Clinical Trial Protocol

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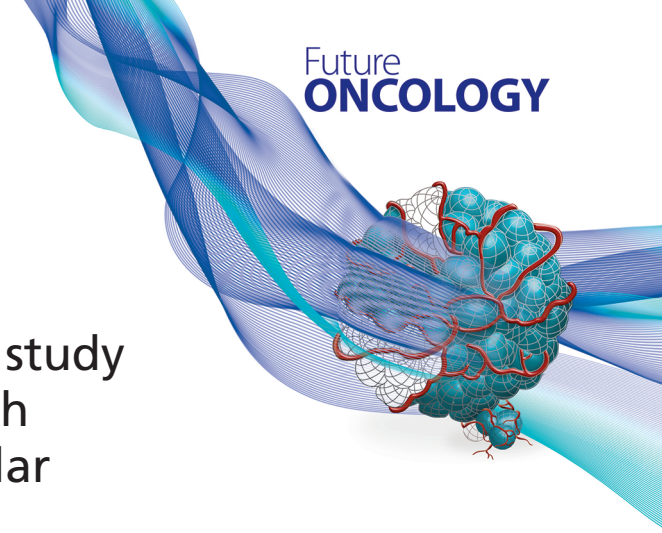

ROSALINE: a phase II, neoadjuvant study targeting ROS1 in combination with endocrine therapy in invasive lobular carcinoma of the breast

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Invasive lobular carcinoma (ILC) is the most common histologic subtype of breast cancer after invasive ductal carcinoma (i.e., no special type [NST]). ILC differs from NST in clinical presentation, site-specific metastases and response to conventional therapies. Loss of E-cadherin protein expression, due to alterations in its encoding gene *CDH1,* is the most frequent oncogenic event in ILC. Synthetic lethality approaches have shown promising antitumor effects of ROS1 inhibitors in models of E-cadherin-defective breast cancer in *in vivo* studies and provide the rationale for testing their clinical activity in patients with ILC. Entrectinib is a tyrosine kinase inhibitor targeting TRK, ROS1 and ALK tyrosine kinases. Here, the authors present ROSALINE (NCT04551495), a phase II study testing neoadjuvant entrectinib and endocrine therapy in women with estrogen receptor-positive, HER2-negative early ILC.

Plain language summary: Breast cancer is the most common cancer among women worldwide. Breast cancer is not a unique disease, but rather a heterogeneous disease, with different subtypes. Lobular breast cancer is the second most common histologic subtype of breast cancer after ductal breast cancer. Lobular breast cancer has some peculiar characteristics that make it a distinct entity in the context of breast cancer. Nevertheless, few clinical studies so far have focused specifically on this subtype. ROSALINE is a clinical study aimed to test entrectinib, a new drug that showed promising activity in preliminary research studies, in combination with endocrine therapy in women with lobular breast cancer before surgery.

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Invasive lobular carcinoma (ILC) accounts for approximately 5–15% of all breast cancer diagnoses and is the second most common histologic subtype after invasive ductal carcinoma (IDC), formally referred to as invasive

