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Original article

Combined effects of neoadjuvant letrozole and zoledronic acid on γδT cells in postmenopausal women with early-stage breast cancer

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ABSTRACT

Introduction: Adjuvant bisphosphonates lead to better prognosis in postmenopausal breast cancer. However, the association between clinical outcomes and immune modulation by them is still unclear.
Methods: In this prospective, open-label phase II study, postmenopausal women with estrogen receptor-positive and human epidermal growth factor receptor 2-negative early-stage breast cancer received neoadjuvant letrozole (LET) for one month, followed by treatment with a single dose of zoledronic acid. The patients underwent an additional 5 months of treatment with LET prior to surgery. The primary endpoint was the tumor objective response rate (ORR) determined by diameter via MRI. The association between the ORR and γδT cell frequencies was assessed as a secondary endpoint.
Results: Out of sixty patients, 55 patients were evaluable for response by MRI. The ORR for LET with zoledronic acid was 38.2% (21/55), which was comparable to that of historical controls (45%). A decrease in the frequency of the Vδ2 T cell subset was observed throughout treatment, and Vδ2 T cells were activated for 6 months. In planned subgroup analyses, patients with low frequencies of Vδ2 T cells prior to zoledronic acid infusion experienced a favorable tumor response compared to those with high frequencies (59.3% [16/27] vs 17.9% [5/28], p = .002). There were no serious adverse events with this treatment regimen.
Conclusion: These results showed that neoadjuvant LET with zoledronic acid could not achieve overall effect for local tumor response. However, patients with a low frequency of γδ T cells would benefit from the treatment including zoledronic acid. (UMIN 000008701).

1. Introductions

Breast cancer is a common disease worldwide, and estrogen receptor (ER)-positive breast cancer accounts for 70% of the total cases. Adjuvant hormone therapy is currently the standard of care for ER-positive breast cancer, and aromatase inhibitors have greater benefits than tamoxifen in the treatment of postmenopausal women with early-stage breast cancer [1]. However, it is still necessary to maximize the efficacy of adjuvant endocrine therapy to decrease the mortality rate in ER-positive breast cancer. Neoadjuvant endocrine therapy is accepted as a feasible option for postmenopausal patients with highly endocrine-responsive disease

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[2]. Moreover, this treatment modality can provide an opportunity to better understand the molecular determinants of neoadjuvant endocrine therapy and identify subgroups that may benefit from this treatment [3].

Accumulating evidence from previous studies has demonstrated that adjuvant bisphosphonates can improve the prognosis of breast cancer [4–6]. The Early Breast Cancer Trialists' Collaborative Group (EBCTCG) meta-analysis shows that adjuvant bisphosphonates improve the survival rate in postmenopausal women [7]. The addition of zoledronic acid to neoadjuvant chemotherapy [8–11] and endocrine therapy [12] achieved better and modest clinical outcomes, respectively. The systemic anti-tumor effect of zoledronic acid was only observed in postmenopausal women, but the precise mechanism remains unclear.

Among immune cells, human γδ T cells account for 1–5% of the total peripheral T cells and play an important role in anti-infectious and anti-tumor immunity [13]. The subset of Vδ2-bearing Vγ9Vδ2 (also known as Vγ2Vδ2) T cells accounts for 50–95% of γδ T cells in healthy adults. Activated Vδ2 T cells exhibit cytotoxic activity against tumors, and the infiltration of Vδ2 T cells into tumor sites has been observed in various tumors, including lung [14], renal [15] and breast cancer [16]. Modest therapeutic effects were reported in breast cancer for both adoptive and active immunotherapies targeting Vγ9Vδ2 T cells [17].

Nitrogen-containing bisphosphonates including zoledronic acid are taken up not only by myeloid lineage cells but also by tumor cells through fluid phase endocytosis. Zoledronic acid inhibits farnesyl diphosphate synthetase (FDPS) in the mevalonate pathway, thereby inducing cell death by blocking prenylation of small GTPases [18]. Inhibition of FDPS also results in the accumulation of its upstream metabolite, isopentenyl pyrophosphate, which stimulates and activates Vγ9Vδ2 T cells in a butyrophilin 3A1-dependent manner [19].

