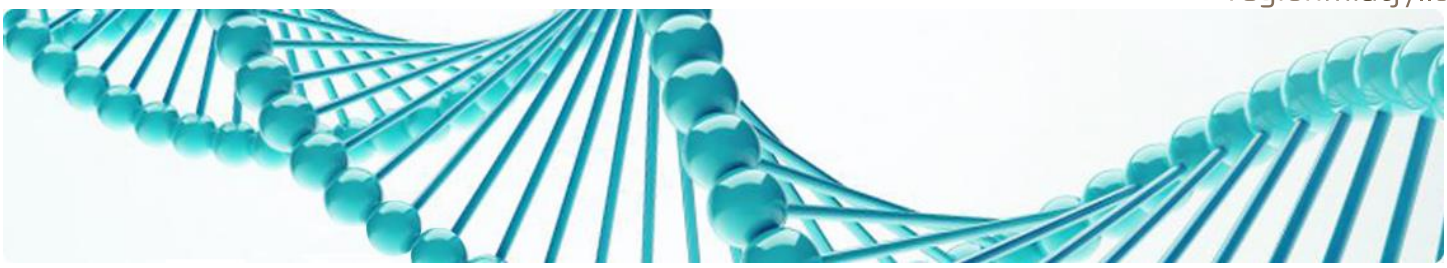

A Randomized, placebo-controlled, Phase III Study of (Neo)Adjuvant Atorvastatin Therapy in Patients with Early Breast Cancer

The MASTER study (MAmmary cancer **ST**atin **ER** positive study)

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TRIAL SUMMARY

The MASTER study (MAmmary cancer **ST**atin **ER** positive study)

A randomized, multicenter, double-blind, placebo-controlled comparison of standard (neo)adjuvant therapy plus placebo versus standard (neo)adjuvant therapy plus atorvastatin in patients with early breast cancer.

Cholesterol-lowering drugs such as statins are currently used to lower cholesterol levels and prevent cardiovascular events. Statins have, however, received substantial scientific attention as cancer-inhibiting drugs. Previous findings were recently supported in a large-scaled study again demonstrating the beneficial effects of statins on breast cancer outcome this time nested within a large, international, randomized clinical trial of modern adjuvant cancer therapy. Given the compelling evidence supporting a protective effect of statins on breast cancer recurrence, calls for prospective clinical trials have been expressed. In this trial – the MASTER trial – we hypothesize that the addition of statin treatment to the current breast cancer treatment will improve the prognosis of women with early breast cancer. Thus, the primary objective of the MASTER trial is to determine the clinical efficacy of the statin – atorvastatin - as measured by invasive disease-free survival among patients with primary breast cancer.

The trial is nationwide throughout Denmark and a total of 3,360 women are to be included in the trial. Women eligible for the trial have been diagnosed with an estrogen receptor positive breast cancer and are candidates for systemic cancer therapy, either prior to or following breast surgery. Upon eligibility and signed informed consent, trial participants will be randomized in a 1:1 manner to either standard treatment and atorvastatin 80 mg/day or standard treatment and placebo. The randomization is blinded. The treatment with atorvastatin or placebo will continue for two years unless side effects are experienced and further treatment with atorvastatin or the placebo is deemed inadequate. The standard treatment will of course continue as planned. The trial participants will follow the standard clinical routines in terms of follow-up and in addition they are asked to fill in questionnaires, i.e. regarding potential side effects, up to ten years following inclusion. Potential breast cancer recurrences are identified through follow-up and a follow-up of at least $6^1/_2$ years will be required for the trial the demonstrate the estimated clinical difference between the randomized groups of patients.

TRIAL SYNOPSIS

TITLE	A randomized, multicenter, double-blind, placebo-controlled comparison of standard (neo)adjuvant therapy plus placebo versus standard (neo)adjuvant therapy plus atorvastatin in patients with early breast cancer.
SPONSOR	Aarhus University Hospital
OBJECTIVES	PRIMARY OBJECTIVES To compare invasive disease-free survival (IDFS) in patients randomized to standard (neo)adjuvant therapy plus placebo or standard (neo)adjuvant therapy plus atorvastatin (80 mg/day for two years). SECONDARY OBJECTIVES - To compare overall survival (OS), recurrence-free interval (RFI), distant recurrence-free interval (DRFI) including associations with first site of recurrence, cardiac death-free interval, and overall safety in the two treatment arms. - To evaluate pathological response (only neoadjuvant treated patients) according to treatment arm - To investigate patient reported outcome measurements. - To address translational endpoints as specified in the translational protocol parts.
TRIAL DESIGN	A prospective, two-armed, randomized (1:1), multicenter, national, double-blind, placebo-controlled study in early breast cancer patients.
NUMBER OF PATIENTS	N=3,360 participants.
TARGET POPULATION	Early cohort; randomization before start of (neo)adjuvant therapy. Late cohort; randomization after start of adjuvant therapy, but within three years of initiation of the endocrine treatment. Observational cohort; non-randomized cohort of patients already on cholesterol-lowering medication at the time of diagnosis.
INCLUSION CRITERIA	Patients must meet ALL of the following criteria to be eligible for randomization: 1. Women with estrogen receptor positive breast cancer who are candidates for (neo)adjuvant systemic therapy OR have received ≤3 years of adjuvant endocrine therapy. 2. Age > 18 years. 3. Performance status of ECOG ≤ 2. 4. Prior to patient registration, written informed consent must be given according to ICH/GCP, and national/local regulations.
EXCLUSION CRITERIA	Patients meeting ANY one of the following criteria are not eligible: 1. History of any prior (ipsi- and/or contralateral) invasive breast carcinoma. 2. Ongoing (prevalent) cholesterol-lowering therapy (statins, fibrates, ezetimibe, PCSK9 inhibitors). If so, the patient can be enrolled in the observational arm given fulfillment of other in- and exclusion criteria.

	3. Evidence of hepatic dysfunction (alanine aminotransferase level more than three times the upper limit of the normal range) or renal dysfunction (creatinine level more than three times the upper limit of the normal range). 4. Predisposing factors for rhabdomyolysis, including hypothyroidism, reduced renal function, any muscle – or liver disease, or excessive alcohol consumption (above 14 drinks/week) AND creatine kinase (CK) measured to ~~less~~ more than five times the upper limit (CK only measured in case of predisposing factors). 5. ~~No~~ Current medication with potent CYP3A4-inhibitors (e.g. ketokonazole, erythromycin) or gemfibrozile, cyclosporin or danazol. 6. Pregnancy or breast-feeding (contraception according to clinical routines for premenopausal, fertile breast cancer patients, that includes non-hormonal contraception such as condom, vaginal diaphragm, or intrauterine device). 7. Psychological, familial, sociological or geographical condition potentially hampering compliance with the study protocol and follow-up schedule; these conditions will be discussed with the patient before registration in the trial. 8. History of allergic reactions attributed to compounds of similar chemical or biological composition to atorvastatin.
STANDARD OF CARE	All study patients will receive (neo)adjuvant therapy according to current regional and nationwide guidelines irrespective of randomization, including chemotherapy, radiotherapy, endocrine treatment and/or HER2 directed therapy.
INVESTIGATIONAL DRUG	Atorvastatin, oral administration, 80 mg daily (evening), two years of duration starting at the day of study enrollment and for two years onwards.
SAFETY	MONITORING Patients are followed according to standard clinical routines. In case of acquired renal or hepatic dysfunction during the on-treatment period, extended monitoring will be initiated and continued enabling sufficient information for potential treatment adjustments. DOSE ADJUSTMENTS AND EARLY STOPPING - Raising levels of hepatic transaminases or creatine kinase to more than three times the upper limit of normal will require discontinuation of STUDY MEDICATION until lowering of levels within normal limits has been reached. - Myopathy (muscle aching or muscle weakness in conjunction with increase in creatine kinase values greater than five times the upper limit of normal) will require permanent discontinuation of STUDY MEDICATION. - Reduced performance status (ECOG>2) will require permanent discontinuation of STUDY MEDICATION.
DURATION OF THERAPY	All patients will receive STUDY MEDICATION (atorvastatin or placebo) for two years.

