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METHOD

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The NEOLETRIB trial: neoadjuvant treatment with Letrozole and Ribociclib in ER-positive, HER2-negative breast cancer

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ABSTRACT

Chemotherapy is used as neoadjuvant therapy for all subgroups of breast cancer, including ER-positive, and HER2-negative cases. However, studies have suggested that using aromatase inhibitors combined with CDK4/6-inhibitors might be an appropriate alternative in selected patients. Thus, the NEOLETRIB trial evaluates the response of ER-positive, HER2-negative luminal A/B breast cancer to the combination of letrozole and ribociclib in the neoadjuvant setting. Comprehensive molecular biology procedures, including sequential single-cell RNA-sequencing of tumor biopsies, are performed during 6 months of treatment with extensive biobanking of blood samples, tumor biopsies and gut microbiome specimens. Our findings will hopefully contribute to an improved selection of patients who may benefit from this drug combination and give new insights into the intra-tumoral changes during this treatment.

Trial registration number: NCT05163106 (ClinicalTrials.gov)

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gut microbiome; HR positive; letrozole; locally advanced breast cancer; neoadjuvant; ribociclib; senescence; single-cell RNA sequencing

1. Background & rationale

Breast cancer is a leading cause of cancer-related deaths among women, affecting approximately about 2.3 million women annually [1]. About 5–10%, will have locally advanced breast cancer when diagnosed [2]. In some cases, presurgical treatment (neoadjuvant therapy) may be necessary to facilitate tumor resection with free margins, allowing for breast conserving surgery. Thus, the rationale for using neoadjuvant therapy in the treatment of early breast cancer is to down-size large, otherwise inoperable tumors, irrespective of the presence of axillary lymph node metastasis. Neoadjuvant treatment, involving different oncological modalities like chemotherapy or targeted therapies, has been shown to increase the chance of curing patients with locally advanced breast cancer [3]. The neoadjuvant setting is also useful for studying endocrine treatment responses *in vivo* in breast cancer patients as it allows for longitudinal clinical evaluation of the tumor during therapy [4]. For exam-

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ple, clinical outcomes may be predicated by evaluating changes in the expression of estrogen-regulated and proliferation genes in the tumor cells before, during and after treatment [5]. Among breast cancer patients undergoing neoadjuvant endocrine therapy, changes in expression of the proliferation marker Ki67 may be useful as a surrogate marker of treatment efficacy [6].

Hormone receptor-positive (HR-positive) breast cancer is the most common subtype and accounts for about 70–80% of all breast cancers [7]. Thus, while chemotherapy is still regarded as the standard of care for many patients with locally advanced, HR-positive, HER2-negative breast cancer, certain patients may benefit from neoadjuvant endocrine therapy, e.g. aromatase inhibitors in postmenopausal women [8–10]. Multiple trials have demonstrated that for a selected group of patients, the efficacy of neoadjuvant monotherapy with third-generation aromatase inhibitors (anastrozole, letrozole, exemestane) [11–17], is comparable with standard neoadjuvant chemotherapy [13].

More recently, the response to traditional endocrine treatment regimens used in the metastatic setting has been revolutionized by the addition of novel agents such as cyclin-dependent kinases (CDK4/6) inhibitors (palbociclib, ribociclib, abemaciclib). In HR-positive, HER2-negative metastatic breast cancer, the combination of an aromatase inhibitor, or SERDs like fulvestrant, with a CDK4/6 inhibitor has been established as the standard of care in first and second-line treatment [18,19]. Multiple pivotal Phase III clinical trials of this combination therapy have documented a doubling of the time to progression and prolonged overall survival [20–29].

The utility of CDK4/6 inhibitors in metastatic breast cancer has led to their experimental application in the neoadjuvant setting for HR-positive, HER2-negative breast cancer, with promising results (Table 1) [30–35]. A selection of key trials are briefly summarized in the following paragraphs.

