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Original article

Ribociclib plus letrozole in early breast cancer: A presurgical, window-of-opportunity study

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ABSTRACT

Objectives: Cyclin D–cyclin-dependent kinase (CDK) 4/6–inhibitor of CDK4/6–retinoblastoma (Rb) pathway hyperactivation is associated with hormone receptor-positive (HR+) breast cancer (BC). This study assessed the biological activity of ribociclib (LEE011; CDK4/6 inhibitor) plus letrozole compared with single-agent letrozole in the presurgical setting.

Materials and methods: Postmenopausal women ($N = 14$) with resectable, HR+, human epidermal growth factor receptor 2-negative (HER2−) early BC were randomized 1:1:1 to receive 2.5 mg/day letrozole alone (Arm 1), or with 400 or 600 mg/day ribociclib (Arm 2 or 3). Circulating tumor DNA and tumor biopsies were collected at baseline and, following 14 days of treatment, prior to or during surgery. The primary objective was to assess antiproliferative response per Ki67 levels in Arms 2 and 3 compared with Arm 1. Additional assessments included safety, pharmacokinetics, and genetic profiling.

Results: Mean decreases in the Ki67-positive cell fraction from baseline were: Arm 1 69% (range 38–100%; $n = 2$), Arm 2 96% (range 78–100%; $n = 6$), Arm 3 92% (range 75–100%; $n = 3$). Decreased phosphorylated Rb levels and *CDK4*, *CDK6*, *CCND2*, *CCND3*, and *CCNE1* gene expression were observed following ribociclib treatment. Ribociclib and letrozole pharmacokinetic parameters were consistent with single-agent data. The ribociclib plus letrozole combination was well tolerated, with no Grade 3/4 adverse events over the treatment.

Conclusion: The results suggest absence of a drug–drug interaction between ribociclib and letrozole and indicate ribociclib plus letrozole may reduce Ki67 expression in HR+, HER2− BC (NCT01919229).

Introduction

Endocrine therapy is a key treatment strategy for hormone receptor-positive (HR+) breast cancer due to the dependency of these tumors on estrogen signaling [1]. Combining endocrine

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therapy with targeted therapies may enhance the effect of treatment by targeting compensatory pathways that act downstream of estrogen signaling. Short-term, window-of-opportunity studies of drug combinations can inform the optimal biological dose [2], enable the investigation of pharmacodynamic (PD) markers, identify biomarkers for patient selection, and may expedite drug development [3]. Moreover, short-term endpoints in window-of-opportunity studies, such as cell proliferation as measured by Ki67, can act as surrogate markers of longer-term patient outcomes [4], and several short-term studies have contributed to treatment decisions for endocrine therapy, including the potential of combination therapies [5] and the preferred patient biomarker profile [6].

The cyclin D–cyclin-dependent kinase (CDK) 4/6–inhibitor of CDK4/6 (INK4)–retinoblastoma (Rb) pathway acts downstream of estrogen receptor (ER) activation to promote cell cycle progression and cell division in response to estrogen signaling [7]. As such, endocrine therapy inhibits activation of this pathway, down-regulating cell proliferation [8]. Recent data demonstrate that endocrine therapy-resistant tumor cells are able to maintain cyclin D–CDK4/6–INK4–Rb pathway activity [1]. Additionally, the cyclin D–CDK4/6–INK4–Rb pathway is frequently disrupted in favor of cell cycle progression in HR+ breast cancer [9–11] and has been associated with poor clinical outcome [1]. Therefore, targeting the cyclin D–CDK4/6–INK4–Rb pathway may present an effective strategy to enhance the efficacy of endocrine therapies.

Ribociclib (LEE011) is an orally bioavailable, selective inhibitor of CDK4/6 that prevents Rb phosphorylation, resulting in G1 cell cycle arrest *in vitro* [12,13]. Ribociclib has exhibited synergistic activity with letrozole in preclinical xenograft models of ER-positive (ER+) breast cancer [14]. In clinical trials, ribociclib has demonstrated clinical activity both as a single agent in patients with advanced solid tumors, and when administered in combination with letrozole to patients with advanced ER+, human epidermal growth factor receptor 2-negative (HER2−) breast cancer [15,16].

We report results of a Phase II, window-of-opportunity, pre-surgical treatment study evaluating the safety, pharmacokinetics (PK), and PD of two clinical doses of ribociclib (400 mg and 600 mg) in combination with letrozole versus single-agent letrozole in HR+ early breast cancer (ClinicalTrials.gov study number: NCT01919229).