	STUDY MEDICATION will be discontinued if: 1. There are any SAFETY concerns. 2. Cholesterol-lowering therapy becomes clinically indicated. 3. Discontinuation is requested by the participant and/or her treating physician. 4. In case of an incident breast cancer event (any invasive breast cancer, including local/regional, contralateral or distant recurrence). Atorvastatin is a well-documented drug prescribed for continuous use over several years and the appearance of other illnesses would not indicate discontinuation of atorvastatin.
CONCOMITANT THERAPY	During STUDY MEDICATION concomitant drugs are permitted. Caution should be taken in case of: 1. Digoxin treated patients should be monitored as co-administration of atorvastatin and digoxin has been shown to increase steady-state plasma digoxin concentrations by approximately 20%. Among digoxin-treated patients, plasma digoxin will be measured at baseline, after 1 week and after 1 month on STUDY MEDICATION and an increase of ≥ 20% at consequently will lead to a reduction in dosage of digoxin. 2. Daily intake of grapefruit juice should be avoided during atorvastatin therapy due to the grape fruit induced inhibition of CYP450 system by which atorvastatin is metabolized. 3. CYP3A4 inhibitors such as macrolide antibiotics (erythromycin), immunosuppressants (cyclosporine), azole antifungal agents (*i.e.,* itraconazole, ketoconazole), and some antidepressants (*i.e.*, nefazodone) should if possible be avoided during atorvastatin therapy as they may have a potential of increasing plasma concentrations of atorvastatin.
PROCEDURES (Summary)	During STUDY MEDICATION, patients will be assessed for safety and efficacy (see Schedule of Assessments tables). Patients will receive STUDY MEDICATION for a total of two years (104 weeks).
ASSESSMENTS	EFFICACY IDFS, defined as the time from randomization until the date of the first occurrence of one of the following events: • Ipsilateral invasive breast tumor recurrence: invasive breast cancer involving the same breast parenchyma as the original primary. • Regional invasive breast cancer recurrence: Invasive breast cancer in the axilla, regional lymph nodes, chest wall, and skin of the ipsilateral breast. • Distant recurrence: Metastatic disease-breast cancer that has either been biopsy confirmed or clinically diagnosed as recurrent invasive breast cancer. • Death attributable to any cause, including breast cancer, non-breast cancer, or unknown cause. • Contralateral invasive breast cancer. • Second primary non-breast invasive cancer. SAFETY

	Any adverse events (AE's) will be recorded according to clinical routine during active treatment and their frequencies reported. Toxic effects will be categorized using a modified version of the NCI Common Terminology Criteria for Adverse Events, Version 5.0.
STATISTICAL ANALYSES	PRIMARY EFFICACY ENDPOINT The primary efficacy variable is IDFS and is defined as the time between randomization and date of first occurrence of an IDFS event (as defined above in efficacy assessments). The intention-to-treat (ITT) population will be used in the primary analysis. The stratified log-rank test will be used to compare IDFS between the two treatment arms. SECONDARY EFFICACY ENDPOINTS Pathological response (neo-adjuvant patients only) Recurrence-Free Interval Distant Recurrence-Free Interval including associations with first site of recurrence Overall Survival Death attributable to any cause, including non-breast cancer or unknown cause, is considered a competing event. Cardiac death-free interval. Cardiac death is defined as: • Definitive cardiac death due to heart failure, myocardial infarction or documented primary arrhythmia. • Probable cardiac death defined as sudden, unexpected death within 24 hours of a definite or probable cardiac event (e.g., syncope, cardiac arrest, chest pain, infarction, arrhythmia) without documented etiology. Overall safety Patient Reported Outcome Translational biomarker endpoints (stated below) SAFETY ANALYSES Randomized patients who receive any amount of STUDY MEDICATION will be included in the safety analyses. Safety will be summarized by the amount of treatment actually received. SAMPLE SIZE The sample size calculations are based on estimates of 10-year IDFS with an annual hazard rate of 0.0223. A sufficient number of patients is sought to provide 80% power using a two-sided 0.05-level test to detect a 25% reduction in the risk of an IDFS event (hazard ratio=0.75). A total of 379 events are required. With an estimated 5% drop-out at 5 years (annual rate 0.0103), this is estimated to be achieved by a total recruitment of 3,360 participants and a 6½ years follow-up. The sample size estimate is

	based on inclusion for 3 years, a median follow-up of 6½ year, that is 5 years of additional follow-up after the 3 years of inclusion.
TRANSLATIONAL STUDIES	BLOOD SAMPLES Trial participants are asked for blood samples collected at baseline, after one and two years of study treatment, respectively, and in case of early withdrawal or recurrence while on study treatment. Particularly for neoadjuvant patients, an additional blood sample will be drawn at the time of stopping chemotherapy/prior to surgery. Samples are stored in a research biobank established for this study. Circulating biomarkers will be analyzed for their predictive and prognostic value. Samples are stored as plasma, serum, buffy coat, and whole blood at minus 80-degree Celsius at the Departments of Biochemistry of participating hospitals and final storage at the Danish Cancer Biobank. TUMOR TISSUE SAMPLES Archived tumor tissue from the primary breast tumor will be sampled in tissue micro arrays, which are then stores in a research biobank. Triplicate cores of 1 mm in diameter will be sampled from the donating tumor block and mounted in a recipient paraffin block, which will be able to host tumor cores from 50-60 tumors per paraffin block. Primary tumor tissue will be analyzed for the expression of cholesterol associated biomarkers, including i.e. HMGCR, LDL-receptor, and CYP27A1. One of the tumor tissue cores will be sampled for RNA-extraction and further gene expression analyses using the Illumina Platform. Tumor tissue analyses will allow for identification of prognostic and treatment predictive factors and pave the way for more personalized (neo)adjuvant treatment.

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1 BACKGROUND AND INTRODUCTION

1.1 Background disease information

Breast cancer is the most common malignancy among women worldwide and accounts for more than one million incident cases each year (1, 2). In Danish terms, that is a yearly incidence of around 4,600 women diagnosed with breast cancer and a lifetime risk of 10-15% (Nordcan, 2019). Over the past 20 years the breast cancer incidence has increased globally (3) which corresponds to an increase of 1.4% per year in Denmark (Nordcan, 2018). Breast cancer treatment includes a wide range of both local and systemic approaches applied in different phases of the disease—surgery, radiotherapy, chemotherapy, endocrine therapy, and anti-HER2 therapy. Neo-adjuvant systemic therapy (NACT) is recommended to patients with large tumors (2-5 cm) and to patients who are technically inoperable (locally advanced breast cancer, LABC). Adjuvant therapy is provided patients with radically operated non-metastatic disease and comprises chemotherapy, radiation therapy, and in the case of estrogen receptor (ER) positive breast cancer, endocrine therapy is prescribed for 5-10 years, tamoxifen to pre-menopausal women, and aromatase inhibitors to postmenopausal patients. Anti-HER2 therapy with trastuzumab/pertuzumab is approved in the (neo)adjuvant setting for patients with HER2 amplified breast cancer.

Breast cancer mortality has improved in developed countries with a decrease of 14% from the 1970s to the 1990s (3). However, breast cancer still remains the leading causes of cancer death among women (2). The prognosis for Danish breast cancer patients corresponds with a relative 1-year survival of 97% and a 5-year survival of 87% (Nordcan, 2015). The risk of breast cancer is indeed age-related and less than 1% of breast cancer patients are younger than 30 years at diagnosis (4). The incidence increases along with age and peaks at the age of 65 years *(*Nordcan, 2015*).*

Clinical evidence of statins beneficial effects in breast cancer

Cholesterol-lowering statins (HMG-CoA reductase inhibitors) have received substantial scientific attention as cancer-inhibiting drugs. One of the first epidemiologic studies on this topic reported an imprecisely-measured protective association between statin use and breast cancer recurrence in a cohort of almost 2,000 women (hazard ratio [HR]=0.67, 95% CI: 0.39, 1.13). In an additional Danish study, Ahern, Cronin-Fenton *et al* measured this association with much higher precision in a cohort of over 18,000 Danish breast cancer patients, finding nearly identical reduction in recurrence rate among women who took statins (HR_{adj}=0.73, 95% CI: 0.60, 0.89). Since publication of these initial studies, several other groups have replicated the protective association in a variety of source populations and with a variety of study conditions (7-9). A recent systematic review and meta-analysis of the evidence base reported a summary relative risk of 0.64 (95% CI: 0.53 to 0.79) (10).

Most recently, Drs. Borgquist, Ejlertsen and colleagues published a study nested in the Breast International Group 1-98 trial (BIG 1-98), in which DBCG was a key partner and important trial contributor. The aims of the study were to (1) characterize patterns of cholesterol-lowering medication (CLM) initiation according to adjuvant endocrine therapy received (tamoxifen or letrozole), and (2) evaluate whether initiation of CLM (predominantly statins) during endocrine therapy was associated with clinical outcome. The incidence of CLM was higher among women taking letrozole, whereas cholesterol levels decreased over time among women taking tamoxifen, irrespective of CLM use. Initiation of CLM during endocrine therapy was associated with improved breast-cancer-free interval (HR=0.76, 95% CI: 0.60 to 0.97) and distant-recurrence free interval (HR=0.74, 95% CI: 0.56 to 0.97) (11). This study was the first to support the beneficial effect of statins on breast cancer outcome that is nested within a large, international, randomized clinical trial of modern adjuvant endocrine therapy.

