

## LAY ABSTRACT

**Clinical problem addressed:** Estrogen receptor positive (ER+) metastatic breast cancer (MBC) is treated with a combination of drugs: antiestrogens and cyclin-dependent kinase 4 and 6 inhibitors (CDK4/6i). Unfortunately, drug resistance to both antiestrogens and CDK4/6i is prevalent. ER+ MBC that is resistant to these standard of care therapies is treated with chemotherapies such as taxanes or capecitabine, or with mTOR inhibitors. However, treatment with chemotherapies show meager success and adverse side effects. Better therapies that kill drug resistant metastatic cancer cells but spare normal cells are urgently needed. In this study, we will determine if BOLD-100, a novel ruthenium-based small molecule drug that targets the DNA damage repair pathway in cancer cells, can be used to effectively treat ER+ MBC.

**Significance:** BOLD-100 is a promising new therapy for metastatic cancer. The clinical studies conducted so far show anticancer effects with no bone marrow suppression. Side effects of BOLD-100 included nausea, vomiting, dehydration or anemia. BOLD-100 is currently being tested in a Phase 1b/2a clinical trial in combination with FOLFOX (fluorouracil, oxaliplatin and leucovorin) in patients with advanced gastrointestinal cancers (NCT04421820). Based on interim outcomes announced at the annual meeting of American Society of Clinical Oncology (ASCO) in June 2022 (personal communication with BOLD Therapeutics), in 19 out of the total of 69 patients, the treatment regimen is being well-tolerated, with no unexpected Grade 3 or 4 treatment-emergent adverse events identified. In this trial, efficacy data for third line or beyond colorectal cancer patients (n=7) showed a PFS of 6.4 months, which represents a 3-times improvement compared to benchmark data of 2.0 months for standard of care treatments. The disease control rate, which measures complete response, partial response or stable disease, was 78%. Through our published data, our lab was the first to show that BOLD-100 interfered with the DNA repair pathway, and boosted the effect of capecitabine in breast cancer cells. As a monotherapy, there is no improvement in overall survival (OS) in patients treated with capecitabine compared with treatment containing no capecitabine. Therefore, a combination of BOLD-100 and capecitabine could be a promising new anticancer therapy for ER+ MBC patients.

**Rationale and Hypothesis:** As monotherapies, capecitabine stops growth of cancer cells by inhibiting DNA/RNA synthesis while BOLD-100 targets proteins associated with the DNA repair pathway. Our preliminary data showed that BOLD-100 improved the efficacy of capecitabine, compared with capecitabine alone, in inhibiting growth of ER+ breast cancer cells that are resistant to antiestrogens and CDK4/6i. Therefore, ***we hypothesize that combination of BOLD-100 will augment the anticancer effects of capecitabine in ER+ metastatic tumors that are resistant to CDK4/6i and antiestrogens.*** Our proposed studies will offer new effective treatment options for ER+ MBC patients.

**Specific aims:** Through two aims, we will obtain further insights into molecular mechanisms and preclinical data in tumor models, which will be crucial for justifying future clinical trials to test BOLD-100 and capecitabine in ER+ MBC patients. In **Aim 1**, we will use antiestrogen and CDK4/6i resistant ER+ breast cancer cell models to uncover molecular pathways that are triggered when cells are treated with BOLD-100 and capecitabine. This mechanistic knowledge is important to improve drug combination regimens and to identify protein biomarkers that corresponds with treatment efficacy. In **Aim 2**, we will use mouse and patient-derived PDX models of antiestrogen and CDK4/6i resistant MBC to test the efficacy of BOLD-100 and capecitabine compared with control, BOLD-100 alone or capecitabine alone. These results will provide preclinical evidence for the efficacy of the combination of BOLD-100 and capecitabine in inhibiting growth of MBC.

**Clinical Impact:** Successful completion of our proposed studies will provide a novel therapeutic option to effectively treat breast cancer patients who progress on antiestrogens and CDK4/6i. Outcomes from this study will enable the discovery of proteins involved in response to the combination of capecitabine and BOLD-100 combination treatment, which could be used as predictive biomarkers in MBC. Moreover, positive outcomes from the studies in tumor models will provide strong preclinical validation to conduct clinical trials with BOLD-100 and capecitabine for patients with advanced MBC. BOLD-100 is already showing encouraging therapeutic benefit in gastrointestinal cancers. Thus, findings from our proposed study have the potential to positively impact clinical treatment of MBC patients.