The NeoPAL trial was a randomized, parallel, non-comparative phase II study including patients with Prosigna-defined luminal A and luminal B, stage II–III breast cancer not suitable for breast-conserving surgery [31]. Overall, 106 patients were randomly assigned to either letrozole (2.5 mg daily) and Palbociclib (125 mg once daily for 3 weeks on, 1 week off) or FEC100 (5-FU 500 mg/m^2, epirubicin 100 mg/m^2, cyclophosphamide 500 mg/m^2) $\times$ 3 in 21-day courses. The Letrozole-Palbociclib (LETPAL) combination therapy was associated with encouraging clinical and biomarker responses. Accordingly, the therapy was suggested as a potential alternative to chemotherapy in early high-risk luminal breast cancer. The 40-month PFS rate of the NeoPAL trial was reported in 2022 by Delaloge et al. [36]. The PFS rate was 86.7 and 89.9% in the LETPAL group and control group, respectively (not statistically significant).

The CORALEEN trial [34] was a parallel-arm, multicenter, randomized, open-label, phase II trial. Postmenopausal women with stage I-IIIA breast cancer that were HR-positive, HER2-negative, luminal B by PAM50 of at least 2 cm in diameter by MRI were included. 106 patients were enrolled and randomized to either six 28-day cycles of ribociclib (600 mg once daily for 3 weeks on, 1 week off) plus daily letrozole (2.5 mg/day) or four cycles of doxorubicin (intravenous 60 mg/m2) and cyclophosphamide (intravenous 600 mg/m^2) every 21 days followed by weekly paclitaxel (intravenous 80 mg/m^2) for 12 weeks. The total duration of neoadjuvant therapy was 24 weeks in both arms. The authors concluded that some patients with high-risk, early-stage, HR-positive, HER2-negative breast cancer could achieve molecular downstaging of their disease using the CDK4/6 inhibitor + letrozole combination.

The FELINE trial [35] was a randomized, placebo-controlled, multicenter, investigator initiated trial that included postmenopausal women with anatomical stage II-III HR-positive, HER2-negative breast cancer. 120 patients were randomized 1:1:1 to receive either letrozole (2.5 mg daily) and placebo, letrozole (2.5 mg daily) and intermittent ribociclib (600 mg once daily for 3 weeks on, 1 week off), or letrozole (2.5 mg daily) and continuous ribiciclib (400 mg once daily), The total duration of treatment was 24 weeks in all three arms. Surgery was performed between days 8 and 21 of cycle 6. The primary objective was to determine if adding ribociclib to letrozole resulted in a higher proportion of patients with preoperative endocrine prognostic index (PEPI) score at the time of surgery, and obviating chemotherapy. The PEPI score combines surgical staging parameters after endocrine neoadjuvant therapy, estrogen receptor status and Ki67 levels to define the risk of recurrence. A PEPI score of 0 is associated with a low risk of recurrence without adjuvant chemotherapy [37]. The results did not show a significant increase in the number of patients with a PEPI score of 0 between groups. However, the addition of ribociclib to letrozole led to a significantly higher proportion of patients achieving complete cell cycle arrest (CCCA) at day 14 cycle 1.

A meta-analysis of neoadjuvant trials involving endocrine therapies and CDK4/6 inhibitors showed that combining an aromatase inhibitor with a CDK4/6 inhibitor offers similar efficacy to neoadjuvant chemotherapy with an improved safety profile. Moreover, a higher rate of CCCA was achieved in the neoadjuvant endocrine combination therapy group compared with neoadjuvant chemotherapy [38].

Table 1. Selected clinical trials using aromatase inhibitors as monotherapy or in combination with CDK4/6-inhibitors in the neoadjuvant setting.

	Involved cancer drugs	Number of patients	Duration (months)	Ref.
Aromatase inhibitors as neoadjuvant monotherapy				
Eiermann et al. (PO24) (2001)	LET vs TAM	337	4	[11]
Tubiana-Hulin et al. (2007)	EXE	42	4	[12]
Semiglazov et al. (2007)	ANA vs EXE vs ChT	239	3	[13]
Takei et al. (SBCCSG-03) (2008)	EXE	44	4	[14]
Fontein et al. (2014)	EXE	102	6	[15]
Skriver et al. (2017)	LET	112	4	[16]
Krainick-Strobel et al. (2017)	LET	33	4–8	[17]
Aromatase inhibitors in combination with CDK4/6- inhibitors (neoadjuvant trials)				
Ma et al. (NeoPalAna) (2017)	ANA + PAL	50	5	[30]
Cottu et al. (NeoPAL) (2018)	LET + PAL vs ChT	106	4,5	[31]
Johnston et al. (PALLET trial) (2019)	LET vs LET + PAL	307	4	[32]
Hurvitz et al. (neoMONARCH) (2020)	ANA + ABE	224	4	[33]
Prat et al. (CORALLEEN) (2020)	LET + RIB vs ChT	106	6	[34]
Khan et al. (FELINE) (2020)	LET + placebo vs LET + RIB continuous dose vs LET + RIB intermittent dose	121	6	[35]

ABE: Abemaciclib; ANA: Anastrazole; ChT: Chemotherapy; EXE: Exemestane; LET: Letrozole; PAL: Palbociclib; RIB: Ribociclib; TAM: Tamoxifen.