Calls for clinical trials of statins in the breast cancer adjuvant setting

Given the compelling evidence supporting a protective effect of statins on breast cancer recurrence, calls for clinical trials have appeared in several prominent journals. One of these calls came from a paper authored by Drs. Ahern, Christiansen, and colleagues, which summarized the preclinical and epidemiologic evidence for a beneficial effect of statins in breast cancer survivors, and recommended design features for a future adjuvant clinical trial (12).

Molecular basis for statin use as breast cancer therapeutic agents

Statins are routinely used to treat hyperlipidemia but may also have antineoplastic effects. The target for

statins, HMGCR, is a transmembrane glycoprotein found in the endoplasmic reticulum in all cells. HMGCR activity controls the mevalonate pathway which, in addition to cholesterol, produces steroid-based hormones and non-sterol isoprenoids (Figure 1) (12, 13). Inhibition of hepatic HMGCR causes reduced intracellular cholesterol levels in hepatocytes. This in turn triggers up-regulation of low-density lipoprotein cholesterol (LDL-C) receptors to scavenge cholesterol from the serum to support cell growth and division. Serum levels of LDL-C consequently plummet, reducing the rate of adverse cardiovascular events in statin-treated individuals (14). Cellular cholesterol levels and HMGCR activity are maintained *via* tightly regulated feedback mechanisms, whereas extracellular serum cholesterol concentrations vary (15). In addition to reducing LDL-C production, blocking the mevalonate pathway interrupts synthesis of the isoprenoids geranylgeranyl pyrophosphate (GGPP) and farnesyl pyrophosphate (FPP) (12, 13). Isoprenylation of proteins by GGPP and FPP enables subcellular localization and intracellular trafficking of membrane-associated proteins that are essential for the cell (16). GGPP and FPP post-translationally prenylate a host of proteins—including the oncogene products Ras, Rac, and Rho—to enable their full function.(16-18) The isoprenoids demonstrate tumor-suppressive properties as regulators of important processes in cancer such as proliferation, migration, and angiogenesis (13, 19). Comprehensive analysis of statin-induced changes in tumor cell lipid profiles may reveal the dominant pathways through which statins attenuate tumor promotion (20).

Highly proliferative cells (such as cancer cells) must rapidly produce lipid bilayer membranes, requiring increased cholesterol biosynthesis (21). While the cholesterol biosynthesis pathway is tightly regulated in normal cells, it may be dysregulated in cancer cells (22). It has been implied that HMGCR is a metabolic oncogene, and that dysregulation of the mevalonate pathway promotes transformation (23). In addition, high mRNA levels of HMGCR and other mevalonate pathway genes were associated with impaired prognosis for breast cancer patients (23). This finding was recently validated in two large breast cancer datasets (24).

The mevalonate pathway is a possible therapeutic target for tumors with mutations of the tumor suppressor p53 (25, 26). The p53 protein, encoded by the *TP53* gene, is dubbed "the guardian of the genome" due its tumor-suppressing activities. These activities, triggered by DNA damage, include activation of DNA repair mechanisms, initiation of growth arrest at the G1/S boundary, and induction of apoptosis if repair fails. The mevalonate pathway is both necessary and sufficient for the phenotypic effects of mutant p53 on breast tissue architecture, and mutant p53 associates with sterol gene promoters (25). In a 3-dimensional culture model, mutant p53 up-regulated mevalonate pathway genes in breast cancer cells, leading to disordered, invasive morphology (25). In the same model, depletion of mutant p53 by RNA interference caused reversion to normal morphology. Remarkably, the addition of clinically achievable concentrations of statin to the culture system resulted in marked reductions in tumor cell growth, induction of apoptosis, and reversion to normal morphology in the various breast cancer cell lines tested.(25) The beneficial effects of statin were negated when the mevalonate pathway products GGPP and FPP were simultaneously added to the culture medium. The p53 effect is likely modulated by sterol regulatory element-binding proteins (SREBPs) and tied to the YAP/TAZ effectors of the Hippo signaling pathway (27). YAP/TAZ activity is also controlled by Rho GTPases, which are dependent on prenylation for activation (28). Therefore, overexpression of genes encoding p53, SREBPs, mevalonate pathway genes, and the YAP/TAZ transcriptional regulators may identify breast tumors that will be sensitive to statin treatment.

A large number of *in vitro* and *in vivo* cancer studies with statins have been performed, with many more underway. So far, these models have shown that statins decrease proliferation and increase apoptosis of breast cancer cells (29, 30). The biological mechanisms for these actions are not yet fully elucidated. Our previous work demonstrated that HMGCR is differentially expressed in human breast cancer samples and holds prognostic value (31, 32). HMGCR may also predict tumor response to endocrine treatment (33), as well as to statin treatment, the latter demonstrated in a recent phase II clinical trial (ClinicalTrials.gov Identifier: NCT00816244) (34). Other *in vitro* studies demonstrated substantial statin-induced increases in HMGCR expression (35), and atorvastatin induced HMGCR up-regulation when assessed with a novel, well-validated monoclonal HMGCR antibody (24). Additionally, *in vivo* studies suggest that HMGCR activity is higher in mammary tumors compared with normal mammary glands, and that tumors are resistant to feedback regulation by sterols (36).

If statins do target the mevalonate pathway in cancer cells within a tumor, the lowering of intracellular cholesterol may lead to lowered intra-tumoral autocrine hormone production, as cholesterol is fundamental for all steroid hormone synthesis. Interestingly, one study has reported that atorvastatin and its metabolites

are detectable in human breast samples following oral administration (37), indicating that direct inhibition of HMGCR may occur in breast tumors. Most relevant for an endocrine responsive disease such as estrogen receptor (ER)-positive breast cancer, statin treatment reduces levels of the cholesterol metabolite 27-hydroxy cholesterol (27HC) (38). 27HC acts as an ER ligand, potentiating ER-dependent tumor growth (39-42). Interestingly, 27HC can agonize both the ER and the liver X receptor (LXR) to drive breast tumor proliferation (39, 40), and it promotes metastasis through interactions with myeloid immune cells.(43) The cholesterol biosynthesis pathway was recently shown to be up-regulated in ER-positive breast cancer cell lines that are resistant to estrogen deprivation (44, 45), suggesting that dysregulation of cholesterol biosynthesis may be a mechanism of endocrine resistance in hormone receptor-positive breast cancer (44, 45). Chronic estrogen deprivation in ER-positive breast cancer cells seems to stabilize the epigenetic activation of the mevalonate pathway and cholesterol biosynthesis,(45) and this leads to the accumulation of other ligands (such as 27HC), which potentiate ER signaling in the absence of estrogen, consequently driving the activation of genes that promote a proliferative and invasive cell phenotype (45).

To summarize, cancer cells depend on cholesterol for continued growth and survival. Therefore, attenuating cholesterol biosynthesis seems to be a promising anti-cancer strategy. Of note, rapidly proliferating cancer cells have an increased cholesterol demand to enable cell membrane synthesis (21, 46). By lowering plasma levels of cholesterol and 27HC, their availability for use by cancer cells is consequently lowered. Additionally, direct inhibition of HMGCR by statins depletes intratumoral reserves of isoprenoids, which are key regulators of cancer cell proliferation and metastasis. Ongoing studies are exploring additional roles that cholesterol, cholesterol metabolites, and statins play in breast tumor promotion.

Predictive factors for statin response in breast cancer

The well-characterized molecular and clinical heterogeneity of breast cancer warrants that for every novel drug showing efficacy against this disease, specific treatment predictive biomarkers should be identified to enable the precise selection and treatment of only those patients who may derive clinical benefit from the treatment. As such, the search for statin treatment predictive markers in breast cancer has been the subject of many studies. HMGCR has been shown to be overexpressed in about 80% of breast tumors, and its expression is correlated with less aggressive tumor phenotype and longer recurrence-free survival.(31, 32, 47) In the aforementioned clinical phase II trial when 50 breast cancer patients were treated pre-operatively with atorvastatin 80mg/day for two weeks, tumor levels of Ki67 (a proliferation marker) decreased from pre-surgical biopsy to resected tumor only if the tumor expressed HMGCR,(34, 48) suggesting a predictive role for HMGCR for efficacy of statin treatment in breast cancer. HMGCR was not only differentially expressed across tumors from breast cancer patients, but was also associated with improved prognosis among ER positive breast cancer patients, whereas ER negative patients seemed to have better outcomes when HMGCR was absent.(31, 32) Several other mevalonate pathway biomarkers have been associated with breast tumor response to statins *in vitro* and in animal models. However, no study has comprehensively evaluated the network comprised of these individual factors, and how it is perturbed by statin exposure to alter recurrence risk. Randomized trials are now warranted to clarify the potential beneficial effects of statins in breast cancer management in the adjuvant and metastatic setting. In October 2016, the first trial with statins in metastatic breast cancer was launched, with the hypothesis that HMGCR expression will identify tumors that will respond to statin treatment (ClinicalTrials.gov Identifier: NCT02958852).

