The NEOLETEXE trial: a neoadjuvant cross-over study exploring the lack of cross resistance between aromatase inhibitors

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The aromatase inhibitor letrozole (Femar®/Femara®) and the aromatase inactivator exemestane (Aromasin®) differ in their biochemical effect on the aromatase enzyme. Letrozole is a competitive aromatase inhibitor while exemestane binds irreversibly to the aromatase enzyme. This pharmacological difference is of clinical interest since a lack of cross-resistance has been documented. It has been demonstrated in several clinical trials that exemestane may cause a disease regression following resistance to nonsteroidal aromatase inhibitors. The exact mechanism(s) behind this phenomenon is yet unknown. Here, we present the NEOLETEXE trial with the aim of exploring the individual mechanisms involved behind the observed lack of cross resistance. Clinical trial registration: The trial has been approved by the Regional Ethics Committee of South-East Norway (project number 2015/84).

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Breast cancer is one of the most common cancer types and the leading cause of cancer-related deaths among women on a global basis [1,2]. Several decades of clinical and basic research have increased our understanding of the heterogeneity of the disease and treatment options have been directed accordingly. Endocrine treatment is vital since about 70% of all breast cancers are hormone-sensitive due to the expression of hormone receptors making the use of antihormonal treatment possible [3–5].

The ‘third-generation’ aromatase inhibitors (letrozole, anastrozole and exemestane) are currently the standard treatment option for postmenopausal women with estrogen receptor positive breast cancer in all stages of the disease, as they have been shown to suppress plasma and tissue estradiol levels by >90% in vivo [6–11].

While the triazoles letrozole and anastrozole belong to the class of nonsteroidal aromatase inhibitors, exemestane belongs to a different entity, called steroidal aromatase-inactivators (Figure 1) [11–13]. The two subgroups of aromatase inhibitors differ in their mode of actions on the aromatase enzyme. Letrozole and anastrozole bind competitively and reversibly, with respect to the androgen substrate, to the heme-containing active site of the aromatase complex, while exemestane is a mechanism-based inactivator and binds irreversibly to the active site of the enzyme (Figure 2A–C) [12–14]. These fundamental differences are of clinical interest as a ‘lack of cross-resistance’ between nonsteroidal aromatase inhibitors and steroidal inactivators has been recognized for some time and is documented by several
clinical trials (Table 1) [15–21], providing the rationale of sequential use of these drugs in the metastatic setting (e.g., use of exemestane following progression on treatment with a nonsteroidal compound) [22]. Despite earlier comprehensive research, the mechanism behind the observed lack of cross-resistance is still unknown [23]. It has also been suggested that a detailed understanding of this clinical phenomenon may in fact provide a new way of treating hormone-sensitive breast cancer [24].

In this neoadjuvant study, we will focus on intratumor mechanisms of adaption to letrozole and exemestane, in other words, basic information on aromatase expression and regulation in individual breast cancer patients treated with both drugs in a randomized and cross-over designed sequence. We believe that this trial will contribute to our basic understanding of these two very important anticancer drugs used by millions of women with breast cancer in the different clinical stages of the disease.

**The NEOLETEXE trial**

**Background & rationale for neoadjuvant endocrine therapy in patients with locally advanced breast cancer**
Locally advanced breast cancer is generally defined as either T3-T4 and/or N2-3 primary breast cancer [25,26]. These tumors are considered to be primarily inoperable, and in need of presurgical therapy [27,28]. The rationale for presurgical therapy is to gain local tumor control, thus avoiding complications which may appear in the long run such as bleeding, wound development and exudation. In addition, presurgical therapy would transform a nonoperable situation into an operable one [29].

Postmenopausal patients with ER-positive locally advanced breast cancer may benefit from primary endocrine therapy as it has been shown to be nearly as effective as standard neoadjuvant chemotherapy [30]. Aromatase inhibitors of the third generation (letrozole, anastrozole and exemestane) are currently the preferable drugs when...
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Clinical Trial Protocol

Figure 2. Interaction of androstenedione and different classes of aromatase inhibitors with the CYP19 complex. (A) Conversion of androstenedione to estrone (untreated situation). (B) Interaction of the steroidal aromatase inactivator exemestane with the aromatase complex. (C) Interaction of the nonsteroidal aromatase inhibitor letrozole with the aromatase complex.

HEME: Heme-containing active site of the aromatase complex; HP: Hydrophobic pocket; NADP: Nicotinamide adenine dinucleotide phosphate; NADPH: Reduced form of NADP; SBS: Substrate binding site.
Box 1. General inclusion and exclusion criteria (NEOLETEXE-trial).