The NEOLETRIB trial described in this paper is primarily designed as an exploratory clinical trial with the aim of optimizing patient selection for CDK4/6 inhibitors and an aromatase inhibitor combination therapy in the neoadjuvant setting.

1.1. Objectives

The trial aims to study the direct and indirect anti-tumor effects of neoadjuvant therapy with letrozole and ribociclib combination therapy for a period of 6 months. The tumor immune environment in patients with locally advanced, luminal A/B breast cancer will be studied at several predefined time points during treatment with letrozole and ribociclib. Blood, tumor tissue and gut microbiome samples (fecal samples) will also be collected multiple times during neoadjuvant therapy (Figure 1) and annually for 5 years following surgery. We hope to be able to answer several remaining key questions related to basic breast cancer biology during simultaneous aromatase- and CDK4/6 inhibition.

1.2. Primary objective

The primary objective is to evaluate the intra-tumor immunological effects of letrozole and ribociclib combination therapy in the neoadjuvant setting. This will be determined mainly through sequential single-cell RNA-sequencing of tumor biopsies in addition to other relevant methods focusing on the composition of immune cells in the peripheral blood etc. However, a broad set of state-of-the-art molecular biology procedures will be used to elucidate both changes in the local tumor environment and systemic effects, as reflected in the secondary objectives outlined below (summarized in Figure 2).

1.3. Secondary objectives

A key secondary objective of the trial is to study early and late mechanisms of adaption and resistance to the combination of letrozole and ribociclib. The evolution of the tumor cell clones at baseline, during neoadjuvant therapy and before surgery will be monitored using whole exome/genome tumor sequencing and single-cell RNA sequencing. The clinical, radiological (MRI) and pathological response of targeted neoadjuvant therapy with letrozole and ribociclib, determined by the treatment effects will be assessed by breast cancer pathologists after surgery, including changes in the Ki67 proliferation marker are evaluated.

An additional secondary objective is to evaluate blood samples for tumor-derived or immune-related markers such as serum cytokine levels, serum metabolite levels and circulating free tumor DNA (ctDNA) as surrogate parameters for estimating the anti-tumor effects from liquid biopsies before and during therapy.

Recent publications have cited the potential significance of the patient's microbiota in breast cancer patients [39]. Accordingly, each patient's gut microbiota will be profiled at baseline and during treatment in the NEOLETRIB trial. Analysis of fecal samples will allow total DNA/RNA screening at baseline, during treatment and the follow-up period after surgery. Fecal samples will be profiled using metagenomic sequencing targeting the V4 region of the 16S rRNA gene.

1.4. Exploratory objectives

We intend to investigate signals mediated by extracellular vesicles (such as exosomes, microvesicles and apoptotic bodies) in an effort to elucidate the intricate relationship