Exploring transcription profiles associated with breast cancer sensitivity to statin treatment is another method that has been explored to discover genes or gene signatures that are predictive of statin treatment sensitivity. In one such study based on *in vitro* experiments with atorvastatin in a collection of breast cancer cell lines, we aimed to uncover transcriptional differences associated with statin response in breast cancer.(24, 48) The strongest discriminants between the breast cancer cells associated with statin sensitivity at the transcriptional level were the expression of the estrogen receptor and a gene set enriched for genes involved in the cholesterol biosynthesis pathway. Following statin treatment, the less-sensitive cells exhibited the classical response of up-regulating the expression of genes in the cholesterol biosynthesis pathway *via* the normal negative feedback loop resulting from the statin-induced inhibition of HMGCR. This classical response was, however, weaker in the sensitive cells, suggesting that these cells may possess an inherent defect in this pathway. This cholesterol biosynthesis gene set showed potential for identifying tumors that experience reduced proliferation upon statin treatment, albeit in a small cohort of patients. Further studies using similar and more advanced approaches are necessary to identify robust biomarkers for identifying patients most likely to derive benefit from the addition of cholesterol-lowering

medications to their therapeutic regimen for controlling breast cancer.

1.2 Background therapeutic information

Atorvastatin Mechanism of Action and Pharmacokinetics

Atorvastatin is a synthetic lipid-lowering agent, and not a pro-drug, but delivered in its active form as the calcium salt of the active hydroxyl acid at oral administration. Atorvastatin is a selective, competitive inhibitor of 3-hydroxy-3-methylglutharyl-coenzyme A reductase (HMGCR) that catalyzes the conversion of HMG-CoA to mevalonate, which is an early and rate-limiting step in the biosynthesis of cholesterol. Of importance for the abilities of atorvastatin to diffuse across membranes, the acid form consists of a lipophilic part and a more hydrophilic part. In humans, the acid form is converted to a lactone form, which is more lipophilic compared with the acid form. The formation of the active metabolites from the acid and the lactone is mediated through CYP3A4.

Atorvastatin is rapidly absorbed after oral administration; maximum plasma concentrations occur within 1 to 2 hours. Atorvastatin tablets are 95-99% bioavailable compared to solutions. The absolute bioavailability is around 14%, whereas the systemic bioavailability of HMGCR inhibitory effect is approximately 30%. The relatively low systemic bioavailability depends on the pre-systemic clearance in the gastrointestinal-tract and/or hepatic first-passage-metabolism. Mean distribution of atorvastatin is approximately 565 L. Atorvastatin is 98% bound to plasma proteins. Approximately 70% of circulating inhibitory activity for HMGCR is attributed to active metabolites. Elimination is predominantly via biliary excretion, whereas the renal route is less important. Mean plasma elimination half-life of atorvastatin is 7 hours, however, the elimination half-life of the inhibitory activity for HMGCR is 20 to 30 hours due to the contribution of longer-lived active metabolites. Atorvastatin can be administrated either as a morning or an evening dose. Apart from grapefruit, food is not considered to affect the extent of absorption. Grapefruit is a CYP3A4 inhibitor, and similarly, pharmaceutical CYP3A4 inhibitors, such as erythromycin and itraconazole can interact with atorvastatin.

Clinical studies, atorvastatin

In phase I trials the tolerated dose of atorvastatin for life-long treatment is soundly documented from cardiovascular studies and doses up to 80 mg per day are generally well tolerated. In phase II trials, atorvastatin is a well-documented drug and regularly used in humans, and large trials enrolling thousands of patients have demonstrated a high degree of safety and tolerability. The side effects of atorvastatin are few, with the most important being:

1) Hepatic effects raising serum transaminases, although not associated with other clinical signs or symptoms.

2) Muscle effects in terms of myopathy in conjunction with elevated creatinine phosphokinase values. Rhabdomyolysis has been reported in very rare cases, and renal dysfunction secondary to myoglobinuria has been reported.

Pharmaceutical Data, atorvastatin

Supplied: Atorvastatin retrieved from the hospital-pharmacy by the study nurse and provided to the patient.

Stability: Good.
Storage: Normal indoor temperature.
Solution Preparation: Tablets.
Route of Administration: Orally administrated.

Cholesterol-related side effects, aromatase inhibitors

In an adjuvant trial, hypercholesterolemia was reported in 52.3% of letrozole-treated patients and 28.6% of tamoxifen-treated patients. Grade 3-4 hypercholesterolemia was reported in 0.4% of letrozole patients and 0.1% of tamoxifen patients. Also, in the adjuvant setting, an increase of ≥1.5 X ULN in total cholesterol (generally non-fasting) was observed in patients on monotherapy who had baseline total serum cholesterol within the normal range (i.e., <=1.5 X ULN) in 151/1843 (8.2%) on letrozole

vs 57/1840 (3.2%). Lipid lowering medications were required for 25% of patients on letrozole and 16% on tamoxifen.

2 OBJECTIVES OF THE TRIAL

2.1 Primary objective

The primary objective of this trial is to determine the clinical efficacy of (neo)adjuvant atorvastatin as measured by invasive disease-free survival (IDFS) among patients with primary breast cancer.

2.2 Secondary objectives

Secondary objectives embrace investigating additional clinical endpoints including: pathological response (only neo-adjuvant treated patients), recurrence-free survival, distant-recurrence-free interval including associations with first site of recurrence, and overall survival and cardiac death-free interval as well as comorbidity, overall safety and patient reported outcome.

Additional translational objectives (described in the translational protocol part) include molecular biological predictors of (neo)adjuvant treatment - including atorvastatin - in breast cancer patients will be studied based on tumor tissue and circulating markers of response to atorvastatin will be correlated with the clinical response.

For patients already on cholesterol-lowering medication, who are included in the observational non-randomized cohort among which prospective data and biological samples are collected, the objective is to evaluate breast cancer prognosis among non-randomized breast cancer patients on cholesterol-lowering medication as "real world data" and include information on prevalent use.

2.3 Endpoints

Primary endpoint:

Invasive disease-free survival (IDFS), defined as the time from randomization until the date of the first occurrence of one of the following events:

- Ipsilateral invasive breast tumor recurrence: invasive breast cancer involving the same breast parenchyma as the original primary.
- Regional invasive breast cancer recurrence: Invasive breast cancer in the axilla, regional lymph nodes, chest wall, and skin of the ipsilateral breast.
- Distant recurrence: Metastatic disease-breast cancer that has either been biopsy confirmed or clinically diagnosed as recurrent invasive breast cancer.
- Death attributable to any cause, including breast cancer, non-breast cancer, or unknown cause.
- Contralateral invasive breast cancer.
- Second primary non-breast invasive cancer.

Secondary endpoints:

A. Pathological response (only neo-adjuvant treated patients)
B. Distant-recurrence free interval defined as time from inclusion to first distant recurrence including associations with first site of recurrence.
C. Recurrence-free interval including associations with first site of recurrence
D. Overall survival.
E. Overall safety.
F. Cardiac death-free interval. Cardiac death is defined as:
    a. Definitive cardiac death due to heart failure, myocardial infarction or documented primary arrhythmia.

b. Probable cardiac death defined as sudden, unexpected death within 24 hours of a definite or probable cardiac event (e.g., syncope, cardiac arrest, chest pain, infarction, arrhythmia) without documented etiology.

G. Co-morbidity incidence beyond cardiac events during follow-up including diagnoses such as diabetes mellitus.

H. Patient reported outcome.

I. Translational endpoints covering i.e.:

a. Baseline tumor- and circulating biomarkers predicting clinical response to atorvastatin

b. Alterations of circulating lipid-associated biomarkers (i.e. cholesterol, LDL, HDL, triglycerides, ApoA, and ApoB) and the association with clinical response to atorvastatin.

c. Alterations of circulating hormone biomarkers (*e.g.*, estradiol) and the association with clinical response to atorvastatin.

All statistical clinical endpoint analyses will be performed for the entire study population as well in exploratory analyses separately for:

- neoadjuvant and adjuvant treated patients in order to detect a potential differentiated effect of statin depending on the time of systemic therapy.

- patients receiving combined systemic therapy with chemotherapy, anti-HER2 therapy, and/or endocrine therapy AND endocrine-only treated patients.

- pre- and postmenopausal patients.

As for the randomized cohort, patients included in the observational cohort will be analyzed separately in supplemental analyses according to corresponding endpoints as specified above. In additional analyses, patients enrolled in the observational cohort will be analyzed as a third treatment arm together with the two randomized arms (atorvastatin treated, and placebo treated) and constitute information on the effects of statin use as "real world data".

