapy in patients with HER2-positive tumors and chemotherapy.[5] It is widely known that cancer treatments concomitantly increase the risk for a range of adverse events and have thus the risk to deteriorate patients QoL. As a strategy to balance both efficacy and tolerability, we have observed in recent years a trend to deescalate the use of chemotherapy while increasing the use of endocrine therapy. This strategy was mostly driven by the aim to reduce short and long-term toxicities of chemotherapy and further guided by the identification of genomically-defined groups of patients that could be spared chemotherapy. However, there is only scarce high-quality data on the long-term QoL impact of chemotherapy and the differential impact of chemotherapy and endocrine therapy, especially using well validated metrics and comparing contemporaneous regimens, thus we took advantage of CANTO study to extensively study QoL 2 years after diagnosis of breast cancer.[213]

Overall, 2 years after diagnosis and compared to before no treatment initiation, QoL was impacted in several domains and in a composite score accommodating several functions and symptoms. Although it is well recognized that local treatments (as surgery and radiotherapy) and adjuvant systemic treatments impact patients' wellbeing, for the systemic part of treatments the longitudinal impact was ill defined[152–154,214,215], and only more recently did we have access to longitudinal descriptions of long-term consequences of systemic treatments, namely cognitive, physical and in sexual function.[144,145,155,156] Our data extends this knowledge by showing that, at diagnosis and thus before any treatment, many patients already report symptoms and function impairment and that there is a larger scope of functions and symptoms that are deteriorated with multimodal cancer treatments. In specific, most functions deteriorate

over time with e.g. poor global health status going from affecting 22.7% at diagnosis to affect 27.8% 2 years after, poor role function from 9.7 to 17.1%, poor physical function from 9.2 to 12.6%, poor cognitive function from 22.9 to 38.4%, poor social function from 16.0 to 24.9%, poor sexual enjoyment from 29.5 to 38.4% and poor body image from 15.2 to 32.7%. Likewise, most symptoms deteriorate over time with e.g. severe pain going from affecting 26.5% at diagnosis to affect 51.0% 2 years after, severe dyspnea from 27.2 to 45.2%, severe constipation from 23.3 to 34.3%, severe systemic therapy side effects from 10.0 to 24.2%, severe breast symptoms from 13.6 to 23.0%, severe arm symptoms from 19.7 to 37.4% and severe upset by hair loss from 59.2 to 72.2 (among those reporting hair loss). Although as age progresses certain functions and symptoms are expected to deteriorate, the collection of outcomes after an interval of 2 years and the substantial changes point to the overall relevant burden of cancer treatments in patients QoL. Interestingly, poor emotional function and poor future perspective (but also severe appetite loss) improve over time showing a positive psychological impact of treatment on patients' wellbeing.

Despite the evidence showing that treatment impacts patients' wellbeing, how different classes of systemic treatment impact QoL is not definitively established. A recent substudy of the TAILORx trial focusing on the relative contribution of endocrine therapy vs. endocrine therapy plus chemotherapy in 3 specific dimensions of QoL (cognitive function, fatigue and endocrine symptoms) up to 36 months after treatment initiation added a piece of evidence to this question.[157] In this study, the chemo-endocrine therapy arm was associated with a short-term (3-6 months) significant greater cognitive impairment, fatigue and endocrine symptoms (during the adjuvant chemotherapy period of time). After this period, despite an absolute lower QoL in patients in the chemo-endocrine therapy arm such differences are not significant. In our study QoL differed over time by class of systemic therapy. Looking at the C30 summary score (composite score of several functions and symptoms) and compared to those not receiving chemotherapy, patients receiving chemotherapy have their QoL correct over time. In contrast, the impact of endocrine therapy was a relevant driver of overall persistent QoL deterioration 2 years after diagnosis. Of note, both endocrine therapy and chemotherapy influenced specific functions and symptoms differently. Endocrine therapy negatively influenced role and social functions, but also pain, insomnia, systemic therapy side effects and breast symptoms. Moreover, it attenuated the recovery of emotional function and future perspectives, two domains that showed to correct over time in the overall cohort and capturing psychological dimensions of the patients' wellbeing. Chemotherapy negatively impacted physical and cognitive function, but also financial difficulties, body image and breast symptoms. With the introduction of extended adjuvant endocrine therapy for up to 10 years, survivorship clinics

should look to these patients as a group at considerable risk for QoL deterioration and downstream risks, as treatment non-adherence.