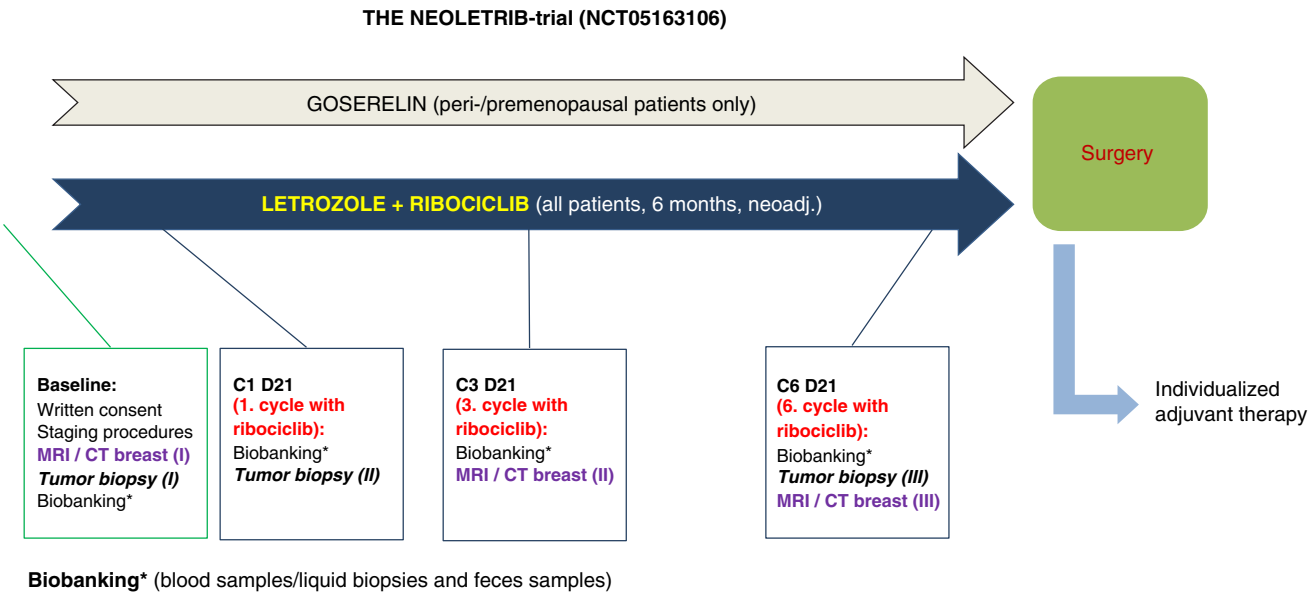

Figure 1. Design and timeline of the NEOLETRIB trial. All patients will be treated with Letrozole and Ribociclib, with the addition of Goserelin to pre- and perimenopausal patients. At baseline written consent will be collected and staging procedures will be performed. The first MRI/CT breast will be done as well as the first tumor biopsy and biobanking of blood samples, liquid biopsies and feces samples.
Biobanking procedures will be repeated on day 21 of cycles 1, 3 and 6 of ribociclib treatment. MRI/CT breast will be repeated on day 21 of cycles 3 and 6 of ribociclib treatment.
C1 D21: Cycle 1 day 21. C3 D21: Cycle 3 day 21. C6 D21: Cycle 6 day 21.

between local breast cancer treatment effects, the local and systemic immune system and the gut microbiota.

In an attempt to build upon the findings of a recent study of the nature of the CDK4/6 inhibitor-induced senescence in liposarcoma [40], we intend to look deeper into the mechanisms behind senescence and cell cycle aberrations in patients treated with letrozole and ribociclib in the neoadjuvant setting.

The study also aims to optimize methods for evaluating neoadjuvant treatment responses in breast cancer patients using spectral CT scans and split-dynamic MRI. All radiological procedures will be performed at baseline, 3 months and 6 months of therapy (Figure 1). The primary and secondary objectives of the NEOLETRIB trial, and a selection of involved procedures, are summarized in Figure 2.

1.5. Trial design

The NEOLETRIB trial is a phase II, multicenter, single-arm, open-label, neoadjuvant study (Figure 1).

2. Methods

2.1. Study setting

Two major Norwegian breast cancer centers will recruit patients to the NEOLETRIB trial: Akershus University Hospital, responsible for the eastern part of the Oslo area and Drammen Hospital responsible for the western part of the Greater Oslo Region.

2.2. Eligibility criteria

Patients diagnosed with locally advanced, ER-positive, HER2-negative, luminal A/B breast cancer suitable for neoadjuvant anti-hormonal treatment will be considered for the study. For this particular trial, ER-positivity is defined by ER-positive IHC staining in $\geq$50% of cancer cells. ER is established as a standard prognostic marker for response to neoadjuvant endocrine therapy [41]. The ER-positive cutoff of 50% was chosen in order to obtain rapid downstaging, which is crucial in the neoadjuvant setting. The protocol defines locally advanced breast cancer as T3-T4 tumors, and/or N2-3, primary breast cancer. However, patients with a large T2 tumor ($\geq$3 cm), lacking other treatment options due to their age, comorbidity, or desire to avoid standard chemotherapy may be included as well if they have a disease with the tumor biology as described above.