breast carcinoma of no special type (NST) [1]. These tumors are characterized by small, discohesive epithelial cells and most are estrogen receptor (ER)-positive and HER2-negative [2]. Within ILC, the classic subtype is the most prevalent whereas other subtypes have been defined on the basis of their architecture (solid, alveolar and trabecular) or cytonuclear characteristics (apocrine, histiocytoid, pleomorphic and signet ring, further grouped into the mixed-nonclassic subtype) [3]. ILCs also have different clinical behavior compared with IDCs. ILCs often present as multifocal disease, with a relatively higher incidence of bilateral disease, although this tendency has not been consistently shown in all studies [4]. Moreover, the discohesive phenotype and the low density of tumor cells explain the difficulty in its clinical and radiological detection [4]. Furthermore, ILCs have a peculiar metastatic pattern to the peritoneum, ovaries, gastrointestinal tract and leptomeninges, besides common bone lesions [5]. From the therapeutic point of view, early ILC seems to exhibit a lower responsivity to chemotherapy [6–9], and some studies show that patients with ILC might derive enhanced benefit from treatment with aromatase inhibitors, as compared to tamoxifen [10]. Interestingly, a recent retrospective analysis conducted on more than 13,000 patients with metastatic breast cancer showed that lobular histology (n = 1804) was an independent adverse prognostic factor (hazard ratio [HR] for overall survival [OS]: 1.31, 95% CI: 1.20–1.42, $p < 0.0001$), and specifically in patients with hormone receptor-positive, HER2-negative disease (HR: 1.17; 95% CI 1.08–1.27, $p < 0.0001$) [11]. Several genomic initiatives have been designed to characterize the mutational landscape of ILC [12,13] and have identified genomic alterations in key oncogenic pathways such as *PIK3CA, PTEN, AKT1, ERBB2, ERBB3, FOX1A* and *ESR1* [12]. Alterations in *CDH1*, the gene coding for the cell adhesion molecule E-cadherin, are responsible for the discohesive phenotype and are almost pathognomonic genomic events in ILC, being reported in up to 90% of cases in some reports [12,14,15]. The loss of E-cadherin protein expression is predominantly caused by *CDH1* somatic mutations (~65% of cases) and loss of heterozygosity (~90%), but it can also be the result of promoter methylation and copy number alterations of *CDH1* [12]. Taken together, all evidence supports that ILC represents a distinct clinical and biological entity. Nevertheless, patients with ILC are treated with the same drugs and in the same clinical trials as subjects with IDC [16], with several trials not even reporting separate outcomes according to histotype, thus making it difficult to extrapolate data for patients with ILC, specifically. Additionally, metastatic ILC is often underrepresented in clinical trials because of its diffuse growth pattern and the lack of measurable disease [17]. Hence, there are several unmet needs and areas of uncertainty in the management of ILC that make necessary to design appropriate clinical studies tailored to this particular subgroup of patients [16].

Introduction to the trial

Synthetic lethality approaches for *CDH1* loss of function

Synthetic lethality occurs when the simultaneous perturbation of two genes results in cellular or organismal death [18]. Synthetic lethality also occurs between genes and small molecules and can be used to elucidate the mechanism of action of drugs [18]. Bajrami and colleagues studied E-cadherin-targeted synthetic lethality approaches [19]. They used breast cancer cell lines and made them E-cadherin-deficient using gene-editing technology. Drug sensitivity screens (testing 80 inhibitors) identified different active inhibitors, including PAK inhibitors, an Aurora kinase inhibitor, PIK3/mTOR inhibitors and the ROS1 inhibitors foretinib and crizotinib [19]. Interestingly, an siRNA sensitivity screen performed on both *CDH1*-deficient and *CDH1*-proficient cells was able to identify ROS1 as a significant hit. These effects were seen on different cell lines and, notably, those with reduced expression of E-cadherin were more sensitive to crizotinib compared with those with higher expression of E-cadherin [19]. Moreover, the restored expression of *CDH1* reduced sensitivity to ROS1 inhibitors [19]. Testing in non-breast cancer cell lines also showed ROS1 synthetic lethality in other *CDH1*-deficient cancers (e.g., gastric cancer). Consistently, in these tumor cell lines, reduced E-cadherin expression (measured by *CDH1* mRNA levels) was associated with higher sensitivity to crizotinib compared with those cells with higher expression of E-cadherin [19]. Furthermore, *in vitro* and *in vivo* experiments with endocrine-resistant cell lines showed sensitivity to ROS1 inhibition, suggesting that its activity is independent of endocrine sensitivity, and that can lead to mitotic catastrophe in *CDH1*-deficient cells. Loss of E-cadherin can impair p120 catenin function and this dysfunction was exacerbated by ROS1 inhibition [19]. The findings were replicated in *in vivo* mouse ILC models and xenografts [19]. Based on these preclinical findings, a trial with crizotinib in combination with fulvestrant for patients with E-cadherin-negative metastatic diffuse gastric cancer and metastatic ILC of the breast is currently ongoing (NCT03620643).

