TECHNICAL ABSTRACT

Background: Bone is the most common site of metastasis for human breast cancer (BCa), which results in significant morbidity and mortality in patients with advanced disease. A vicious cycle, arising due to the interaction of BCa cells and cells in the bone microenvironment results in the activation of osteoclasts and increased osteolytic bone destruction. The major treatment to reduce the burden of bone metastasis in BCa patients is bisphosphonate therapy. Despite significant efforts to improve the potency of bisphosphonates, the complications are only retarded but not prevented. Thus, development of newer therapies that can both ameliorate the threshold of bone destruction and increase survival of patients with metastatic breast disease will be highly beneficial.

A better understanding of the molecular events in BCa osteolytic bone destruction indicates that the receptor activator of nuclear factor kappa-B ligand (RANKL) stimulates the recruitment, differentiation, and activation of osteoclasts by binding to RANK. Osteoprotegerin (OPG) is a "decoy" receptor that competes with RANK for RANKL, thus, modulating the effects of RANKL. However, during the metastatic event involving cancer and stromal cell interaction, endogenous OPG levels are markedly reduced. Thus, OPG remains as an effective molecule for future therapies for bone metastasis. Recently, we developed a recombinant vector expressing OPG and tested its potential in reducing bone destruction in a xenograft model using mesenchymal stem cells (MSC), genetically modified to overexpress OPG in localized osteolytic bone lesion in the tibia. Despite the potential of genetically-engineered MSC in bone regeneration, a major limitation in their use is poor homing of transplanted MSC to the bone. In order to overcome this, we further developed a strategy by transient, ectopic expression of a bone homing signal on MSC prior to *in vivo* administration. This led to a marked increase in bone homing of MSC and importantly, also reduced their lung entrapment. These efforts on the potential of MSC, modified to express OPG, in remodeling damaged bones and a unique strategy for targeted delivery of MSC to the bone position us to now test the therapeutic potential of this approach in an immunocompetent mouse model, with osteolytic damage in all major bones as in the human patients. Further, combining this approach with stable anti-angiogenic therapy, targeted to both tumor cells and tumor endothelium, we envision long-term survival in this preclinical model, which will allow us to initiate phase-1 trials for human patients in our cancer center.

Objective/Hypothesis: The central hypothesis of the proposed work is bone-targeted delivery of genetically-engineered MSC, over-expressing OPG, will prevent osteolytic bone damage and restore skeletal remodeling. Further, based on the requirement of angiogenesis for tumor growth in primary and metastatic sites, in combination with a systemically stable anti-angiogenic therapy, long-term survival will significantly increase. These hypotheses will be tested in this proposal using an immnocompetent, preclinical mouse model of BCa dissemination to all major bones as in human patients.

Specific Aims: ***1)*** To determine therapeutic effects of genetically-modified MSC, overexpressing OPG, for osteolytic bone damage using a bone-targeted delivery, in an immunocompetent mouse model of BCa dissemination to the bone, ***and 2)*** To determine the combined effect of MSC-OPG therapy with stable anti-angiogenic therapy for long-term survival.

Study Design: Bone dissemination model of syngeneic breast cancer cell line 4T1, constitutively expressing luciferase for non-invasive imaging, will be developed in immunocompetent BALB/c mice. Upon establishment of tumors in total body skeleton, MSC, genetically-engineered to overexpress OPG will be further modified to transiently express alpha-4, beta-1 integrin for enriched bone homing. The modified stem cells will be systemically transplanted. Synergistic effects of this therapy to tumor angiogenesis will be determined using a systemically stable anti-angiogenic therapy in the same immunocompetent BALB/c mouse model.

Impact: The development of MSC-OPG therapy, and in combination with anti-angiogenic therapy for BCa bone metastasis will benefit both as a primary, and as an adjuvant therapy. Successful completion of this study in a preclinical model will allow us to translate the outcome to phase-1 human clinical trial in future.