3 PATIENT SELECTION CRITERIA

3.1 Inclusion criteria

- Women with primary, estrogen receptor positive breast cancer who are candidates for (neo)adjuvant systemic therapy OR have received ≤3 years of adjuvant endocrine therapy.
- Age > 18 years.
- Performance status of ECOG ≤ 2.
- Prior to patient registration, written informed consent must be given according to ICH/GCP, and national/local regulations.

3.2 Exclusion criteria

- History of any prior (ipsi- and/or contralateral) invasive breast carcinoma
- Prevalent cholesterol lowering therapy (statins, fibrates, ezetimibe, PCSK9 inhibitors). These patients can be enrolled in the observational cohort given fulfillment of other in- and exclusion criteria.
- Evidence of hepatic dysfunction (alanine aminotransferase level more than three times the upper limit of the normal range) or renal dysfunction (creatinine level more than three times the upper limit of the normal range).
- Predisposing factors for rhabdomyolysis, including hypothyroidism, reduced renal function, any muscle – or liver disease, or excessive alcohol consumption above 14 drinks/week AND creatine kinase (CK) measured to more than five times the upper limit (CK only measured in case of predisposing factors).

- Current medication with potent CYP3A4-inhibitors (e.g. ketokonazole, erythromycin) or gemfibrozile, ciclosporin or danazol.
- Pregnancy or breast-feeding (contraception according to clinical routines for premenopausal, fertile breast cancer patients, that includes non-hormonal contraception such as condom, vaginal diaphragm, or intrauterine device).
- Psychological, familial, sociological or geographical condition potentially hampering compliance with the study protocol and follow-up schedule; these conditions will be discussed with the patient before registration in the trial.
- History of allergic reactions attributed to compounds of similar chemical or biological composition to atorvastatin.

4 TRIAL DESIGN

A multi-center randomized 1:1 placebo-controlled, double-blind phase III trial investigating the effects of atorvastatin on breast cancer in the (neo)adjuvant setting.

5 THERAPEUTIC REGIMENS, EXPECTED TOXICITY, DOSE MODIFICATIONS, DRUG ADMINISTRATION

5.1 Treatment plan

5.1.1 Drug administration

STUDY MEDICATION (atorvastatin, oral administration, 80 mg daily (evening) OR placebo) two years of duration starting at study enrollment and for two years onwards.

The Glostrup pharmacy will be responsible for the following regarding medication:

1. Buy atorvatastin 80 mg (TEVA)
2. Produce the placebo medication
3. Create documentation for the Medical Product Agency (catalogue scheme)
4. Randomization list in collaboration with the DBCG secretary
5. Be responsible for labelling and packing (in glasses of 100 tablets/glas) of both active medication (atorvastatin 80 mg) and placebo
6. Distribution to all trial sites

Control of receiving of STUDY MEDICATION:

Glostrup Apotek ensures that the temperature range is adhered to when dispatching test drugs. This is ensured through the use of Distributor (BHS Logistics) who works under the rules of Good Distribution Practice. When dispatching medication, enclose a receipt for receipt stating the quantity delivered. The medication received is checked against this receipt, which is signed and returned to Glostrup Pharmacy. Furthermore, the medicine delivered must be compared with what has been ordered. This should be described in a short instruction, where everyone who receives the medicine must have completed this instruction, thus ensuring proper training in the function.

5.1.2 Premedication

No premedication will be required.

5.1.3 Patient monitoring

The patients will be monitored according to standard clinical routines. Renal function in terms of creatinine is analyzed at baseline and hepatic function will be measured at baseline and after 3, 12 and 24 months. In case of renal or hepatic dysfunction above the described limits measured in blood samples during monitoring period, extended monitoring will be initiated. See 5.1.4 for further information.

Adherence to STUDY MEDICATION is monitored and is registered in the MASTER folder in the DBCG CRF database.

5.1.4 Early stopping rules

- Raising levels of hepatic transaminases to three times the upper limit of normal will require discontinuation of STUDY MEDICATION therapy until normalized hepatic function has returned. Upon normalization, the STUDY MEDICATION can be re-induced.

- Intolerable adverse events deemed related to STUDY MEDICATION (answered "Probable" or "Certain" in CRF), will require discontinuation of STUDY MEDICATION. Upon normalization, the STUDY MEDICATION can be re-induced.

- Myopathy (muscle aching or muscle weakness in conjunction with increase in creatinine phosphokinase values greater than five times the upper limit of normal) will require permanent discontinuation of STUDY MEDICATION.

- Reduced performance status (ECOG>2) will require permanent discontinuation of STUDY MEDICATION.

In case of early stopping of STUDY MEDICATION, all other procedures will continue apart from STUDY MEDICATION. It should be registered in the CRF folder "Master End of Trial" if the patient stops medication (End of treatment) and if the patients for any reasons stops participating in this study (End of trial).

5.1.5 Duration of therapy

All patients will receive atorvastatin or placebo for two years with the exception mentioned in section 5.1.4 or in case of discontinuation requested by the patient. Atorvastatin is a well-documented drug prescribed for continuous use over several years and intercurrent illness would not indicate discontinuation of atorvastatin, unless symptoms are related to those mentioned in section 5.1.4.

5.1.6 Concomitant therapy

Concomitant drugs during atorvastatin therapy are permitted.

Caution should be taken in case of:

● Digoxin treated patients should be monitored as co-administration of atorvastatin and digoxin has been shown to increase steady-state plasma digoxin concentrations by approximately 20%. Among digoxin-treated patients, plasma digoxin will be measured at baseline, after 1 week and after 1 month on STUDY MEDICATION and an increase of ≥ 20% at consequently will lead to a reduction in dosage of digoxin.
For patients on digoxin, a note to the physician prescribing digoxin should be sent indicating that the patient participates in a randomized trial with either atorvastatin 80 mg or placebo.

● Daily intake of grapefruit juice should be avoided during atorvastatin therapy due to inhibition of the CYP450 system by which atorvastatin is metabolized.

● CYP3A4 inhibitors such as macrolide antibiotics (erythromycin), immunosuppressants (cyclosporine), azole antifungal agents (i.e. itraconazole, ketoconazole), and some antidepressants (i.e. nefazodone) should if possible be avoided during atorvastatin therapy as they may have a potential of increasing plasma concentrations of atorvastatin.

6 CLINICAL EVALUATION, LABORATORY TESTS, FOLLOW-UP

6.1 Before treatment start

Before treatment start the patient´s eligibility should be evaluated which will include:

● Social and psychological conditions according to section 3.

● Medical history and clinical examination.

● Blood samples (hepatic (ALT) and renal (creatinine) parameters, (creatine phosphokinase if predisposing factors, including hypothyroidism, reduced renal function, any muscle – or liver disease, and excessive alcohol consumption)).

Case Report Forms (CRF) in the MASTER folder in the DBCG system will be filled in at baseline.

6.2 Observational cohort

Patients that are not able to participate in the randomized placebo-controlled part of the trial due to prevalent cholesterol lowering therapy (statins, fibrates, ezetimibe, PCSK9 inhibitors), but otherwise fulfil in- and exclusion criteria can be included in the observational cohort. Patients can participate in the observational cohort regardless of the dose of cholesterol lowering therapy. In this cohort no randomization or medical intervention is relevant, otherwise these patients will with a few exceptions follow the study schedule in accordance with the randomized patients.

Adverse events will be registered in the Master folder in the DBCG CRF according to the schedule above. However, SAEs are not to be reported for patients in the observational cohort.

In case the patient terminates cholesterol-lowering medication for any reason, the patient is still followed according to the trial protocol. It should be registered in the CRF folder "Master End of Trial" if the patient stops medication (End of treatment) and if the patients for any reasons stops participating in this study (End of trial).

6.3 During treatment

Adverse events will be registered in the MASTER folder in the DBCG CRF. Herein, protocol compliance will also be assessed and reported.

In addition, patients are instructed to report information addressing patient reported outcome including compliance to STUDY MEDICATION into a REDCap research database at the time points specified below.

Blood samples assessing renal function at baseline and hepatic function at baseline and at the 3, 12, and 24-month visits are collected. Further therapeutic decisions are based on section 5.1.4.

6.4 After the end of treatment (follow-up)

The follow-up includes assessment of clinical events (recurrences) and overall survival at a yearly basis up to 10 years after inclusion in the study. Patients are followed in the clinic according to the time points for clinical routine visits (up to 10 years depending on menopausal status). Clinical events are recorded at a yearly basis into the DBCG CRF database.

To ensure detection of potential long-term effects and side effects of STUDY TREATMENT, linkage to medical records and the national cancer - and death registries up to 20 years after diagnosis will facilitate long-term follow-up and detection of late recurrences and other secondary cancers. Other possible late side effects will be identified through linkage to the Hospitalization Registry.