Entrectinib

Entrectinib is a potent inhibitor of tyrosine receptor kinases, in particular, tropomyosin receptor kinases A, B and C (TRKA, TRKB and TRKC, encoded by the *NTRK* genes *NTRK1, NTRK2* and *NTRK3*, respectively), ROS1 (encoded by the gene *ROS1*) and ALK (encoded by the gene *ALK*) [20]. The major human active metabolite of entrectinib, M5, showed similar *in vitro* potency and activity. Entrectinib has antitumor potency in *NTRK* and *ROS1* fusion-driven tumor models driving tumor regressions across multiple tumor types, including non-small-cell lung carcinoma (NSCLC), colorectal cancer (CRC), sarcomas, head and neck carcinoma, acute myeloid leukemia (AML) and gliomas. Entrectinib inhibits the autophosphorylation of the target kinases hyperactivated in cancer cells harboring *NTRK, ROS1* or *ALK* gene fusions, representing multiple tumor indications [21]. Suppression of TRK, ROS1 or ALK kinase fusion activity leads to inhibition of key downstream transducers, including phospholipase C gamma, MAPK and PI3K/PI3K/AKT. Inhibition of these oncogenic targets leads to selective antitumor activity in nonclinical models harboring *NTRK, ROS1* or *ALK* gene fusions via induction of cell cycle arrest and tumor cell apoptosis. Entrectinib has demonstrated *in vivo* antitumor activity in nine *TRK*-driven tumor models and three *ROS1*-driven tumor models, representative of six tumor types (NSCLC, CRC, sarcoma, head and neck squamous cell carcinoma, glioma and AML) and eight gene fusions (*TPM3-NTRK1, MPRIP-NTRK1, ETV6-NTRK3, LMNA-NTRK1, BCAN1-NTRK1, ETV6-ROS1, SCD4-ROS1* and *CD74-ROS1*). The antitumor activity of entrectinib has been studied in four clinical studies in adult (ALKA, STARTRK-1 or GO40784, STARTRK-2 or GO40782) and pediatric (STARTRK-NG or CO40778) cancer patients. An integrated analysis of three ongoing phase I and II trials of entrectinib (ALKA-372-001 GO40783, STARTRK-1 and STARTRK-2) was reported [22]. The population included adult patients (aged ≥18 years) with locally advanced or metastatic tumors with *NTRK* fusions or *ROS1* fusion-positive NSCLC who received oral entrectinib at a dose of at least 600 mg every day, with a follow-up of at least 12 months. Across the four main entrectinib clinical studies, the most common (≥20% incidence) adverse events (AEs) were dysgeusia (42%), dizziness (32%), constipation (33%), diarrhea (26%) and fatigue (24%). Most were of grade 1 or 2 severity according to the Common Terminology Criteria for Adverse Events (CTCAE) developed by the National Cancer Institute (NCI). For those patients with NSCLC and *ROS1* fusions, 41 (77%; 95% CI: 64–88) of 53 patients in the efficacy-evaluable population had an objective response (OR). The median follow-up was 15.5 months (interquartile range [IQR]: 13.4–20.2) and the median duration of response was 24.6 months (95% CI: 11.4–34.8) [22]. On 15 August 2019, the FDA granted accelerated approval to entrectinib (ROZLYTREK, Genentech Inc.) for adults and pediatric patients of 12 years of age and older with solid tumors that have an *NTRK* gene fusion without a known acquired resistance mutation, are metastatic or where surgical resection is likely to result in severe morbidity, and have progressed following treatment or have no satisfactory standard therapy. Both the FDA and EMA approved entrectinib for adults with metastatic NSCLC whose tumors are ROS1-positive.

Neoadjuvant endocrine therapy in estrogen receptor-positive HER2-negative early breast cancer

Aromatase inhibitors have been tested in patients with early breast cancer in both the adjuvant and neoadjuvant settings with positive results [23,24]. Aromatase inhibitors act by radically diminishing the levels of estrogens in postmenopausal women, where estrogen production relies almost exclusively on the conversion of androgens into estrogens by aromatase enzymes [25]. Historically, neoadjuvant hormonotherapy was first studied in fragile populations, such as elderly women, where its activity was demonstrated both in terms of disease response and survival rates [26]. Nowadays, neoadjuvant endocrine therapy is considered a standard treatment option for selected patients with ER-positive, HER2-negative early breast cancer. Several studies have demonstrated that it yields similar response rates, comparable rates of breast-conserving surgery and lower rates of AEs compared with neoadjuvant chemotherapy. In a meta-analysis including data from over 3400 patients enrolled in 20 randomized clinical trials, neoadjuvant endocrine therapy was associated with similar response rates but lower toxicity compared with neoadjuvant chemotherapy [27]. The use of hormonal agents as neoadjuvant therapies is particularly interesting for patients with ILC, who are known to have poor response rates to neoadjuvant chemotherapy. A trial enrolling 61 patients with ER-positive, large operable or locally advanced cancers, or who were initially unfit for surgery, evaluated the efficacy of neoadjuvant therapy with letrozole and demonstrated a mean reduction in tumor volume at three months of 66% and a rate of successful breast conservation of 81% [28].