All patients who are considered for the NEOLETRIB trial must undergo comprehensive clinical staging with CT scans (lungs, abdomen and pelvis), bone scintigraphy and a baseline MRI of the breasts, as well as routine blood samples, prior to inclusion. Additional MRIs of

Molecular biology procedures and techniques to be used in the NEOLETRIB trial.
Abbreviations: PDECs: Patient-derived explant cultures. WGS: Whole genome sequencing. WES: Whole exosome sequencing

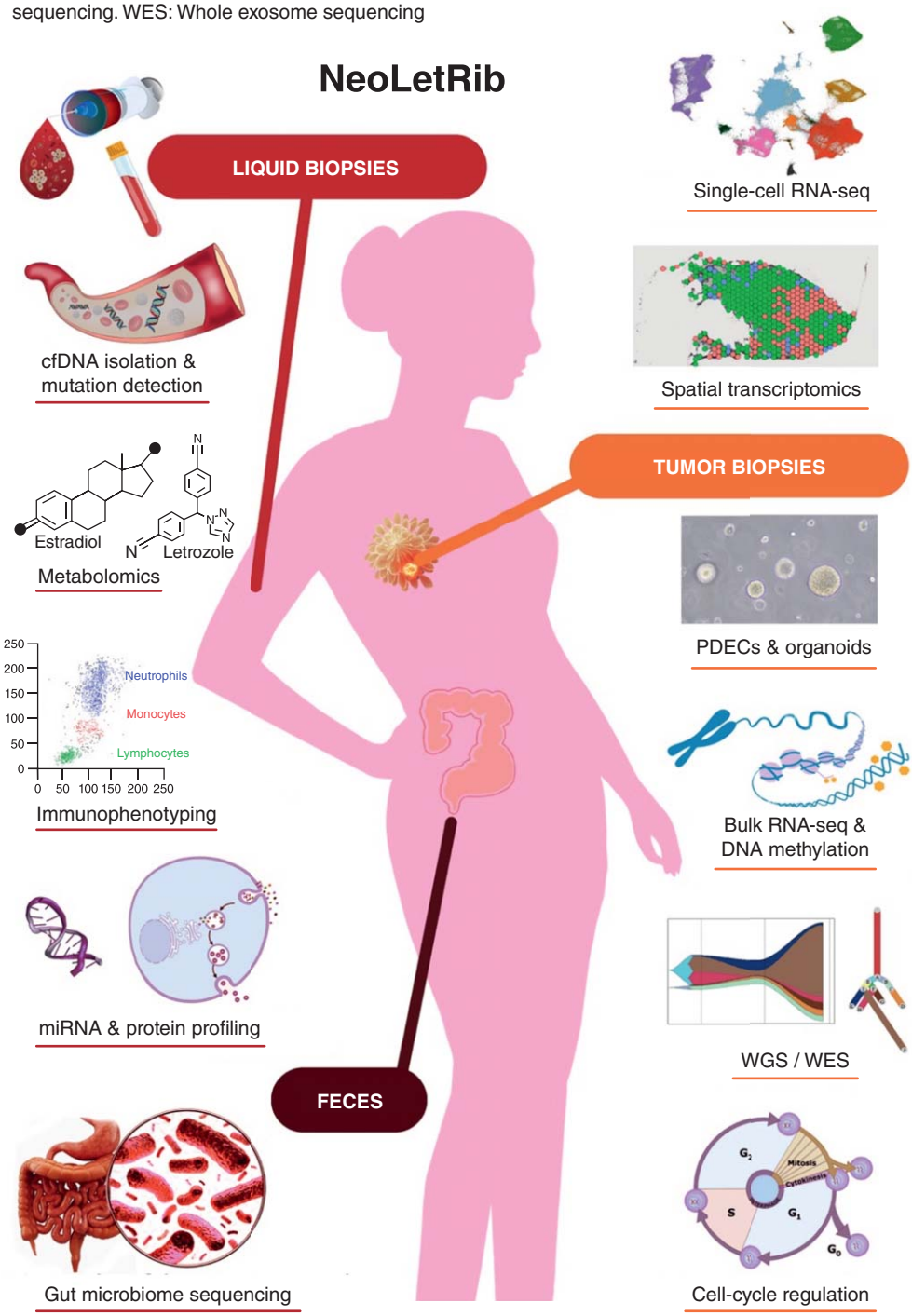

Figure 2. Molecular biology procedures and techniques to be used in the NEOLETRIB trial.
PDEC: Patient-derived explant culture; WES: Whole exosome sequencing; WGS: Whole genome sequencing.

the spine/pelvis may be performed as well (physician's choice). Patients with triple-negative breast cancer, HER2-positive breast cancer (IHC 2+ and amplified or IHC 3+) and patients with any type of distant metastasis are ineligible.