Importantly, patient reported outcomes are to be reported yearly through a RedCap-based model up to 10 years after study inclusion.

6.5 Summary table 1

Study procedures for the randomized cohort described in the following table:

Required Investigations	Baseline (T1)	3 months (+/- 14 days) (T2)	6 months (+/- 14 days) (T3)	1 year (+/- 14 days) (T4)	2 years (+/- 14 days) (T5)	Yearly, (+/- 28 days) 3-10 years (T6)
Inclusion - exclusion criteria (DBCG)	X					
Informed consent	X					
Randomization (DBCG)	X					
CRF Master flow (DBCG)	X	X[3]	x[34]	x[34]	x[34]	X[4]
CRF Master AE (DBCG)	X	X[34]	X[3]	X[34]	X[3]	
STUDY MEDICATION START/ADHERENCE (DBCG)	X	X	X	X	X	
Patient Reported Outcome (RedCap)	X	X	X	X	X	X
Pathological response (Only patients in neo-adjuvant cohort)			X			
HCG[5](serum or urine)	X					
Hepatic (ALT) function	X[2]	X		X	X	
Renal (creatinine) function	X[2]					
Creatine kinase[1]	(X)					
Translational blood samples	X		(X neoadjuvant cohort patients)	X	X	
Translational tumor tissue samples from existing tissue	X		(X neoadjuvant cohort patients)			

1. Predisposing factors for rhabdomyolysis, including hypothyroidism, reduced renal function, any muscle – or liver disease, or excessive alcohol consumption above 14 drinks/week AND creatine kinase (CK) measured to more than five times the upper limit (CK only measured in case of predisposing factors).

2. Blood samples are allowed to be taken up to 28 days before T1

3. Either done by phone interview or attendance at the clinic

4. Data collected from the patient file

5. Only fertile women

6.6 Summary table 2

Study procedures for the observational cohort are described in the following table:

Required Investigations	Baseline (T1)	3 months (+/- 14 days) (T2)	6 months (+/- 14 days) (T3)	1 year (+/- 14 days) (T4)	2 years (+/- 14 days) (T5)	3-10 years (+/- 28 days) (T6)
Inclusion-exclusion criteria (DBCG)	X					
Informed consent	X					
Registration (DBCG)	X					
CRF Master Flow (DBCG)	X	X[2]	X[2]	X[2]	X[2]	X[3]
CRF Master AE (DBCG)	X	X[2]	X[2]	X[2]	X[2]	
Patient reported outcome (REDCap)	X	X	X	X	X	X
Hepatic function (ALT)	X[1]					
Renal function (creatinine)	X[1]					
Translational blood samples	X			X	X	
Translational tumor tissue samples from existing tissue	X					

1. Blood samples are allowed to be taken up to 28 days before T1
2. Either done by phone interview or attendance at the clinic
3. Data collected from the patient file

7 CRITERIA OF EVALUATION

Evaluable for toxicity

All patients will be evaluable for toxicity from the time of their initiated treatment with atorvastatin (T1) and until discontinued treatment (T5).

Evaluable for response

All eligible patients will be included in the response rate calculation. The subset that will be assigned a response category (see definitions below) are all patients who have received two years of minimum 80% of full dosage atorvastatin.

Response and Evaluation Endpoints

- Response according to primary endpoint:

Atorvastatin response according to invasive disease-free-survival (IDFS) will be assigned significant in case of a significant improved IDFS. Recurrences cover all invasive recurrences.

- Response according to secondary endpoints:

- Clinical survival endpoints will be evaluated by follow-up within the trial and annual linkage to the national death registry.

All statistical analyses will be performed for the entire study population as well as in exploratory analyses separately as stated in section 2.3 order to detect a potential differentiated effect of atorvastatin depending on the clinical setting.

- Translational endpoints are described in section 10.

- Patients reported outcome are evaluated and related to differences between treatment arms in incidence of recorded symptoms.

8 STATISTICAL CONSIDERATIONS

8.1 Statistical design

8.1.1 Sample size

Design

A multi-center randomized placebo-controlled double-blind phase III trial investigating the effects of atorvastatin on breast cancer in the (neo)adjuvant setting.

Sample size

The sample size calculations are based on estimates of 10-year IDFS with an annual hazard rate of 0.0223. A sufficient number of patients is sought to provide 80% power using a two-sided 0.05-level test to detect a 25% reduction in the risk of an IDFS event (hazard ratio=0.75). A total of 379 events are required. With an estimated 5% drop-out at 5 years (annual rate 0.0103), this is estimated to be achieved by a total recruitment of 3,360 participants and a 6½ years follow-up. The sample size estimate is based on inclusion for 3 years, a median follow-up of 6½ year, that is 5 years of additional follow-up after the 3 years of inclusion.

Hypothesis

H0: (Neo)adjuvant atorvastatin treatment has no impact on invasive disease-free survival for breast cancer patients and survival rates do not differ between randomized groups; atorvastatin versus placebo.

Ha: Atorvastatin treatment influence invasive disease-free survival in breast cancer.

8.1.2 Randomization and stratification

Patients will be randomized electronically in a 1:1 manner through the DBCG CRF system. Access to randomize patients will require eligibility according to recorded in- and exclusion criteria. Randomization will be based on stratification for 1) trial site, 2) clinical setting (neoadjuvant/adjuvant early entry/adjuvant late entry).

8.2 Statistical analysis

Statistical and Analytical Plan

This is a phase III placebo-controlled adjuvant study to investigate the efficacy of atorvastatin in patients with operable early breast cancer. The protocol will accrue 3,360 patients all together randomized in a 1:1 ratio. The treatment phase will be two years followed by an observational phase of 6½ years follow-up for the initial data analysis. Patients will be followed in the clinic according to the current clinical guidelines in Denmark, and in addition patients will be asked to reply to questions regarding patient reported outcome in a RedCap-based questionnaire until year 10, please see Table 1. Follow-up for clinical events will be possible through clinical visits, medical records and the DBCG database, which will allow for detection of early as well as late recurrences, and long-term analysis executed as for 10-, 15- and 20-year survival rates. The data will be stored for 25 years after termination of the study.

A recurrence will be defined as any invasive breast cancer recurrence irrespective of localization. Information on overall survival will be retrieved by linkage to the national death registry.

Invasive disease-free survival among treatment groups will be analyzed in crude analysis using the Kaplan-Meier and Log-Rank test as well as the Cox regression hazards analysis with the latter allowing for confounder-controlled multivariable analysis.

Study Design

Significance level and power

The sample size calculations are based on estimates of 10-year IDFS with an annual hazard rate of 0.0223. A sufficient number of patients is sought to provide 80% power using a two-sided 0.05-level test to detect a 25% reduction in the risk of an IDFS event (hazard ratio=0.75). A total of 379 events are required. With an estimated 5% drop-out at 5 years (annual rate 0.0103), this can be achieved by a total recruitment of 3,360 participants and a 6½ years follow-up. The sample size estimate is based on inclusion for 3 years, a median follow-up of 6½ year, that is 5 years of additional follow-up after the 3 years of inclusion.

Accrual and Duration of Study

The study was initiated in January 2021. The estimated nation-wide accrual rate is 90 patients per month. Thus, patient accrual is expectedly completed within 4 years. Treatment duration time adds two years and the initial observational period adds another 5 years. In total, the time frame from study start to 5-year recurrence data is estimated to 10 years.

Given the bulk of patients with ongoing endocrine therapy who are eligible for the late entry stratum of the trial, this will be very pronounced within the beginning of the trial accrual.

All of the patients registered in the study will be accounted for besides patients already on a cholesterol-lowering drug who are enrolled in the observational cohort. The number of patients who were not evaluable, or who died or withdrew before treatment with STUDY MEDICATION began, will be specified. The distribution of follow-up time will be described, and the number of patients lost to follow-up will be provided.

Safety Monitoring

Adverse events will be recorded in the MASTER trial CRF in the DBCG database and monitored on an ongoing basis during active treatment and their frequencies reported to the sponsor equal to the coordinating national trial site, Department of Oncology, Aarhus University Hospital. Toxic effects will be categorized using a modified version of the NCI Common Terminology Criteria for Adverse Events, Version 5.0.

For reporting of adverse events, please see protocol chapter 14.

9 PHARMACOKINETICS

Atorvastatin is a well-documented drug and additional pharmacokinetics will not be performed within this trial.

10 TRANSLATIONAL RESEARCH

Translational research will be performed in co-operation with the laboratory units at the trial sites participating in this protocol.