The neoadjuvant setting represents an attractive scenario for clinical trials for several reasons, as it offers the possibility of direct evaluation of treatment effect on tumor size, better surgical results as well as possible research opportunities via the comparative analysis of tumor biology before and after treatment [26,29]. This has led to the

development of potential surrogate end points for long-term clinical outcomes in women receiving preoperative endocrine treatment, such as early changes in tumor Ki67 expression (a protein that measures cell proliferation) and the preoperative endocrine prognostic index (PEPI) score that is evaluated at surgery [30]. The PEPI score is calculated based on tumor and lymph node stage, ER status according to Allred score and Ki67 status, all evaluated on the surgical specimen obtained post-endocrine neoadjuvant therapy [30,31]. Both end points have been associated with event-free survival in this setting. The combination of aromatase inhibitors and goserelin has been tested in the neoadjuvant setting in multiple trials [26,31–35].

Residual cancer burden

Despite the fact that pathological complete response (pCR) is approved by regulatory guidance as a surrogate end point in neoadjuvant clinical trials for patients with early breast cancer [36], its role is being increasingly questioned in some settings. Of note, pCR does not provide granularity in evaluating the extent of residual disease, since it allows only a dichotomous distinction between pCR and non-pCR. Moreover, the rate of pCR achieved after neoadjuvant treatment in hormone receptor-positive, HER2-negative breast cancer is lower compared with other breast cancer subtypes [37], making it even more challenging to consider pCR as an optimal predictor of long-term survival in this setting. As long-term prognosis might also depend on the extent of residual disease, the residual cancer burden (RCB) index is being increasingly used to measure residual disease in pathologic resection specimens. RCB is a continuous index that combines pathologic measurements of the primary tumor (primary tumor bed dimensions and cellularity fraction of invasive cancer) and of nodal metastases (size of largest positive lymph node and number of positive lymph nodes) [38]. Based on these parameters, the RCB index can result in one of four classes with an increasing amount of residual disease: RCB 0 (pCR), RCB-I, RCB-II and RCB-III. Symmans and colleagues prospectively validated the RCB index in breast cancer and demonstrated that it is an accurate predictor of long-term survival after neoadjuvant treatment in all breast cancer subtypes (hormone receptor-positive/HER2-negative, HER2-positive or triple receptor-negative) independent of other clinical-pathologic variables [39]. These findings were further validated externally in retrospective cohorts [40]. Hence, the measurement of the RCB index can provide important, long-term prognostic data that add relevant information to pretreatment clinical-pathological information and to post-treatment disease stage; thus, it represents an effective clinical end point to assess the efficacy of neoadjuvant treatments in early breast cancer.

ROSALINE

The ROSALINE study was designed based on the rationale just described. Targeting ROS1 in breast ILC is already being studied in the advanced setting (NCT03620643). Nonetheless, the neoadjuvant setting provides a higher probability for patients to derive benefit compared with the metastatic setting, since in the early setting, tumors are treatment-naive, possibly resulting in less disease heterogeneity and treatment resistance. Thus, the efficacy of the combination of endocrine treatment with entrectinib will be studied in patients with early breast cancer. ROSALINE (NCT04551495) is a single-arm, multicenter, phase II study evaluating the efficacy and safety of combining entrectinib and endocrine therapy in patients with stage IIA–IIIA, ER-positive/HER2-negative ILC in the neoadjuvant setting.

Eligibility criteria

Table 1 lists the key inclusion and exclusion criteria. Briefly, pre- or postmenopausal women aged 18 years or older are eligible if they have a local, histological diagnosis of ILC that is ER-positive, HER2-negative and larger than 20 mm as measured by local breast MRI, clinical nodal stage (cN) 0 or 1 (i.e., stage IIA–IIIA), without prior treatment, and candidate for preoperative treatment. Patients with clinical T4 disease including inflammatory breast cancer and/or cN2 or cN3 will be excluded. Additional exclusion criteria were defined according to the entrectinib safety profile (e.g., cardiotoxicity, neurotoxicity, lung toxicity). Significant cardiac disease, including recent myocardial infarction, congestive heart failure, unstable angina and bradyarrhythmias are exclusion criteria, as well as left ventricular ejection fraction (LVEF) $\leq$55% measured by echocardiography or multigated radionuclide angiography (MUGA), or a personal or family history of corrected QT (QTc) interval prolongation. Patients with peripheral neuropathy of grade 2 or higher and patients with interstitial lung disease or interstitial fibrosis are not eligible. To avoid drug–drug interactions, patients receiving concurrent treatment with strong or moderate CYP3A inhibitors or drugs known to cause QTc interval prolongation are excluded. To avoid a notable impact

Table 1. Key eligibility criteria.