1. Inclusion criteria
   (A) Patients diagnosed with locally advanced primary breast cancer (defined by T3-T4 and/or N2-N3 status)
   (B) "Large T2 tumors"; primary tumors above 4 cm in diameter
   (C) Postmenopausal status: age above 55 years or age above 50 years and at least 2 years of amenorrhea in addition to LH-, FSH- and plasma estradiol levels in the postmenopausal range
   (D) Estrogen receptor positive status (at least 10% of cancer cells)
   (E) HER-2 negative status
   (F) No or distant limited distant metastasis

2. Exclusion criteria
   (A) Triple-negative breast cancer
   (B) HER-2 positive breast cancer
   (C) Life-threatening metastasis (advanced visceral metastasis, brain-metastasis, elevated liver enzymes; defined as above three-times above normal values)
   (D) Any previous therapy for breast cancer within the last 12 months
   (E) Treatment with drugs that may interfere with endocrine therapy of breast cancer

neoadjuvant endocrine treatment in postmenopausal women is considered [31]. In addition to the clinical effects as standard care for breast cancer patients, neoadjuvant therapy is widely used to study the endocrinology of breast cancer in general [32,33]. It is recognized as one of the best model systems to predict responses in other clinical settings (early or metastatic breast cancer) allowing for adjustments to be made at a relatively early stage [34].

Due to the large interpatient variation of hormone-dependence and other factors, an inpatient cross-over study is a good model to study different effects of these compounds in a single patient allowing all patients to function as their own controls for selected comparisons. This concept has previously been used successfully to compare anastrozole and letrozole in breast cancer [6]. In this study, we will mainly focus on intratumor mechanisms of adaption to letrozole and exemestane studying the direct effects of the two compounds on tumor biology.

Study design
The NEOLETEXE trial is a neoadjuvant, randomized, open-label, inpatient and cross-over single center clinical trial.

Primary & secondary objectives
The main scientific objective of this trial is to explore the potential mechanisms of adaption and resistance to the two chosen endocrine treatment options in sequence. To reach this goal and to explore significant differences between letrozole and exemestane in vivo, we plan to measure the total estrogenic activity in blood samples using the AroER tri-screen scan [35]. During this particular spin-off, the plasma samples will be assayed both in the presence as well as the absence of letrozole to estimate relative contributions of different steroid fractions to the overall estrogenicity. In addition, the individual influence of letrozole and exemestane on plasma levels of adipocytokines like leptin and others will be performed using LumineX xMAP technology (multiple ELISA) [36]. We believe that differential effects of letrozole and exemestane on adipocytokines may play a key role in the mentioned lack of cross-resistance.

Finally, marked changes in DNA variant burden and RNA expression will be studied in vitro by whole exome and transcriptome sequencing. Based on the final results of these first spin-off trials, we will plan further investigations.

Eligibility criteria
Postmenopausal patients diagnosed with locally advanced, ER-positive breast cancer (defined by ER-positive in ≥10% of cancer cells) suitable for neoadjuvant antihormonal therapy will be considered for this protocol (Box 1).

Locally advanced breast cancer is in general defined as either T3 or T4, and/or N2-3 primary breast cancer. Patients with primary tumors above 4 cm in diameter (large T2-tumors) may also be recruited in accordance with the international trend to provide neoadjuvant therapies to these patients in clinical trials. Patients have to be postmenopausal to be able to benefit from treatment with aromatase inhibitors. The definition of postmenopausal status: age above 55 years or age above 50 years and at least 2 years of amenorrhea in addition to LH-, FSH- and plasma estradiol levels in the postmenopausal range. Limited, non-life-threatening distant metastasis suitable for systemic antihormonal therapy is allowed.
Dose & schedule of therapy
Patients enrolled in the NEOLETEXE trial are randomized to one of the two following treatment arms (Figure 3):

- Letrozole 2.5 mg od. for at least 8 weeks thereafter continuing with exemestane 25 mg od. for another 8 weeks prior to surgery.
- Exemestane 25 mg od. for at least 8 weeks thereafter continuing with letrozole 2.5 mg od. for 8 weeks prior to surgery.

Planned study period
We plan to enroll 100 patients in this study that is currently recruiting. A total of 59 patients have been enrolled so far. End of study inclusion is expected in Q4 2020.

Study procedures
Patients participating in the trial will be followed at the outpatient clinic by a medical breast cancer oncologist every 4 weeks during the entire study. Caliper measurements of maximum tumor diameters will be registered.

Breast MRIs will be performed at study entry after 2 months and after 4 months of therapy (prior to biopsies and final surgery). Maximum tumor diameter is registered.

Blood samples include: 20 ml of heparin plasma, 20 ml EDTA plasma, 20 ml serum and 20 ml of citrate plasma and 10 ml of EDTA full blood. These scientific blood samples will be processed as usual and stored at -80°C until processing.