The key eligibility criteria are briefly summarized in Table 2.

2.3. Interventions

Patients enrolled in the NEOLETRIB trial will receive continuous treatment with letrozole 2.5 mg orally daily (o.d.) in combination with ribociclib 600 mg o.d. given for 21 days followed by a seven-day break (28 days = 1 cycle) (Figure 1). Six neoadjuvant cycles of ribociclib (corresponding to 6 months of neoadjuvant therapy)

Table 2. Key inclusion and exclusion criteria for the NEOLETRIB trial.

Key inclusion and exclusion criteria NEOLETRIB trial
Inclusion criteria
• Histologically confirmed locally advanced breast carcinoma, defined as T3-T4, and/or N2-3 primary breast cancer
• Large T2 tumors (>3 cm in diameter);
• ER-positive (defined by ER-pos. in >50% of cancer cells) and HER-2 negative, luminal A/B breast cancer
• Postmenopausal status (natural status or induced by treatment with the LHRH-analogue goserelin 3.6 mg implant s.c. given every 4 weeks)
• Adequate bone marrow and organ function as defined by standard laboratory values (as assessed by a central laboratory for eligibility)
• Standard 12-lead ECG values are defined as the mean of the triplicate ECGs
○ QTc interval at screening <450 msec (QT interval using Fridericia's correction)
○ Mean resting heart rate 50–90 bpm (determined from the ECG)
• Performance Status: Eastern Cooperative Oncology Group (ECOG) score 0-1
• Ability and willingness to comply with study visits, treatment, testing and to comply with the protocol
• Informed written consent
Exclusion criteria
• Any prior treatment for primary invasive breast cancer during the last 2 years
• Patient with a known hypersensitivity to any of the excipients of ribociclib or letrozole
• Triple-negative breast cancer
• HER-2 positive disease, suitable for neoadjuvant therapy with trastuzumab, pertuzumab and taxanes etc.
• Other conditions rendering patients in need of other treatment options with immediate effect like chemotherapy
• Concomitant medications that are known strong inducers of CYP3A4/5.
• Clinically significant, uncontrolled heart disease and/or cardiac repolarization abnormality
• Pregnant or breast-feeding (lactating) women
• Premenopausal women who do not comply with established pregnancy preventing measures during the trial

will be given. Manufacturer (Novartis Pharmaceuticals) recommendations for drug dose reduction for ribociclib, blood samples and ECG assessments will be followed in this trial. However, individual dose adaptations in response to patient comorbidities, advanced age and possibly other factors may be made at the clinician's discretion. Additional cancer therapy is not permitted.

Clinical evaluation of all patients will be performed using tumor palpation and caliper measurement of the tumor every 4–6 weeks. Additionally, breast MRIs will be performed at baseline and following 3 and 6 months of neoadjuvant therapy.

In addition to the regular breast MRIs, subgroups of patients will be evaluated with a spectral CT scan (breast) at baseline and following 3 and 6 months of therapy.

Following standard treatment protocol, blood will be drawn to evaluate liver function, kidney function, bone marrow function, among other parameters before initiation of every cycle with ribociclib. Additional scientific blood samples will be drawn at baseline and on day 21 of the first, third and sixth cycle of ribociclib (Figure 1). ECG will be performed at baseline and following 14, 28 and 42 days of therapy as usual with standard ribociclib therapy.

After surgery, study samples (blood and feces) will be obtained annually for 5 years and at the time of relapse/metastasis.

Response to aromatase inhibitors such as letrozole is dependent upon the patient being postmenopausal, either naturally or induced by goserelin. In this protocol, postmenopausal status is defined as age >55 years, or age >50 years and at least 2 years of amenorrhea, in addition to LH, FSH and plasma estradiol levels in the postmenopausal range. Pre- and perimenopausal women may also be included as they will be treated with goserelin (3.6 mg every 28 days s.c.) starting at least 1 week prior to initialization of letrozole and ribociclib.

