A Randomized Phase II Trial Investigating the Effect of Platelet Function Inhibition on Circulating Tumor Cells in Patients With Metastatic Breast Cancer

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Abstract

A randomized phase II clinical trial that investigated the effect of platelet inhibition on circulating tumor cells (CTCs) in patients with metastatic breast cancer was performed. The negative results from this translational study highlight the challenges of biomarker-driven trials and provide data pertinent to designing future related studies.

Background: Blockade of platelet activation and aggregation can inhibit metastasis in preclinical models and is associated with cancer prevention. To test whether disruption of platelet function with clopidogrel and aspirin would decrease the number of circulating tumor cells (CTCs) in patients with metastatic breast cancer, a randomized phase II study was performed. **Methods:** Patients with metastatic breast cancer who were not currently receiving cytotoxic chemotherapy were eligible. Patients were randomized to receive either clopidogrel and aspirin or to a control group receiving no treatment. Phlebotomy was performed at baseline, at 2 and 4 weeks, and monthly thereafter to obtain specimens to assess CTC, platelet aggregation, and thrombin activity. The primary end point was the proportion of patients with detectable CTCs at 1 month. **Results:** Forty-eight patients were enrolled and 42 were evaluable at 1 month. Baseline CTC numbers were ≥ 5 in 13% and ≥ 1 in 65% of patients. Despite adequate platelet function inhibition in the treatment group, the proportion of patients with detectable CTCs was similar between the clopidogrel/aspirin and control groups at baseline ($P = .21$) and 4 weeks ($P = .75$), showing no treatment effect. Measured endogenous thrombin potential did not correlate with CTC number. No bleeding-related serious adverse events (SAEs) occurred. **Conclusion:** The baseline CTC numbers were lower than expected, decreasing the ability to detect an impact of platelet inhibition on CTCs. Clopidogrel and aspirin were well tolerated. Future studies evaluating the potential therapeutic role of antiplatelet therapy in breast cancer remain of interest, and they may be informed by these results.

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Introduction

The metastatic process is complex and involves migration from the primary tumor site, vascular invasion, evasion of host immune defenses, dissemination (often as microemboli with tumor cells, platelets, and other blood cells), extravasation, and proliferation. Cancer can be associated with a prothrombotic state, increasing the risk of venous thromboembolic events.[1,2] Blood components have been implicated in playing a direct role in the development of

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metastatic disease.[3] Platelets may play a crucial role in the metastatic process by affecting tumor cell arrest and adhesion at distant metastatic sites and can protect tumor cells from immune attack.[4] Gasic et al. demonstrated that lowering the platelet count in mice resulted in decreased lung invasion after intravenous injection of tumor cell lines.[5] Furthermore, antibodies directed against platelet antigens involved in tumor cell adhesion decreased lung tumors in mice after intravenous administration.[6-8] Preclinical models have demonstrated that platelets together with tumor-secreted proteins influence the premetastatic bone microenvironment and may promote the metastatic process.[9] In vivo experiments have shown that thrombin, a platelet activator, increases pulmonary metastasis, whereas a thrombin inhibitor (r-hirudin), decreases melanoma pulmonary metastases.[6,10-13] Clinically, it has been shown that the addition of antiplatelet therapy to chemoradiotherapy in the treatment of patients with small-cell lung cancer prolongs duration of remission and overall survival.[14]

Studies that have used single-agent antiplatelet therapy in cancer have been promising. It is of note that in the clinical management of cardiovascular diseases, dual-platelet therapies have shown superior results. Circulating tumor cell (CTC) number has been associated with overall and progression-free survival in women with metastatic breast cancer.[15] To investigate the effect of dual-antiplatelet therapy on CTCs in women with metastatic breast cancer, a randomized controlled study was conducted using clopidogrel and aspirin to test the hypothesis that platelet inhibition decreases the number of CTCs, a surrogate for cancer outcomes.