Taken together, we hypothesize that the clinical efficacy of adjuvant bisphosphonates may be related to the immune modulatory effects on γδ T cells. In the present study, we performed a single-arm prospective trial to investigate the association between the clinical efficacy and immune modulation of γδ T cells for neoadjuvant letrozole (LET) combined with zoledronic acid in postmenopausal women with early-stage breast cancer.

2. Methods

2.1. Study design, patients and intervention

The study design and patient population were previously described [20]. Briefly, the patients enrolled in the present study were postmenopausal women with ER-positive, human epidermal growth factor receptor 2 (HER2)-negative, and clinical T1 or T2 N0M0 breast cancer. After registration, patients were started on 2.5 mg/day of LET orally and received a single dose of zoledronic acid at one month. The dose of zoledronic acid was reduced based on renal function. The patients continued to take LET every day for the following 5 months until undergoing breast surgery within two weeks.

2.2. Measurements

The patients were clinically evaluated according to the trial schedule summarized in supplementary Table 1. The primary endpoint is the objective response rate (ORR) based on the MRI diameter. The secondary endpoints are the changes in tumor volume via MRI, and the ORR evaluated using a caliper and ultrasonography (US). The frequencies of immune cells, including γδ T cells, were measured throughout the treatment. MRI diameter and volume were assessed at baseline and at 3 and 6 months by an independent central committee. Investigators evaluated the ORR using a caliper and US. Serum interferon (IFN)-γ was subject to enzyme-linked immunosorbent assays (ELISA) within hours after the administration of zoledronic acid. The frequencies of immune cells were analyzed by two-color flow cytometry prior to the administration of zoledronic acid and at 2 and 6 months. The following monoclonal antibodies (mAbs) were used: anti-CD3, anti-Vδ1, anti-Vδ2, anti-CD4, anti-CD8, anti-CD25, and anti-NKG2D mAb as previously described [20].

2.3. Statistical analysis

The present study was a single-arm phase II study. The ORR of neoadjuvant LET was reported as 45%, whereas the ORR of zoledronic acid-combined neoadjuvant LET was expected to be 60%. A sample size of 69 was chosen using the Bayesian predictive probability criterion based on a previous design [21], with an expected ORR of 60%, a null hypothesis of 45% [22], and non-informative analysis prior and pre-specified probability thresholds of 95% (akin to a significance level of 5%) and 80% (akin to power). Therefore, a sample size of 75 was determined, considering ineligible patients.

The primary endpoint was analyzed using the Bayesian method as previously described. As a reference, Clopper-Pearson's exact confidence intervals (CIs) were provided for the ORR. For the pre-planned subgroup analyses, the γδ T cell frequency and immune-related properties were assessed in context with the ORR using Fisher's exact test. Associations between the γδ T cell frequency and clinical pathological and immune-related factors were planned as exploratory analyses. A two-sided significance level of 5% was used to calculate CIs throughout the exploratory analyses. No adjustment was made for multiple comparisons for any of the endpoints or subgroups analyzed. All analyses were performed using SAS statistical software, version 9.4 (SAS Institute, Cary, NC).

3. Results

3.1. Patient characteristics

The first patient was enrolled in August 2013, and data collection ended in October 2015. The trial was planned to recruite 75 patients, of which 61 postmenopausal women with ER-positive early-stage breast cancer recruited from 6 centers in Japan were enrolled to receive neoadjuvant LET. Patient disposition is summarized in supplementary Table 2. One patient who received an overdose of zoledronic acid was excluded from the full analysis set due to a major protocol violation. Five of the 60 patients did not receive either zoledronic acid or surgery. Fifty-one patients received surgery and were provided the surgical specimen. The safety analysis set comprised 56 patients. Patient demographics and baseline tumor characteristics are summarized in Table 1.

3.2. Objective response rate

Out of 60 patients, 55 individuals underwent MRI assessment of tumor response. The calculated ORR was 38.2% (21/55; 95% CI, 25.4 to 52.3), and the Bayesian posterior probability of exceeding the threshold of 45% was 16%; this value did not meet the pre-specified probability threshold of 95%. The ORR at the end of treatment, measured by caliper (n = 56) and by ultrasound (n = 58) was 50.0% (28/56; 95% CI, 36.3 to 63.7) and 51.7% (30/58; 95% CI, 38.2 to 65.0), respectively (Table 2). The median volume reduction on MRI from the baseline value was 51.0% (95% CI, 34.7 to 59.3) at 6 months. A