Nell-1 Enhances Bone Formation in an Osteoporotic Sheep Model

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Introduction
Osteoporosis, a disease characterized by a decrease in bone mineral density and increased bone fracture risk, affects 200 million women worldwide. It results in more than a million cases of hip, wrist, and vertebrae fractures and 50,000 deaths each year in the United States alone. Traditional treatments for osteoporosis utilize antiresorptive agents such as bisphosphonate therapy to reduce the incidence of fracture. Newly developed drug treatments focus on increasing bone formation (i.e., anabolic agents) to restore bone mass and structural integrity that has been lost. Recombinant human Nel-like molecule-1 (rhNELL-1), a novel osteoinductive growth factor that specifically aims to promote osteoblast activity, has previously been shown as a promising agent for bone regeneration. In our study, we investigate the effect of rhNELL-1 in an osteoporosis bone model and its potential to act as an anabolic agent in the setting of osteoporosis.

Methods
Animal Model. A sheep osteoporosis model was selected for the close resemblance to the human spine in constitution and architecture. Osteoporosis was induced through ovariectomy (OVX), controlled diet, and steroid induction in six adult ewes. Post-ovariectomy, three intramuscular injections of 500 mg methylprednisolone acetate (Depomedrol) were administered at three-week intervals starting two weeks post-operation. Special low calcium and low vitamin D osteoporosis diets were formulated in cooperation with Purina LabDiet and were fed to the sheep for eight months post-ovariectomy. Successful osteoporosis induction was confirmed using DEXA scan quantification. Injection Material. The control injection material was composed of 0.5 mL hyaluronic acid and 50 mg tricalcium phosphate (TCP). The treatment injections consisted of two doses of rhNELL-1 protein (0.75 mg, 1.5 mg) mixed into a solution of 0.5 mL hyaluronic acid and 50 mg TCP for the sustained release of rhNELL-1. Surgical procedure. Surgery was performed four months post-ovariectomy after successful osteoporosis induction was confirmed. Delivery vehicles were injected into the lumbar vertebrae at two different vertebral levels (L3 and L5). Three sheep were injected with the control vehicle and three sheep were injected with the treatment vehicle. Nell-1 efficacy in vivo. Both CT and DEXA scans were performed at 1, 2, and 3 months post-injection. CT 3D image reconstructions were performed using the trabecular bone area of the sheep vertebra.

Results
Four months after ovariectomy, sheep osteoporosis was established successfully (Fig. 1). DEXA scanning and quantification of BMD showed significant reductions among OVX treatment groups (p < 0.05) in all six sheep. Next, the efficacy of rhNELL-1 injection was assessed by CT imaging and analysis. Results showed that rhNELL-1 had significant effects in increasing both BMD (Bone Mineral Density) and BV/TV (Bone Volume/Tissue Volume) (Fig. 3). Reconstruction of representative images (Fig. 2) also demonstrated an increase in trabecular bone among rhNELL-1 treated groups.

Conclusion
The significant increase in percent bone volume in osteoporotic sheep vertebrae with rhNELL-1 treatment suggests that rhNELL-1 acts as a successful anabolic agent in the setting of osteoporosis. rhNELL-1 injection resulted in the formation of new trabecular bone throughout the vertebrae, indicating its effectiveness in rebuilding the microarchitecture of osteoporotic bone. Application of such treatment is feasible at the time of surgery to augment bone repair in osteoporotic fractures of the hip or vertebrae. In addition, injection at the time of surgery to risk sites for future fractures (such as the contralateral hip or adjacent vertebral body) may reduce the risk of subsequent additional fractures.

Significance
Vertebral compression fracture is a major risk factor of osteoporosis. Local injection of rhNELL-1 may be an appropriate treatment to augment osteogenesis in the setting of osteoporosis or to reduce the risk of fractures in osteoporotic individuals.