Tissue samples of the primary tumor will be obtained at baseline and on day 21 of ribociclib cycles number 1 and 6 (Figure 1). Consequently, the time point for tumor biopsies is somewhat different from previous trials that obtained tumor biopsies at day 14 of ribociclib treatment. As the treatment with ribociclib continues for 21 days in this trial, we believe that day 21 of each treatment cycle is the most appropriate day for biobanking. Surgery should occur no later than 1–2 weeks following the completion of the 6th cycle of ribociclib. All tumor biopsies will be obtained using an ultrasound-guided, vacuum-assisted needle technique (BD Inc., USA) ensuring multiple, high-quality biopsies at each time point. Only highly experienced breast radiologists will be involved in tumor biopsy procedures.

Insufficient response to the neoadjuvant medication protocol (e.g. stable disease at 3 months or worse), will result in the immediate termination of the treatment, and standard chemotherapy for locally advanced breast cancer will be considered, if applicable. We will strive to obtain a tumor biopsy, blood samples and feces samples at this time point.

The result of the final pathological report post-surgery will be evaluated according to the guidelines of the Norwegian Breast Cancer Group, and all

patients will be considered for adjuvant chemotherapy, if indicated.

2.4. Participant timeline

Trial visits will take place by the time of screening, at baseline, on days 14, 21 and 28 during the first ribociclib cycle and on days 14 and 28 during the second ribociclib cycle. In cycle 3, there are trial visits on days 21 and 28 while there is only a visit on day 28 during cycles 4 and 5. During the last treatment cycle with ribociclib, patients will be scheduled for a trial visit on day 21 (biobanking and termination of ribociclib therapy.) Every patient included in the trial will be followed up by a medical oncologist for up to 10 years after surgery. Biobanking (blood and feces collection) will be performed annually for the first 5 years after surgery, while routine clinical examinations by a breast cancer medical oncologist will be done every 6 months.

2.5. Sample size & recruitment

Based on the results of the CORALLEEN trial [34], treatment response (24 weeks of treatment) based on MRI was 14% Complete Response (CR), 43% Partial Response (PR), 33% Stable Disease (SD) and 10% N/A. We propose a minimum sample size in either group to be at least >30 patients, thus an inclusion goal of 100 patients in total would be sufficient for hypothesis-generating results.

However, the primary aim of this trial is to deliver basic research results, rather than to evaluate the efficacy or compare treatments. Given the main end points and the expected data variation, the data will be sufficient to yield reliable results.

The study was opened for inclusion on 1 April 2021, and will remain open until 31 December 2024, or until a minimum of 100 patients have completed all study procedures. At the time of manuscript submission, 85 patients have already been enrolled in the NEOLETRIB trial.

3. Data collection, management & analysis

The clinical dataset is stored locally at the Department of Oncology at Akershus University Hospital (the electronic patient journal, and data management software; located on the secure hospital server). Data from specialized procedures will be generated and stored on secure research servers at each of the respective institutes. All clinical data are anonymized prior to use by collaborators. Response assessments including clinical caliper measurements, MRIs during therapy and pathological evaluations after surgery, will be carried out following guidelines provided by the Royal Society of Pathologists (UK). Tumor response will subsequently be categorized as above 50% remaining tumor; 10–50% remaining tumor; below 10% remaining tumor or complete responses.

3.1. Data management

Patients will be given a study ID to avoid personal identification. The corresponding principal investigator is responsible for manually providing each study participant with a unique study identification number. The participant log will be stored securely at the Department of Oncology at Akershus University Hospital.

4. Ethics

The treatment given in the NEOLETRIB trial (letrozole and ribociclib) is known to be highly effective in metastatic breast cancer and has been approved for use in this setting in Norway. However, it is not approved as a standard option in the neoadjuvant setting in Norway. Accordingly, the NEOLETRIB protocol presented here was approved by the regional ethical committee of South-East Norway (No: 193780) before the initiation of the study (ClinicalTrials.gov Identifier: NCT05163106). There are no ethical conflicts connected to this study.

5. Conclusion

Following several encouraging publications of neoadjuvant trials in locally advanced luminal A/B breast cancer populations, the NEOLETRIB trial was designed to answer some crucial remaining questions regarding the combination of an aromatase inhibitor (letrozole) and a CDK4/6 inhibitor (ribociclib) in the neoadjuvant setting.

The NEOLETRIB trial is planned as a multicenter, single-arm, open-label, neoadjuvant, phase II study. The trial aims to study the tumor response to the highly potent drug combination of letrozole and ribociclib in women with locally advanced, ER-positive, luminal A/B breast cancer.