Key inclusion criteria
• Female • Age ≥18 years • Histological diagnosis of invasive lobular breast adenocarcinoma that is ER-positive, and HER2-negative per updated ASCO-CAP guidelines according to local testing • Multifocal unilateral or bilateral breast adenocarcinoma tumors are allowed if all tested foci are lobular, ER-positive and HER2-negative • A primary non-metastatic or locally advanced tumor of >20 mm as measured by breast MRI, cN0 or cN1, without prior treatment, candidate for preoperative treatment • ECOG performance status 0 or 1 • Adequate bone marrow, renal and liver function • Signed informed consent obtained prior to any study-related procedure • Negative pregnancy test prior to enrollment for premenopausal women • Highly effective form of contraception for women of childbearing potential from registration date until at least 5 weeks after last administration of entrectinib. Use of oral hormonal contraceptive agents is not permitted
Key exclusion criteria
• Clinical T4 disease including inflammatory breast cancer and/or cN2 or cN3 • Prior history of invasive cancer in the past 5 years except basal or squamous cell carcinoma of skin that has been definitively treated • Known hypersensitivity to study drugs or excipients • Hyperuricemia > grade 1 • Any illness or medical condition that is unstable or could jeopardize patient safety or compliance with study requirements • Subjects unable to swallow oral medications • Prior intake of letrozole, any ROS1 inhibitor, any TRK inhibitor or anticancer therapy (including endocrine therapy) • Concurrent treatment with strong or moderate CYP3A inhibitor • Concurrent treatment with any of the drugs not permitted (i.e., strong CYP3A inducers and drugs known to cause QTc interval prolongation) • Significant cardiac disease, including recent (less than 6 months) myocardial infarction, congestive heart failure, unstable angina, and bradyarrhythmias • LVEF ≤55% measured by ECHO or MUGA • QTc exceeding 450 msec; history of QTc interval prolongation; risk factors for torsade de pointes; other concomitant medications that may prolong QTc; family or personal history of long or short QT syndrome, Brugada syndrome, or Torsade de Pointes; uncorrected electrolyte imbalances • Pregnant or lactating women • Known interstitial lung disease, interstitial fibrosis or history of tyrosine kinase inhibitor-induced pneumonitis • Peripheral neuropathy ≥ grade 2 • Active gastrointestinal disease (e.g., Crohn's disease, ulcerative colitis or short gut syndrome) or other malabsorption syndromes that could reasonably impact drug absorption

ASCO-CAP: American Society of Clinical Oncology-College of American Pathologists; ECOG: Eastern Cooperative Oncology Group; ER: Estrogen receptor; LVEF: Left ventricular ejection fraction; QTc: Corrected QT interval.

on drug absorption, patients with active gastrointestinal disease (e.g., Crohn's disease, ulcerative colitis or short gut syndrome) or other malabsorption syndromes are not eligible.

Study design

Figure 1 shows the ROSALINE study design. Forty-five patients (target inclusion) will receive four 28-day cycles of letrozole (2.5 mg *per os* [p.o.] daily) in combination with entrectinib (600 mg p.o. daily). Premenopausal women will receive goserelin (3.6 mg intramuscular every 28 days). Subjects' responses to therapy will be evaluated at screening, after two cycles and after four cycles of treatment by breast MRI. An LVEF assessment (by echocardiography or MUGA) will be performed at baseline. A triplicate ECG will be performed at screening and before the start of every treatment cycle. Surgery will take place after at least 16 weeks of treatment, during week 18 (+7-day window). Breast and axillary surgery will follow local practice. Postoperative therapy will be at the discretion of the investigator and will follow local practice. In case of progressive disease at the MRI after two cycles of therapy, patients will discontinue the study treatment and will be treated according to the standard of care.