Moreover, our results further reveal that the effect of endocrine therapy is especially detrimental in postmenopausal women. Of note, in our study of adjuvant tamoxifen compared to adjuvant AI in postmenopausal women, when looking at patients receiving a switch strategy (tamoxifen – AI or AI – tamoxifen), of the 5% of patients that started with an AI (95% of patients started with tamoxifen) it was striking to observe that the median time on an AI was of 1.3 years and that 25% of these patients took an AI for less than 2 months.[200] This is not a typical switch strategy and may inform about tolerability challenges that some patients face with AI. Conversely, in those patients starting with tamoxifen median time on first agent was of 2.7 years. With the recent intensification of adjuvant treatments in premenopausal women using OFS (in our study in around 25% after 2014[201]) and the growing use of AIs (in our study in around 30% of those receiving OFS[201]) we need to be vigilant on the QoL implications of such options.

In our work we identified detrimental QoL signals that merit consideration when discussing and planning optimal survivorship care, adjuvant treatment options and when designing studies of escalation of endocrine therapy. These data are especially relevant in the setting of improving patients' survival after the diagnosis of breast cancer and longer treatment duration, particularly those based on endocrine therapy that, despite improving oncologic outcomes, also impose persistent changes in QoL. The interplay between different classes of treatment is however complex and future research should deepen the knowledge on the QoL impact of the multimodal treatments of breast cancer, namely the interaction between locoregional and systemic treatments. With the aim of improving patients' wellbeing and cancer outcomes, tolerability issues are cornerstone, as these are a recognized barrier to treatment adherence and ultimately to the overall principle of helping to live longer and better lives.[186,216,217]

### 6.3 One year after diagnosis 1 in 6 premenopausal women are not adherent to adjuvant tamoxifen with disease recurrence implications

A well-known obstacle to the improvement of cancer outcomes in the setting of oral adjuvant treatments is the adherence and persistence to treatment.[71] However, the true magnitude of the problem is unclear given the intrinsic challenges of studying the field as there is no gold standard method to quantify treatment non-adherence.[72] In this setting we took advantage of the CANTO study to develop a substudy quantifying the prevalence of treatment non-adherence to adjuvant tamoxifen using 1) a self-evaluation questionnaire and 2) by directly quantifying tamoxifen levels in the blood (serum assessment). A surprising proportion of 16% of

patients (1 in 6) was non-adherent 1 year after treatment prescription when measuring tamoxifen serum levels. Another relevant observation was that, when using a questionnaire, the proportion of patients declaring to be non-adherent was 12.3%, an estimate 23.1% lower than that using the serum assessment method and missing 104 patients that stated to be adherent but were classified as non-adherent using the serum assessment. While associated, only a moderate association between the two methods was found (concordance: 86% [95% CI 84 to 88%]; Cramer V = 0.429). This finding is especially relevant, because when looking to cancer outcomes, while the cohort with serum-defined non-adherence had a higher risk for distant recurrence those with self-reported non-adherence had not (non-significant trend). This informs about the classification power of the serum assessment method to identify a group of patients not only taking tamoxifen in a sub-optimal way, but most importantly at higher risk of recurrence and thus in need of tailored interventions to optimize treatment adherence. Moreover, given the dichotomous nature of the serum assessment results, it is clinical actionable even when quantified as early as 1 year after initiation of adjuvant tamoxifen. Indeed, for every 17 patients undergoing interventions able to restore optimal adherence we could avoid one distant recurrence.