Treatment response will be assessed using multiple state-of-the-art approaches including liquid biopsies, tumor biopsies and gut microbiome specimens. Single-cell RNA-sequence analyses will be performed at several time points to study the intra-tumoral changes over time including changes in the microenvironment of the tumor. Whole-genome sequencing analysis of serial tumor samples, evaluation of peripheral immune cells in the bloodstream and multiple additional molecular evaluations, including micro-RNA and exosomes in blood samples, will be performed. Our findings will increase our basic understanding of intra-tumor gene expression and regulation in response to modern combinational endocrine therapy. In contrast to the previous comparable studies as CORALLEEN and FELINE, our trial places significant emphasis on patient gut microbiota and its

importance in this setting. The NEOLETRIB trial will contribute to building a comprehensive biobank with extended analysis at several time points.

Furthermore, the study will enable us to analyze how CDK4/6 inhibitors impact the host microbiota, in addition to determining if variations in gut microbiota in pre-treated patients might influence the response to treatment.

The treatment duration of 6 months is based on data from multiple neoadjuvant breast cancer trials and follows the current recommendations from the Norwegian Breast Cancer Group [9].

The decision to use the non-steroidal aromatase inhibitor letrozole for this trial is based on our previous findings, comparing letrozole with anastrozole head-to-head *in vivo* [42,43]. Our results clearly showed that letrozole was the superior compound considering estrogen suppression in blood samples and tissue samples of breast cancer patients. The same results were found for suppression of total body aromatase inhibition. Exemestane was not considered as the suitable aromatase inhibitor in the present trial due to its effects in second-line treatment for metastatic breast cancer, following progression on a non-steroidal aromatase inhibitor [44].

Ribociclib was chosen as the CDK4/6 inhibitor in the NEOLETRIB trial based on the convincing results of ribociclib in the metastatic setting, demonstrating significant overall survival benefit in both pre-and postmenopausal women [29,45,46]. It also has a favorable toxicity profile and was well tolerated in the MONALEESA trial [47–49]. The dose of ribociclib (600 mg once daily for 21 days on and 7 days off) was chosen given the severity of the clinical situation, requiring rapid tumor response prior to surgery. Dose reductions of ribociclib are, however, allowed in accordance with established clinic protocols, whenever necessary.

In conclusion, the findings of the NEOLETRIB trial will hopefully contribute to the reduction of the use of traditional chemotherapy in the neoadjuvant setting and help to identify those breast cancer patients who may benefit most from the use of letrozole and the CDK4/6 inhibitor ribociclib in the neoadjuvant setting.

Article highlights

Background & rationale
- Hormone receptor-positive breast cancer is the most common subtype and accounts for about 70–80% of all breast cancer patients.
- The rationale for using neoadjuvant therapy in the treatment of early breast cancer is to down-size large, otherwise inoperable tumors, with or without axillary lymph node metastasis, before definite surgery.
- Chemotherapy is still widely used as neoadjuvant therapy for all subgroups of breast cancer, including ER-positive, and HER2-negative cases.
- Neoadjuvant endocrine treatment with aromatase inhibitors and a CDK4/6 inhibitor in combination is currently tested in the neoadjuvant setting with promising results.

Trial design
- The NEOLETRIB trial is a Phase-II, multicenter, single-arm, open-label, neoadjuvant trial.

Objectives
- The trial aims to comprehensively study the tumor response to the highly potent drug combination of letrozole and ribociclib in women with locally advanced, ER-positive, luminal A/B breast cancer. In premenopausal women, goserelin will be added to letrozole and ribociclib.
- Comprehensive molecular biology procedures, including sequential single-cell RNA-sequencing of tumor biopsies, are performed during 6 months of treatment with extensive biobanking of blood samples, tumor biopsies and gut microbiome specimens.

Conclusion
- The findings will hopefully contribute to an improved selection of patients who may benefit from this drug combination, and give new insights into the intra-tumoral changes during this treatment.

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Competing interests disclosure

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Writing disclosure

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

Ethical conduct of research

The NEOLETRIB protocol presented here was approved by the regional ethical committee of South-East Norway (No: 193780) before the initiation of the study (ClinicalTrials.gov Identifier: NCT05163106). There are no ethical conflicts connected to this study.

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