Study procedures

Objectives & end points

Table 2 reports the study objectives and end points. The primary end point of the study is the rate of RCB 0/1 assessed by local evaluation in all evaluable subjects. RCB on postneoadjuvant specimen will be assessed locally according to the following parameters: primary tumor bed dimensions, cellularity fraction of invasive cancer, size of largest positive lymph node and number of positive lymph nodes (calculator: www3.mdanderson.org/app/medcalc/index.cfm?pagename=jsconvert3). Secondary end points are the rate of pCR in breast and axilla (ypT0/Tis ypN0) by local evaluation, the tumor OR assessed by local breast MRI via modified Response Evaluation Criteria in Solid Tumours (RECIST) 1.1 and safety and tolerability of the treatment combination. Exploratory end points include translational analyses performed on baseline and surgical samples investigating genomic, transcriptomic and assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-seq) profiles.

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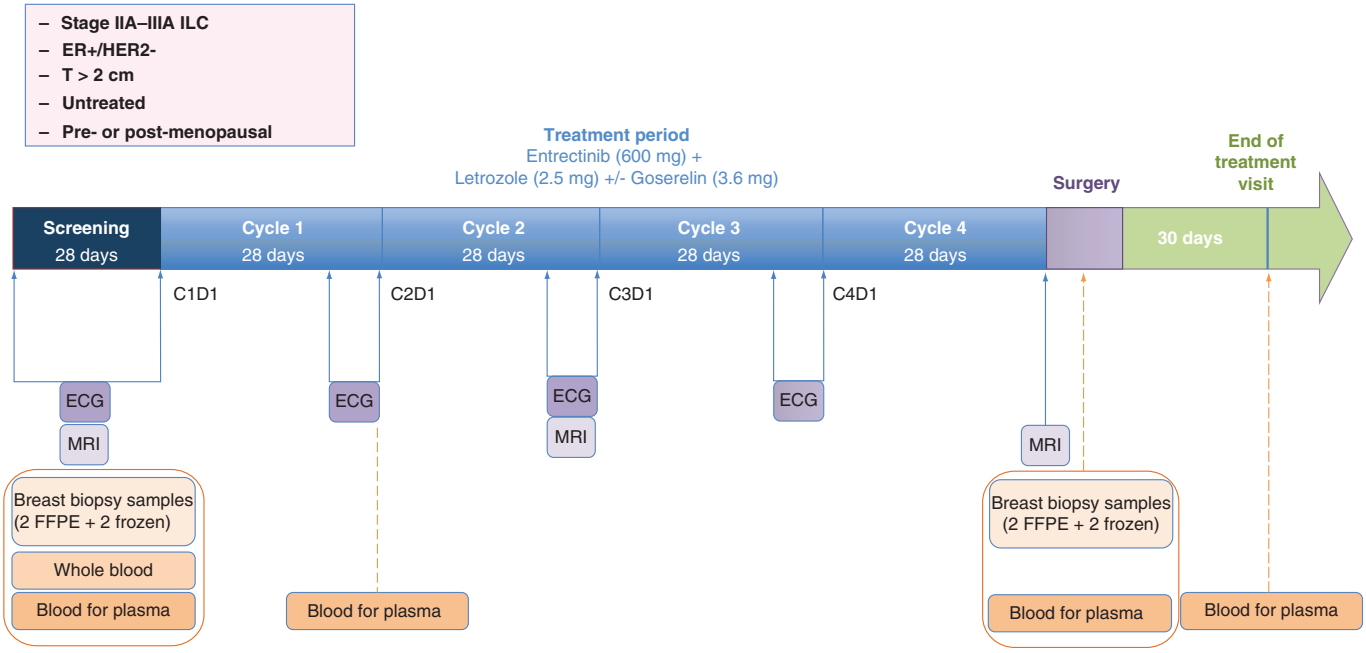

Figure 1. ROSALINE study design.
ER+: Estrogen receptor-positive; FFPE: Formalin-fixed paraffin-embedded; ILC: Invasive lobular breast cancer.

Table 2. Study objectives and end points.

Primary objective	Primary end point
• To evaluate the efficacy of endocrine therapy + entrectinib in women with ER+/HER2- early breast cancer, lobular subtype	• RCB 0/1 by local assessment in all evaluable subjects
Secondary objectives	**Secondary end points**
• To further evaluate the efficacy of the combination by pathology • To further evaluate the efficacy of the combination by imaging • To evaluate the safety of endocrine therapy and entrectinib	• pCR in breast and axilla (ypT0/Tis ypN0) by local evaluation. • Tumor OR assessed by local breast MRI via modified RECIST v. 1.1 • Incidence, nature and severity of AEs graded according to NCI CTCAE, v. 5.0
Exploratory objectives	**Exploratory end points**
• To identify differences between responders vs nonresponders in terms of genomic aberrations, transcriptional programs and transcription factors at baseline (pre-) and surgery (post-treatment)	• Baseline and surgery genomic, transcriptomic and ATAC-seq profiles • Baseline and surgery circulating tumor DNA detection

AE: Adverse event; ATAC-seq: Assay for transposase-accessible chromatin with high-throughput sequencing; ER: Estrogen receptor; NCI CTCAE: National Cancer Institute Common Terminology Criteria for Adverse Events; OR: Objective response; pCR: pathological complete response; RCB: Residual cancer burden; RECIST: Response Evaluation Criteria in Solid Tumors.