Tumor samples will be treated and stored according to current research ethical practice in Denmark in a research biobank assigned for this trial. Blood samples of a total of 30 ml/time are drawn at three time points: at baseline and at 1 and 2 years after inclusion. And for patients in neo-adjuvant cohort also a

blood sample at 6 months. Frozen blood samples will be stored in a research biobank at the Danish Cancer Biobank. Potential left-over blood after the analyses for this study, are stored in the research biobank in the Danish Cancer Biobank for future, not yet specified, research.
Pathological samples are stored according to clinical routines at the Departments of Pathology affiliated with the trial sites although one tissue block is set aside for the MASTER study, and upon completion of trial enrollment, triplet sample cores of 1 mm in diameter are arranged in a tissue microarray (TMA) and stored in a research biobank at the DBCG biobank for future research (RH-2015-122, I-Suite nr: 03903). The biological samples will be used for analyses in relation to the following translational endpoints:

- Tumor tissue sampled at baseline (for neoadjuvant patients both at diagnosis and at surgery) will be analysed for biomarkers of prognosis and treatment prediction; i.e. HMGCR, LDL-R expression along with HMGCR down-stream products in the mevalonate pathway (e.g. farnesyl-pyrophosphatase, geranylgeranyl-pyrophosphatase and phosphorylated-ERK, the latter indicating an activated Ras pathway).

Serum, plasma and whole-blood collected at baseline and during follow-up will be analyzed for prognostic and predictive markers, i.e. LDL, HDL, cholesterol, triglycerides, Apo-A, and Apo-B, HMGCR SNP's, circulating antibodies and circulating tumor-DNA (ctDNA), the latter to monitor the burden of circulating ctDNA according to treatment response as well as to investigate specific mutations for their potential treatment predictive value, i.e. TP53 mutations.

The data center for the translational parts of this trial is housed at the Department of Oncology, Aarhus University.

11 INVESTIGATOR AUTHORIZATION PROCEDURE

This is a multi-center trial and investigator authorization procedures are limited to the sponsor, the national PI and delegated investigators of this trial. The trial will be initiated following the approval of the regional ethical committee and medical products agency.

12 PATIENT REGISTRATION AND RANDOMIZATION

Patients eligible for the trial are offered inclusion into the study at the Departments of Oncology according to this schedule:

1) Neo. adjuvant behandling

Ved behandling med EC-TAX

Cohort:	Early Neo	Early adj.			Late adj.	Ingen inkl.
Behandling:	EC	TAX	Operation	Til og med mdr. 3	Mdr. 4 til og med år 3	År 4+5
				Endokrin behandling		

Ved behandling med TAX-EC

Cohort:	Early Neo	Early adj.			Late adj.	Ingen inkl.
Behandling:	TAX	EC	Operation	Til og med mdr. 3	Mdr. 4 til og med år 3	År 4+5
				Endokrin behandling		

2) Adjuvant behandling med kemoterapi

Ved behandling med EC-TAX

Cohort:		Early adj.	Late adj.			Ingen inkl.
Behandling:	Operation	EC	TAX	Til og med mdr. 3	Mdr. 4 til og med år 3	År 4+5
				Endokrin behandling		

Ved behandling med TAX-EC

Cohort:		Early adj.	Late adj.			Ingen inkl.
Behandling:	Operation	TAX	EC	Til og med mdr. 3	Mdr. 4 til og med år 3	År 4+5
				Endokrin behandling		

Ved behandling med DC

Cohort:		Early adj.	Late adj.			Ingen inkl.
Behandling:	Operation	Første 3 serier DC	Sidste 3 serier DC	Til og med mdr. 3	Mdr. 4 til og med år 3	År 4+5
				Endokrin behandling		

3) Adjuvant behandling uden kemoterapi

Cohort:		Early adj.	Late adj.	Ingen inkl.
Behandling:	Operation	Til og med mdr. 3	Mdr. 4 til og med år 3	År 4+5
		Endokrin behandling		

The patient will receive oral and written information about the trial. Given a positive interest for participation in the trial, the patient will be offered time to consider participation prior to signing the informed consent. After signing, registration of in- and exclusion criteria in the DBCG database will be done, the randomization is performed, blood samples are taken, and STUDY MEDICATION provided to the patient along with practical information regarding RedCap access.

13 FORMS AND PROCEDURES FOR COLLECTING DATA

13.1 Case report forms and schedule for completion

All clinical data including adverse and clinical events are collected from clinical visits/medical records and arranged in the nation-wide DBCG CRF database according to Table 1. The DBCG CRF system is well-established in clinical routine for all breast cancer patients in Denmark, and collects data on imaging, pathology, surgery, and oncological treatment as well as follow-up data. In addition to the standard clinical data, which is also part of the trial data, there will be an added folder in the DBCG CRF for specific trial data not covered by the standard clinical data, i.e. adverse events and compliance regarding STUDY MEDICATION. Potential adverse events are evaluated at the clinical visits and reported into the DBCG CRF.

Patient reported outcomes (PRO) are sampled from RedCap-questionnaires (based on PRO-CTCAE, version 1.0, Danish) for which a separate link is sent out via email corresponding to each of the time points stated in Table 1, and patient replies are gathered in a RedCap database hosted at Aarhus University and subsequently copied into the DBCG database. The emails are sent automatically from the RedCap database system to the email address the patient has stated at the informed consent. Upon end of trial inclusion, data from patient reported outcome are copied into the DBCG database. Patients will be clearly informed that the PRO-data are collected for evaluation upon completion of study enrolment, which implies that the reported outcomes are not evaluated during the trial and thus, all potential AE´s are to be reported to their oncological doctor, who will decide on potential further actions.

Results from the translational blood tests and tumor biological data will subsequently be added into the MASTER trial database folder within the DBCG database.

The database will be stored at the data center at DBCG. Access to the full MASTER database will be limited to the national PI (sponsor), the steering committee, delegated investigators, trial nurses, and delegated monitors.

13.2 Data flow

Forms evaluating patient data including adverse events, blood samples, as well as pathological and translational parameters are handled by the PI, steering committee, delegated investigators, and site-specific trial nurses. In the case of missing or inconsistent data updating on data can only be required by the investigators, the steering committee, or trial nurses.

14 ADVERSE EVENTS

14.1 Definitions

An **Adverse Event (AE)** is defined as any untoward medical occurrence or experience in a patient or clinical investigation subject which occurs following the administration of the STUDY MEDICATION regardless of the dose or causal relationship. This can include any unfavorable and unintended signs (such as rash or enlarged liver), or symptoms (such as nausea or chest pain), an abnormal laboratory finding (including blood tests, x-rays or scans) or a disease temporarily associated with the use of the protocol treatment.

Common side effects (affects 1 to 10 users in 100) include:

- inflammation of the nasal passages, pain in the throat, nose bleed
- allergic reactions
- increases in blood sugar levels
- increase in blood creatine kinase
- headache
- nausea, constipation, wind, indigestion, diarrhea
- joint pain, muscle pain and back pain
- blood test results that show your liver function can become abnormal

Side effects are registered in the MASTER folder in the DBCG CRF database.

An **Adverse Drug Reaction (ADR)** is defined as any response to a medical product, that is noxious and/or unexpected, related to any dose.

> **Response to a medicinal product** (used in the above definition) means that a causal relationship between the medicinal product and the adverse event is at least a reasonable possibility, *i.e.* the relationship cannot be ruled out.

An **Unexpected Adverse Drug Reaction** is any adverse reaction for which the nature or severity is not consistent with the applicable product information (*e.g.*, Investigators' Brochure).

A **Serious Adverse Event (SAE)** is defined as any undesirable experience occurring to a patient, whether or not considered related to the protocol treatment. A Serious Adverse Event (SAE) which is considered related to the protocol treatment is defined as a **Serious Adverse Drug Reaction (SADR)**. The product resume for atorvastatin (TEVA) is the reference document used for evaluation of whether an SAE is expected or unexpected and thus may be classified as a Suspected Unexpected Serious Adverse Reaction (SUSAR).

Adverse events and adverse drug reactions which are considered as **serious** are those which result in:

- death
- a life-threatening event (i.e. the patient was at immediate risk of death at the time the reaction was observed)
- hospitalization or prolongation of hospitalization
- persistent or significant disability/incapacity
- events that lead to congenital malformation
- any other medically important condition (i.e. important adverse reactions that are not immediately life threatening or do not result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed above).

Events not to be reported as SAE

Due to the severity of the primary disease and the standard treatment hereof in patients participating in this trial, a number of events, where SAE reporting can be omitted, have been defined.
The following will not be considered for SAE in this study:
An event resulting in hospitalization or prolongation of hospitalization, if the only reason for hospitalization or prolongation are as follows:
Hospitalization secondary to breast surgery or expected chemotherapy toxicity such as:

- Fever
- Nausea and vomiting
- Neutropenia

Hospitalization secondary to expected cancer morbidity such as:

- DVT and pulmonary embolism
- Electrolyte disturbances
- Pain treatment
- Anxiety
- Reference to any (neo)adjuvant treatment and procedures
- Reference to chemotherapy
- Operation for IV access (ex. Port-a-cath)

The above events can be recorded in the adverse event CRF in the DBCG database.