Material and methods

Study design

The primary objective of this multicenter, randomized study (Fig. 1) was to assess the difference in antiproliferative activity of ribociclib in combination with letrozole versus single-agent letrozole, as measured by changes in expression level of the proliferative marker Ki67 from baseline to time of surgery. Secondary objectives included the assessment of safety, tolerability, and PK of ribociclib and letrozole in combination, and the evaluation of PD markers related to ribociclib activity in breast cancer. The study also evaluated potential correlations between ribociclib exposure and major safety and biomarker parameters, changes in biomarkers related to the cyclin D–CDK4/6–INK4–Rb pathway, and the role of circulating tumor DNA (ctDNA) as a potential platform for molecular characterization. Patients were treated with once-daily letrozole 2.5 mg (Arm 1), with or without once-daily ribociclib 400 mg (Arm 2) or 600 mg (Arm 3) for 14 days (±3 days) prior to surgery.

Patient population

Adult postmenopausal women with treatment-naïve, newly diagnosed, surgically resectable, Grade II/III HR+, HER2− invasive breast cancer were included in this study. Patients were required to have at least one breast lesion with a diameter of ≥1.0 cm confirmed by ultrasound, mammography, computed tomography, or magnetic resonance imaging. All patients had an Eastern Cooperative Oncology Group performance status of 0 or 1 and adequate bone marrow and organ function. Patients were excluded based on the presence of a concurrent malignancy or a history of malignancy within 3 years of randomization, with the exception of adequately treated basal cell skin cancer, squamous cell carcinoma, non-melanomatous skin cancer, or curatively resected cervical cancer. Key exclusion criteria also included active cardiac disease or a history of cardiac dysfunction, including having a left ventricular ejection fraction of <50% as determined by a multiple-gated acquisition scan or an echocardiogram, and a QT corrected using Fridericia's formula (QTcF) of >450 ms. Patients were excluded if they were receiving medications that are known strong inducers or inhibitors of cytochrome P450 3A4 (CYP3A4), have a narrow therapeutic window and are predominantly metabolized through CYP3A4, or have a known risk of prolonging the QT interval or inducing Torsades de Pointes.

Safety assessments

Safety assessments were conducted at baseline and at scheduled intervals throughout the study. Hematology, blood chemistry, thyroid function, vital signs, and physical condition were regularly monitored. Cardiac function was monitored by performing triplicate electrocardiograms (ECGs) within 72 h prior to randomization and again on Days 1, 8, and 14 at the following time points: pre-dose and 2, 4, and 6 h after treatment dose. In each case, the ECG measurements were collected prior to PK sampling. In addition, patients were fitted with a Mortara H12+ Holter (Mortara Instrument, Milwaukee, WI, USA) instrument to carry out continuous ECG recordings over a 24-h period both at baseline (within 1 day prior to the first dose) and on Day 14 approximately 24 h prior to surgery. Adverse events (AEs) were assessed continuously according to the Common Terminology Criteria for Adverse Events version 4.03.

Pharmacokinetic assessments

Blood samples for the analysis of ribociclib and letrozole plasma concentrations were collected on Days 1, 8, and 14 at pre-dose and 2, 4, and 6 h after treatment dose. An additional PK blood sample was collected on Day 15 approximately 24 h after the last treatment dose on Day 14 and immediately prior to surgery. Plasma concentrations were measured using validated liquid chromatography–tandem mass spectrometry with a lower limit of quantification (LLOQ) of approximately 1.0 ng/mL for ribociclib and 2.0 ng/mL for letrozole. PK parameters were derived from individual plasma concentration–time profiles using non-compartmental analysis (Phoenix®; Pharsight, Mountain View, CA, USA) and were summarized using descriptive statistics.

Pharmacodynamic and biomarker assessments

Both tumor tissue samples and plasma samples for ctDNA were collected at baseline prior to the first dose of treatment and on Day 15 (±3 days) at, or immediately prior to, surgery. Blood samples for estradiol assessment were collected prior to the first dose of study treatment and prior to surgery on Day 14 (±3 days). Immunohistochemistry (IHC) detection of Ki67-positive tumor cells was performed on baseline and surgery tumor tissue samples to assess changes in the percentage of positive tumor cells. To assess the PD activity of ribociclib, changes in S780-phosphorylated Rb (pRb) levels in tumor samples were evaluated by IHC with H-score values