Statistical considerations

Sample size

Sample size has been estimated to statistically test a hypothesis about the rate of RCB 0/1 (primary end point). The design used is a two-step optimal Simon design. A rate of RCB 0/1 of 3% is considered the minimum required level of activity, while the targeted study power to conclude treatment activity (and to warrant further investigation) should be reached in case of a rate of RCB 0/1 of 15% or more. One-sided levels of alpha and beta of 10% were used for sample size determination. To test the statistical hypothesis, 39 evaluable subjects are required. A subject will be considered evaluable if all following conditions are met: therapy with the combined schedule of letrozole and entrectinib has started, the subject did not miss more than 18 days of entrectinib, the subject was surgically treated and the criteria for assessing RCB can be applied. Considering a rate of nonevaluability of about 10%, 45 subjects are expected to be included in the study.

Study duration

Based on the anticipated accrual rate, it is expected that the last subject will be included 18 months after the first subject. Adding the length of the screening period for the first subject, the treatment period and the follow-up

period for the last subject, the last subject's last visit is expected to occur 24 months after the first subject will have signed informed consent.

Statistical plan

Efficacy will be assessed on all evaluable subjects. Safety will be assessed on all eligible subjects who started treatment with letrozole and entrectinib. An interim analysis will be performed on the first 17 evaluable subjects. If none out of 17 subjects present RCB 0/1, then the study will be stopped for futility. If at least one subject out of 17 evaluable subjects presents RCB 0/1 then the study will continue enrolling until 39 evaluable subjects are included. For the combination to be considered worthy of further investigation, at least three subjects out of 39 need to present an RCB 0/1. However, if a clear radiological benefit (defined as a partial or complete response) is observed at the presurgical MRI, enrolment into stage 2 could be allowed in the absence of RCB 0/1, after discussion with the sponsor.

The final analysis will be completed either at the time the study is closed for futility or at the time 39 evaluable subjects have been included. A formal statistical hypothesis test will be conducted on the RCB 0/I rate according to the Simon design. A 95% CI for the RCB 0/I rate will also be reported (with the use of an exact binomial distribution). The whole distribution of RCB will be descriptively analyzed. For the analysis of OR, the estimated OR rate (ORR) will be provided together with a 95% CI also obtained with the use of a binomial distribution. Logistic regression models could be used to identify factors predictive of RCB 0/I or OR (if the numbers of events are sufficient to do so). For safety, frequency tabulations will be done for all grades of AEs and for ≥ grade 3 AEs. Grade 5 AEs will be reported separately. Tabulations will be done twice: once for all AEs and once for AEs related to treatment. Rates of subjects with at least one AE and one ≥grade 3 AE will also be estimated.

Translational research

The aim of translational research is to identify differences between responders and nonresponders in terms of genomic aberrations, transcriptional programs and transcription factors at baseline (pretreatment) and at surgery (post-treatment). Tumor biopsies will be collected before and after four months of neoadjuvant hormonotherapy and entrectinib (namely, at baseline and at surgery). In these samples, a comprehensive analysis of the genomic and transcriptomic landscape will be performed using whole-genome sequencing (WGS) or whole-exome sequencing (WES), RNA-sequencing and single-cell analyses. Moreover, normal DNA will be sequenced to exclude germline variants. The gene regulatory landscape will also be studied using ATAC-seq. ATAC-seq will allow the identification of transcriptional factors at a genome-wide level that orchestrate the transcriptional programs linked to response/resistance to hormonotherapy and entrectinib. By performing a genome-wide chromatin accessibility landscape profiling, interactions between distal regulatory elements, gene promoters and gene expression may be identified for key oncogenes. Plasma samples will be used for the analysis of circulating tumor DNA with the best available technology at the time of the analysis. All ER-positive, HER2-negative ILCs are potentially eligible for ROSALINE, regardless of E-cadherin status. E-cadherin status will be assessed in the context of translational analyses to explore its prognostic/predictive role. The sample size is larger than needed by statistical considerations to account for eventual rare CDH1-proficient ILC. All translational analyses on postneoadjuvant samples will be performed centrally at Institut Jules Bordet (Brussels, Belgium) after sample repatriation.

