Background

The recent introduction of CDK4/6 inhibitors has been one of the most pivotal breakthroughs in breast cancer therapy in the last decades. A growing body of evidence is now proposing that novel targeting inhibitors of MAPK, PI3K and CDK4/6 may have anti-tumor effects beyond their direct cytostatic effects, such as immunomodulatory properties. Specifically, the CDK4/6 inhibitors have shown evidence of having immunomodulatory and immunogenic effects, the ability to induce a senescent-like phenotype in the tumor cells and exert changes in the metabolism of the tumor cell.

Patients and methods

Trial: NeoLeteRib is a multicenter, single-arm, open-label, neoadjuvant, phase II trial.

Eligibility criteria: Patients suffering from locally advanced breast cancer defined as either large T2, or T3/T4, and/or N2-3 are suitable for inclusion. Both, luminal-A and luminal-B tumors are allowed. HER-2 positive and triple-negative patients are excluded from this trial. Patients with any signs of distant metastasis are also excluded from participation.

Treatment: All patients receive neoadjuvant therapy for at least 6 months with ribociclib (starting dose: 600 mg daily; 21 days on/7 days off) and letrozole (2.5 mg daily). Premenopausal women also receive goserelin (3.6 mg sc. every 28 days) according to clinical practice.

Primary point: (Translational research): to study the direct and indirect anti-tumor effects of letrozole and ribociclib given in combination in individual patients suffering from locally advanced breast cancer.

Objectives:

1. To study potential mechanisms of adaptation and resistance to aromatase inhibitor (letrozole) in combination with the CDK4/6 inhibitor.
2. To study the microenvironment of the tumor and changes during neoadjuvant therapy with letrozole and ribociclib.
3. To study the relationship between treatment effects and the gut microbiota.
4. To search for surrogate parameters (tumor- or immune related markers etc.) allowing to estimate the anti-tumor effects in liquid biopsies during therapy compared to the baseline situation.

The trial has had its date (11.11.2021) included 15 patients (planned goal: 100).

Pathological and diagnostic related analyses have been conducted according to standard patient assessment at the two recruiting centers in Norway. In addition, single cell RNA-sequencing (scRNA-seq) analyses on the fresh tumor tissue have been conducted.

Perspectives: Our preliminary results from single cell analyses indicate that the final results will allow us to identify different cell types composing the tumors at the three different time points during therapy: baseline (before treatment), 21 days after start of treatment and before surgery at the end of the neoadjuvant treatment (6 months). Furthermore, with all endpoints combined, the study will give detailed information on immunogenic and immuno-modulatory effects of ribociclib in treatment-naive patients, including effects on the gut microbiota as well as senescence.

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**Figure 1:** Biorepot collected from patients included in the clinical trial and analyses planned.

**Figure 2:** The NeoLeteRib trial: design, timelines and selected procedures.

**Figure 3:** UMAP showing NeoLeteRib (NRx samples) evaluated at different time points. Numbers represent the recruitment of patient in the trial and letters the time point at which the sample has been collected. **a:** cells on the UMAP are color coded according to the sample they belong to **b** time point, **c:** whether the cells have a successful TCR sequencing, **d:** cell type

**Table 1:** UMAP showing NeoLeteRib (NRx samples) evaluated at different time points. Numbers represent the recruitment of patient in the trial and letters the time point at which the sample has been collected. **a:** cells on the UMAP are color coded according to the sample they belong to **b** time point, **c:** whether the cells have a successful TCR sequencing, **d:** cell type