

# Targeting T cell – osteoblast crosstalk for bone homeostasis: a novel application of low-dose staphylococcal enterotoxin C2 mutant in osteoporosis

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**INTRODUCTION:** Previous studies suggest that T cells play a critical role in maintaining bone homeostasis, making them a potential therapeutic target for osteoporosis. Staphylococcal enterotoxins (SEs) type C (SECs) are a group of highly conserved proteins that retain T cell stimulation ability but with lower toxicity. Among SECs, SEC2 has been developed as a drug for immunotherapy or fracture repair. Recently, a mutant form of wild type SECs protein named SEC2 2M-118 with reduced toxicity and higher affinity to major histocompatibility complex class II (MHC II) molecule and T cell receptor (TCR) has been developed. This study is to investigate the safety and efficacy of this SEC2 mutant in the treatment of osteoporosis.

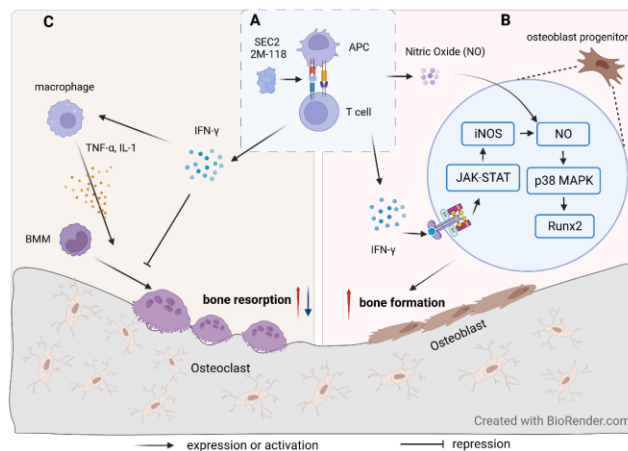
**METHODS:** For the animal experiment, female 12-week-old C57BL/6 mice, BALB/c mice, and T cell-deficient nude mice (BALB/c-Foxn1nu/Arc) were used in this study with institutional ethical approval. Ovariectomy (OVX) was conducted to induce bone loss in these mice and SEC2 2M-118 was given intraperitoneally to the mice twice a week. Pure T cells were isolated from BALB/c wild type mice and transferred into another batch of T cell deficient nude mice. Bone mass, mechanical properties, bone morphology, and bone remodeling were determined by micro-CT, three-point bending, histomorphometry, immunohistochemistry and ELISA assays after treatment. Major organs were also harvested for safety observation. For the cellular experiments, splenic lymphocytes or pure T cells were isolated from C57BL/6 mice, BALB/c mice, and T cell-deficient nude mice and primed with SEC2 2M-118. Osteogenic differentiation potential of osteoblastic cells MC-3T3-E1 was determined after treated with the conditioned medium. Cytokines in the medium were measured by qRT-PCR and ELISA. To clarify the potential mechanism of the selected cytokine IFN- $\gamma$  on osteoblastic cells, RNA sequencing and bioinformatic analysis were conducted. Nitric oxide (NO) radical scavenger, JAK-STAT signaling inhibitor and p38 MAPK inhibitor were applied to further verify the potential signaling pathway involved.

**RESULTS SECTION:** Systemic administration of low-dose SEC2 2M-118 dramatically alleviates OVX-induced bone loss via modulating T cells. Specially, SEC2 2M-118 treatment increases trabecular bone mass significantly via promoting bone formation in OVX mice. These beneficial effects are largely diminished in T cell-deficient nude mice and can be rescued by T cell reconstruction. Neutralizing assays determine IFN- $\gamma$  as the key factor that mediates the beneficial effects of SEC2 2M-118 on bone. Mechanistic studies demonstrate that IFN- $\gamma$  stimulates JAK-STAT signaling, leading to enhanced production of NO, which further activates p38 MAPK-Runx2 signaling and promotes osteogenic differentiation. IFN- $\gamma$  also directly inhibits osteoclast differentiation, but this effect is counteracted by pro-absorptive factors TNF- $\alpha$  and IL-1 $\beta$  secreted from IFN- $\gamma$  stimulated macrophages.

**DISCUSSION:** This study reported a novel application of mutant SEC2 2M-118 on maintaining bone homeostasis in OVX mice. The anabolic effects of SEC2 2M-118 treatment result from T cell-derived IFN- $\gamma$ , and JAK/STAT-NO-p38 MAPK-Runx2 signaling pathway may be the potential mechanism for the promotion of bone formation. The current work provides evidence and insights for targeting T cells as a potential new therapeutic approach for osteoporosis treatment. Nevertheless, more investigations are needed to verify our findings in large animal models and clinical trials. Future investigations into the crosstalk between immune and skeletal systems may foster the development of novel treatment strategies for bone metabolic disorders such as osteoporosis.

**SIGNIFICANCE/CLINICAL RELEVANCE:** This study provides insights for developing new therapeutics which target T cells for the treatment of osteoporosis.

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Schematic figure of this study showing the mechanism of SEC2 2M-118 in regulating bone homeostasis. A) Bone marrow T cells stimulated by SEC2 2M-118 secrete IFN- $\gamma$  and nitric oxide (NO) into microenvironment. B) IFN- $\gamma$  promotes bone formation via JAK/STAT-NO-p38 MAPK-Runx2 signaling pathway. C) SEC2 2M-118 modulates bone resorption via factors secreted from T cells and macrophages.