Ethical considerations

Written approval by the Ethics Committee and compliance with local regulatory conditions are required prior to the start of the study. All study procedures will be performed according to the ethical principles of the Declaration of Helsinki and in adherence to the principles of Good Clinical Practice. Patients will sign a written informed consent before any study-related procedures are conducted. All personal data will be treated in accordance with data protection laws, including the General Data Protection Regulation (GDPR).

Study status

There are ten participating sites in the ROSALINE study, in Belgium (Institut Jules Bordet, Brussels; UZ Leuven, Leuven; CHU UCL Namur Sainte Elisabeth, Namur; UZ Brussels, Brussels; Cliniques universitaires Saint-Luc, Brussels; Grand Hôpital de Charleroi, Charleroi; and UZ Gent, Gent) and France (Institut Curie, Paris; Institut Bergonié, Bordeaux; and Institut Gustave Roussy, Villejuif). The ROSALINE study began following approval by

the Ethical Committee and authorities and, as of 22 April 2022, 28 patients have been enrolled and 13 are already evaluable.

Conclusion

Although ILC is a distinct clinical and biological entity, patients with ILC are treated similarly to those with IDC in clinical practice and are included in the same clinical trials. The optimal treatment of patients with ILC represents an unmet need in modern oncology, and there is an urgent need for clinical studies appropriately designed and tailored to this specific subgroup of patients. ROSALINE (NCT04551495) is a phase II, neoadjuvant study targeting ROS1 in combination with endocrine therapy in patients with ILC. Of note, the loss of E-cadherin expression due to genomic alterations in its encoding gene, *CDH1*, is the most frequent oncogenic event in ILC. *In vivo* studies investigating synthetic lethality approaches have shown promising antitumor effects of ROS1 inhibitors in models of E-cadherin-defective breast cancer, providing a strong preclinical rationale for assessing their activity in this disease.

Executive summary

Background

- Invasive lobular breast cancer (ILC) represents 5–15% of all breast cancers and is the second most frequent histologic subtype after invasive ductal breast cancer (IDC).
- From the clinical, biological and pathological perspectives, ILCs have peculiar characteristics compared with IDCs.
- *CDH1* alterations, leading to the loss of the cell adhesion molecule E-cadherin, are the most common and almost pathognomonic genomic events in ILC.
- Nevertheless, patients with ILC are treated with the same drugs and in the same clinical trials as patients with IDC.
- Several unmet needs and areas of uncertainty exist in ILC and require the design of appropriate clinical studies specifically tailored to this subgroup of patients.

Entrectinib

- *In vivo* studies have shown the profound antitumor effects of ROS1 inhibitors in models of E-cadherin-defective breast cancer.
- Entrectinib is a potent small-molecule tyrosine kinase inhibitor that targets TRK, ROS1 and ALK tyrosine kinases, which are constitutively active in tumors with NTRK, ROS1 and ALK gene fusion.

ROSALINE

- ROSALINE is a single-arm, multicenter, phase II study aimed to evaluate the efficacy and safety of combining entrectinib and endocrine therapy in patients with stage IIA–IIIA, estrogen receptor (ER)-positive/HER2-negative ILC in the neoadjuvant setting (NCT04551495).
- Eligible patients are pre- or postmenopausal women aged 18 years or older with a histological diagnosis of ILC that is ER-positive, HER2-negative and >20 mm as measured by local breast MRI and cN0 or cN1 (i.e., stage IIA–IIIA).
- Patients will receive four 28-day cycles of letrozole (2.5 mg *per os* daily) in combination with entrectinib (600 mg *per os* daily) with or without goserelin (3.6 mg intramuscular every 28 days, only in premenopausal women) before surgery.
- The primary end point is the rate of residual cancer burden 0/1 by local assessment in all evaluable patients.
- Secondary end points are the rate of pathological complete response in the breast and axilla (ypT0/Tis ypN0), tumor objective response by local MRI and safety.
- A total of 45 patients are expected to be enrolled.

Supplementary data

An infographic accompanies this paper. To view or download this infographic in your browser please click here: https://www.futuremedicine.com/doi/10.2217/fon-2022-0358

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