Collection of blood samples will be performed at three time points:

- Before the first treatment after protocol (baseline)
- Following 2 months of therapy (while still on treatment with the first selected drug and prior to biopsy and cross-over)
- Following 4 months of therapy (while still on treatment with the second drug and prior to definite surgery)

Tumor tissue samples by open biopsy will be performed at baseline after 2 months and after 4 months of treatment. In general, all biopsies will be obtained by surgical procedures in local anesthesia. All scientific biopsies will be examined by a pathologist to ensure that the biopsy is consisting of tumor tissue in addition to ER-status, PGR status, HER-2 status and level of Ki67 expression. About 500–1000 mg of tumor tissue will be obtained at each timepoint and further divided into three sample portions:

- Sample will be snap-frozen in liquid nitrogen before storage in -80°C
- Sample will be treated ‘RNA later’ over night following storage at -80°C
- Sample will be prepared for histology using standard paraffin embedding
Outcome measures/end points
Upon completion of neoadjuvant endocrine therapy, all patients will be evaluated by a breast surgeon concerning the best local surgical treatment option (breast conservative surgery, mastectomy, procedures including the ipsilateral axilla). During final surgery, the third tumor biopsy will also be obtained through the skin to avoid manipulation of the posterior surface of the breast allowing evaluation of free margins, distance to tumor tissue, etc.

If patients need additional neoadjuvant treatment following 4 months of therapy according to this protocol, the biopsy scheduled after 4 months is giving critical information and has to be obtained for all patients. MRI (ax, T1, T2, DWI dynamic study and spectroscopy) will be performed at baseline and following 2 and 4 months of therapy. Patients with progressive disease or stable disease during neoadjuvant therapy will be able to obtain additional therapies without any restrictions to allow tumor downstaging by other means.

Statistical evaluations
Adequate statistical procedures will be used for the individual evaluations during the planned spin-off studies according to the aims and design of the trial. Comprehensive descriptive statistics, such as means, counts, percentages and standard deviations, of data at baseline and follow-up time points will be presented. Estimates of longitudinal within- and between patient effects will be analyzed using the appropriate modeling techniques, such as t-tests and linear or logistic regression. An experienced clinical statistician is supervising all statistical evaluations.

Conclusion
The NEOLETEXE study is a randomized, open-label, intrapatient, cross-over trial exploring the phenomenon of a lack of cross-resistance between steroidal and nonsteroidal aromatase inhibitors in postmenopausal women with ER-positive and HER-2 negative, locally advanced breast cancer. Performing a comprehensive exploration of the consequences of therapy in vivo with letrozole and exemestane will hopefully clarify the mechanisms involved in

Executive summary
- Breast cancer is one of the most common cancer types and the leading cause of cancer-related deaths among women on a global basis.
- Endocrine treatment is vital since about 70% of all breast cancers are hormone-sensitive due to the expression of hormone receptors.

The 'third-generation' aromatase inhibitors
- The current standard treatment option for postmenopausal women with estrogen receptor positive breast cancer as they have been shown to suppress plasma and tissue estradiol levels by >90% in vivo.
- Letrozole belongs to the class of nonsteroidal aromatase inhibitors.
- Exemestane belongs to a different entity called steroidal aromatase-inactivators.
- Letrozole and anastrozole bind competitively and reversibly, with respect to the androgen substrate, to the heme-containing active site of the aromatase complex, while exemestane is a mechanism-based inactivator and binds irreversibly to the active site of the enzyme.

The NEOLETEXE trial
- The NEOLETEXE trial is a neoadjuvant, randomized, open-label, intrapatient, cross-over single-center clinical trial running at Akershus University Hospital in Norway.
- A total of 100 postmenopausal women with locally advanced, ER+, HER-2 negative breast cancer will be enrolled.
- The patients are randomized to two treatment arms:
  - Arm 1: letrozole 2.5 mg od. for at least 8 weeks, thereafter continuing with exemestane 25 mg od. for another 8 weeks prior to surgery.
  - Arm 2: exemestane 25 mg od. for at least 8 weeks, thereafter continuing with letrozole 2.5 mg od. for 8 weeks prior to surgery.
- During treatment collection of blood samples, open tumor biopsies and MRI examinations will be performed at three timepoints.
- Through the planned spin-off studies, a comprehensive exploration of the consequences of therapy in vivo with letrozole and exemestane will hopefully clarify the mechanisms involved in the differential effects of nonsteroidal and steroidal aromatase inhibitors.

Conclusion
- The NEOLETEXE study is a randomized, open-label, intrapatient, cross-over trial exploring the phenomenon of a lack of cross-resistance between steroidal and nonsteroidal aromatase inhibitors in postmenopausal women with ER-positive and HER-2 negative, locally advanced breast cancer.
the differential effects of nonsteroidal and steroidal aromatase inhibitors and may eventually lead to novel strategies to treat hormone sensitive breast cancer.

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No writing assistance was utilized in the production of this manuscript.

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References
Papers of special note have been highlighted as: • of interest; •• of considerable interest


•• Pivotal trial confirming ‘lack of cross-resistance’ between different classes of aromatase inhibitors.


**Important research confirming efficacy of exemestane following nonsteroidal.**


**Pivotal study on the sequential use of aromatase inhibitors.**


**Important overview publication presenting a need for a direct head-to-head comparison between steroidal and nonsteroidal aromatase inhibitors.**