Patients and Methods

Patients and Treatment

Women without actively progressing metastatic breast cancer who were not currently receiving chemotherapy were eligible. Previous chemotherapy (adjuvant or metastatic) was permitted. Concurrent endocrine therapy (for at least 2 months before enrollment), bisphosphonate therapy, and/or trastuzumab were permitted. Patient care was directed by the treating physician as clinically indicated. Eligible patients had an estimated survival $\geq$ 3 months, no platelet inhibitor therapy within 1 month of study, platelet count $\geq$ 100,000/mm^3, international normalized ratio within 0.81 to 1.20, and normal kidney and liver function by institutional standard laboratory evaluation. Exclusion criteria were planned surgery, serious bleeding disorders, history of significant bleeding related to peptic ulcer disease, standing therapy with nonsteroidal antiinflammatory drugs or other platelet inhibitors, and anticoagulant therapy. The institutional review board approved the trial and written consent was obtained from all patients before enrollment.

Patients were randomized to receive either clopidogrel (300 mg loading dose followed by 75 mg orally daily) and aspirin (325 mg orally daily) combination therapy, or no study treatment. Initially, study therapy could be continued until tumor progression, discontinuation of therapy because of an adverse event (AE), or withdrawal of consent. The protocol was later amended to change the maximum study duration to 6 months.

Measurement of Circulating Tumor Cells

CTCs were measured at baseline, at 2 weeks and 4 weeks, and then monthly thereafter. Patient blood samples were collected into 10-mL CellSave (Veridex, Raritan, NJ) tubes and were stored at room temperature and subsequently processed within 72 to 96 hours of collection. CTCs were isolated and measured using the CellSearch assay (Veridex, Raritan, NJ). Samples were analyzed at Washington University with the exception of those collected on Fridays, which were sent to Quest Diagnostics (Madison, NJ) to avoid a processing delay. CTC numbers are reported per 7.5 mL of blood, and the limit of detection for the CellSearch assay is 1 CTC/7.5 mL of blood. The technical details of CellSearch have previously been described.[16]

Platelet Function Testing

To show study treatment pharmacologic efficacy, blood samples were collected for platelet function testing using the VerifyNow system (Accumetrics, San Diego, CA) with P2Y12 and aspirin cartridges. VerifyNow is a turbidimetric-based optical detection system that measures aspirin or clopidogrel inhibition of platelet aggregation.[17] Citrated whole blood is added to a test cartridge containing fibrinogen-coated beads and a platelet activator: adenosine diphosphate (ADP) with prostaglandin E (to specifically activate the ADP P2Y12 receptor in the case of clopidogrel) or arachidonic acid (to synthesize thromboxane A_2 in the case of aspirin). Aggregation of activated platelets to fibrinogen-coated beads increases light transmittance, which is reported in aspirin reaction units (ARUs) or P2Y12 reaction units (PRUs). Lower ARU and PRU results are expected when patients take aspirin and clopidogrel.[17] Percent inhibition of platelet function by clopidogrel is determined by dividing ADP-induced platelet aggregation (PRU) by thrombin-induced platelet aggregation.

Given the association of malignancy and hypercoagulability and the possible activating effect of CTCs on platelets, the global hemostatic function was evaluated. Thrombin generation, which measures the overall thrombogenic potential of the plasma sample, was measured in patient plasma samples using the Calibrated Automated Thrombogram system (CAT) (Diagnostica Stago, Parsippany, NJ) and the endogenous thrombin potential percentage (ETP%) was calculated. ETP% is a global measure of the thrombogenic potential, as opposed to the more traditional tests such as prothrombin time and the activated partial thromboplastin time, which measure the activity of the extrinsic pathway of coagulation or the intrinsic pathway, respectively. The CAT system is based on the principles for automated thrombin generation measurement as described previously.[18] Briefly, platelet-poor plasma is combined with an activator containing phospholipids and human recombinant tissue factor. Generated thrombin cleaves a peptidyl substrate specific for thrombin, which releases fluorescent 7-amino-4-methylcoumarin. Thrombin-generation curves are plotted as thrombin concentration vs. time, with endogenous thrombin time (ETP) being the area under the curve. ETP% is then generated by normalizing the experimental sample with the ETP from a normal plasma sample.

Statistical Analysis

The primary end point was proportion of patients with detectable CTCs at 1 month. It has been shown previously that more than 50% of patients with metastatic breast cancer have detectable numbers of CTCs in the blood.[15] Power analysis determined that