Guidelines for connection between adverse events and treatment

> The causal relationship between one of the test drugs and the adverse event judged by the investigator as either YES (related) or NO (not related). If there is a reasonable suspicion of a causal relationship to the study drug, i.e. that there is data (evidence) or arguments to suggest a causal link, the causal relationship between the drug and the incident is

assessed as YES. The criteria for evaluation of the correlation between drug and event will include consideration of potential interactions between medications in the regimen and the drugs, which the patient is also treated with.

The following criteria must be considered in the assessment of causality as YES:
• Reasonable temporal association with administration of the drug.
• It might be caused by the patient's clinical condition, environmental or toxic factors or other treatments that patients receive.
• Known response pattern of the suspected STUDY MEDICATION.
• Loss or decrease upon discontinuation of STUDY MEDICATION.
• Returns on resumption of STUDY MEDICATION.

The following criteria must be considered in the assessment of causality as NO:
• It does not follow a reasonable temporal sequence from administration of the drug.
• It can easily be caused by the patient's clinical condition, environmental or toxic factors or other treatments that patients receive.
• It does not follow a known pattern of response to the STUDY MEDICATION.
• It does not return or become worse when the STUDY MEDICATION is administered again.

14.2 Reporting procedure

Any SAE occurring from start of STUDY MEDICATION until 30 days after termination of STUDY MEDICATION should be reported to the sponsor center, Department of Oncology, Aarhus University Hospital, within 24 hours from the time the investigator became aware of the event (expedited reporting). A template for reporting serious adverse events (SAE) is provided all trial sites and upon an SAE, the filled SAE template is to be email to the sponsor site (SAE@auh.rm.dk). The SAE is implemented into the database and reported according to national regulations. Related SAEs MUST be recorded and reported, regardless of the time elapsed between the last study drug administration, even if the trial is completed. Non-related SAEs occurring during the trial until 28 days after the last dose of STUDY MEDICATION must be recorded and reported. Related SAEs are to be recorded and reported up to 24 months after completion of STUDY MEDICATION. Sponsor will report serious adverse and/or unexpected serious and suspected adverse reactions (SUSARs) as needed to investigators, the Medical Products Agency and the ethics committee in accordance with the relevant legal requirements and ICH's guidelines for Good Clinical Practice (GCP). Hereof, SUSARs, which are deadly or life threatening, are to be registered and reported to the Medical Product Agency as quickly as possible and at the latest 7 days after sponsor has been aware of such a presumed adverse event. All other SUSARs are to be reported to the Medical Product Agency later than 15 days after the sponsor has been notified.

Unblinding: In case an emergency in which unblinding of STUDY MEDICATION is deemed necessary, the procedure is as follows:
1. The patient, relatives or health personnel will find and can call the phone number stated at the trial specific card, which the participants receive upon inclusion, indicating the phone number to the Department of Oncology at their specific site, i.e. for Aarhus University Hospital:

Patienten deltager i et videnskabeligt forsøg:
KFE-XXXX – KALDENAVN
Forsøgets titel: xxxxxxxxx
Forsøgsansvarlig læge: xxxlæge xxxxx xxxxx

Inden for 24 timer skal Klinisk Forsknings Enhed have besked om indlæggelsen på e-mail SAE@auh.rm.dk.

Vagthavende på Kræftafdelingen: Tlf. 7845 0000

2. Each site has staff at the hospital, who can unblind patients immediately, and each site must make a local, written procedure for unblinding.

Annual Safety Report: On a yearly basis during the trial, it is the responsibility of the sponsor to develop a list of all serious adverse events (SAEs), which have occurred during the trial period, together with a report of the trial participants safety.

15 QUALITY ASSURANCE

15.1 Control of data consistency

Trial data are collected and stored in a uniform way during the years of presumed patient enrollment as well as the time following enrollment. The internal consistency will be ensured by the regulations of each site-specific hospital. Monitoring of trial data is performed according to the monitoring plan developed in collaboration with the GCP-units for all participating trial sites.

15.2 Central review of pathology

All clinical pathological assessments will be performed according to local standard procedures. Submission to external investigators is not expected to be necessary.

15.3 Other central review procedures

Blood samples are reviewed according to standard clinical practice. Blood samples collected for the translational research parts will be stored at each site. When enrollment is completed the samples will be centrally stored at the biobank, Department of Oncology, Aarhus University Hospital.

16 ETHICAL CONSIDERATIONS

• Potential advantages

- (Neo)adjuvant therapy with atorvastatin might improve invasive disease-free and overall survival following breast cancer.

- Participating patients on atorvastatin may gain improved cardio-vascular parameters.

• Potential disadvantages:

- The participating patients will not be eligible for other (neo)adjuvant systemic intervention trials during treatment with STUDY MEDICATION.

- The participating patients on atorvastatin might experience side effects.

- Additional venous blood tests will be taken.

16.1 Patient protection

The DBCG, the national PI (sponsor), the steering committee, and delegated investigators will ensure that this trial is conducted in agreement with either the Declaration of Helsinki and the laws and regulations of Denmark, whichever provides the greatest protection of the patient.

The protocol has been written, and the study will be conducted according to the ICH Harmonized Tripartite Guideline for Good Clinical Practice (ref: http://www.ifpma.org/pdfifpma/e6.pdf).

The protocol is sent for approval by the Ethics Committee in Denmark.

The project adheres to the Danish Data Protection Law and the Danish Data Protection Regulation.

The project will be registered at the internal list of research projects in Region Midt.

16.2 Subject identification

All patients included in the trial are provided with a study number. The link between study number and id number will be stored at a central computer at the datacenter (DBCG).

16.3 Procedure of inclusion and informed consent

Information about the trial to a patient can be done by either a doctor, medicine student or nurse. All must be delegated to the task by sponsor or PI at sub-sites.

It must always be a medical doctor that evaluates if the patient is eligible for the study by assessing if the patient fulfills in- and exclusions criteria.

All patients will be informed of the aims of the study, the possible adverse events, the procedures and possible hazards to which she will be exposed, and the mechanism of treatment allocation. Patients will be informed as to the strict confidentiality of their patient data, but that their medical records and other source data may be reviewed for trial purposes by authorized individuals other than their treating physician. This includes monitors from affiliated GCP-units, the Medical Product Agency and other relevant health authorities.

It is the responsibility of the individual investigator to ensure patient comprehension of the enclosed informed consent document. The consent will be dated- and version controlled. If informed consent is provided by others than the site-specific investigator, it is required that the informing person is familiar with Good Clinical Practice and is familiar with breast cancer. Should the patient require an assessor in association with the information, this will be ensured through the clinical trial unit at the current site. Patients will be offered reflection time after information has be given and prior to signing the informed consent, and if needed a follow-up visit can be scheduled up to a week after information has be given.

The informed consent form is part of the documents to be submitted to the ethics committee for approval.

It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever she wants. This will not prejudice the patient's subsequent care. Documented informed consent must be obtained for all patients included in the study before they are registered in the study and any study specific actions are taken. This will be done in accordance with the national and local regulatory requirements.

The written informed consent form should be signed and personally dated by the patient or by the patient's legally acceptable representative.

17 ADMINISTRATIVE RESPONSIBILITIES

Coordinating PI/sponsor: Signe Borgquist, Department of Oncology, Aarhus University Hospital

Steering Board: Bent Ejlertsen, Peer Christiansen, Maj-Britt Raaby Jensen, Marianne Ewertz, Anders Bonde Jensen, Deirdre Cronin-Fenton, Thomas Ahern, Signe Borgquist

Trial Committee: consists of the sponsor, the steering board, and all principal investigators from participating centers.

18 TRIAL SPONSORSHIP AND FINANCING

The trial is not commercially sponsored. Financing is provided by a research grant from The NOVO Nordisk Foundation of 10.977.175 DKK. The grant is used to cover trial-related expenses such as placebo medication and other pharmacy expenses, as well as expenses for the trial units involved, and administrative and statistical support. The grant is paid to the Aarhus University Hospital (PI of the grant is sponsor Signe Borgquist) and managed by Aarhus University Hospital. None of the researchers affiliated to the trial has any association to the funder.

19 TRIAL INSURANCE

Patients enrolled in this clinical trial will be covered by the usual patient insurance.

20 PUBLICATION POLICY

The main trial results will be submitted for publication within twelve months following end of follow-up as described above. Both positive, negative and inconclusive results will be submitted for publication. The lead- and last authorship of the main publication will be shared within the steering board, and active co-investigators will be co-authors. Additional and translational results shall not be published until the main results of the trial have been published. Projects and publications based on this trial are to be approved by the steering board who will also be considered authors on future publications derived from the trial. The DBCG and trial nurses will be acknowledged in all publications. Trial specific funding is acknowledged in all publications.

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