In our work we looked for the demographic, social and disease features associated with non-adherence to facilitate the identification of a group at higher risk for non-adherence. In this setting, patients not living as a couple, more symptomatic (as with severe fatigue and musculoskeletal symptoms), with other comorbidities and not treated with adjuvant chemotherapy were more likely to be non-adherent to therapy. We have already discussed how these features might impact adherence, but social support, poor tolerability, other comedications and the perceived risk of recurrence might be in the causal pathway to non-adherence in these cases. While helping to select patients for specific tailored supportive interventions, in places where serum drug quantification is not readily available, these features might further support the development of a clinical score that triages patients at higher clinical risk of non-adherence to serum assessment.

In our study of postmenopausal women receiving adjuvant tamoxifen or AI, while median duration of treatment in monotherapy was close to a total of 5 years, the lower boundary of the IQR was as low as of 38 months (3.2 years).[200] This observation highlights, from the prescription side, that in this cohort 25% of the patients completed less than 3.2 years of adjuvant treatment. While the reasons for this observation were not possible to retrieve given the design of this study it is also a concerning finding. Interestingly, there were no substantial differences in the lower boundary of the IQR of the time on adjuvant endocrine treatment between tamoxifen and AIs (3.2 years and 3.5 years for tamoxifen and AI, respectively). A similar

observation can be seen for patients opting for a switch strategy (tamoxifen – AI or AI – tamoxifen). In our study of premenopausal women receiving or not adjuvant OFS the median time on OFS was 2.1 years with the lower boundary of the IQR of the time on OFS of 1.7 years.[201] The optimal duration of OFS is not definitely established and evolved over time, with recent guidelines recommending 5 years of treatment.[14] That said, several clinical trials used a shorter duration of 2 years of adjuvant OFS which might explain the findings of our study. Assuming an intended duration of treatment of about 2 years, our results show that also with OFS some patients struggled to complete the intended duration of treatment.

### 6.4 Future steps

Throughout the development and implementation of this body of work the PhD candidate developed technical expertise in handling real-world data and dealing with various typologies of outcomes research projects. With these tools and the body of work generated, several paths of future research and collaboration were opened.

With a focus on the comparative effectiveness of medical interventions, a natural collaboration would come from the continued interaction with RON, and possibly with Infarmed, to contribute towards an ever improving mechanism to monitor the real-world effectiveness of new cancer treatments. With the growing portfolio of treatments available for cancer patients and the persistent gap between patients recruited for clinical trials and those composing the large bulk of patients followed in routine clinical practice, there is a huge need for real-world evidence to support health technologies assessment.

With a focus on the impact of breast cancer treatments on QoL, we aim at further dissecting the impact of systemic and local treatments on the QoL of breast cancer patients. Such next steps would come from the granular look towards different types of endocrine therapy and chemotherapy, the study of the interaction between local and systemic treatments, but also from the addition of longer follow-up to the current analyses. Moreover, with an additional focus on tolerability to medical interventions, we aim at characterizing the downstream actions of health professionals after the occurrence of adverse events and to use this information to build interventions aiming at improving the management of adverse events.

Finally, we aim at identifying opportunities to translate advanced analytics, as artificial intelligence, to the field of survivorship to, e.g., develop tools to predict the occurrence of adverse events that will ultimately improve patients QoL and cancer outcomes.

## 6.5 Conclusions

Endocrine therapy is a powerful tool that helped generations of patients with hormone receptor positive breast tumors to improve their cancer outcomes. Each of the innovations in the field led to incremental gains. In our work we found that such innovations were translated to clinical practice and that results observed in clinical trials were also recorded in the real-world, importantly in terms of overall survival. In this setting we need to be vigilant and make local and national efforts to move treatment innovation to clinical practice so that our patients can benefit from the latest achievements in cancer care. The improvements in efficacy obtained through adjuvant endocrine therapy seem to be however counterbalanced by a relevant deterioration in patients' QoL that may harm treatment adherence and ultimately cancer outcomes. In the era of endocrine therapy escalation, a relevant research effort should be allocated to balance efficacy and QoL considering the continuum of recurrence risk. Moreover, identifying and overcoming barriers to optimal survivorship care might facilitate the management of tolerability troubles and thus help to maximize adherence/persistence to treatment. While endocrine therapy improves survival, the QoL impact is palpable. Focusing our efforts as health professionals in understanding this interaction and optimizing its balance is the challenge that lays ahead of us.

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