

# Romozumab Promoted Bone Regeneration of Critical-Size Ulnar Defect Filled with Demineralized Bone Matrix in a Non-Human Primate Model

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**Introduction:** Romozumab (Romo), a humanized sclerostin antibody, is a bone forming agent that exerts a dual action on bone by increasing bone formation and decreasing bone resorption.<sup>1</sup> Histomorphometric analyses revealed that Romo primarily increases modeling-based bone formation. Romo has been approved for the treatment of postmenopausal women with osteoporosis at high risk for fracture and has demonstrated greater efficacy in reducing fracture risk in comparison with alendronate and greater efficacy in increasing BMD and estimated bone strength in comparison with teriparatide. However, similar to other bone-forming agents,<sup>2,3</sup> Romo failed to show accelerated radiographic healing of hip and distal tibia fractures in clinical studies.<sup>4,5</sup> These results suggest it may not be possible to significantly accelerate the normal fracture healing process; however, it remains unclear if Romo alone or in combination with bone graft substitute can promote bone regeneration in clinically challenging conditions, such as critical-size bone defects, which are too large to heal spontaneously and often require bone graft or bone graft substitutes. The objective of this study was to investigate the effect of Romo on bone regeneration response in a critical-size ulnar defect filled with demineralized bone matrix (DBM), an osteoconductive bone graft substitute, in non-human primates.

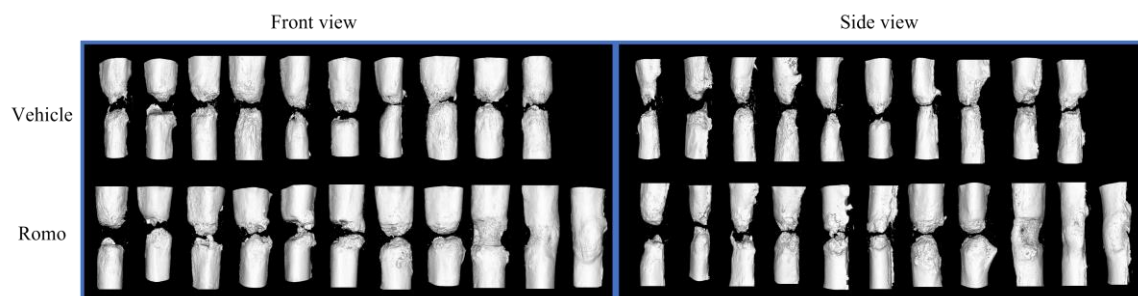
**Methods:** In cynomolgus monkeys (n=22, male, 10–12 years old), a full-cortex bone defect in the length of 0.5 cm was created in the shaft of left ulna that was stabilized with a titanium plate and 6 screws (3 screws on each side of defect). The bone defect was filled with a fixed amount of DBM (Grafton), and a fiberglass cast was added to the left forelimb. Surgeries were performed under general anesthesia by an experienced orthopedic surgeon according to a standard operation protocol. After surgery, animals were randomized into two groups, receiving vehicle (vehicle; n=10) or Romo (n=12; 30 mg/kg) subcutaneously, once every 2 weeks for 28 weeks. Blood samples were collected to assess serum Romo level. Radiographs of the left forelimb were taken every 2 weeks to monitor bone regeneration response. The ulnae were excised and analyzed by micro-computed tomography (micro-CT). To confirm the expected effects of Romo on non-surgical bone, lumbar vertebrae were excised for bone histomorphometric analysis. Unpaired student t-test was used for statistical analysis. The protocol and procedures were approved by the Institutional Animal Care and Use Committees of the sponsor and the contract research organization.

**Results:** Serum Romo levels were above 60 µg/ml from week 2 to 28 in monkeys treated with Romo except for one animal (1.5 µg/ml at week 4 and undetectable level from week 6 through the end of the study), which was excluded from the analysis. Serum Romo was undetectable in vehicle-treated monkeys at all time points examined. At the end of the study, at week 28, in vehicle-treated monkeys, the x-ray images of forelimbs showed that the critical-size ulnar defect did not fully bridge; in contrast, the critical-size ulnar defect fully bridged in 3 monkeys treated with Romo, which was confirmed by Micro-CT analysis (Figure). Micro-CT analysis demonstrated that the average new bone volume and new bone area within the defect region were 118% and 105% greater in the Romo group compared with the vehicle group, respectively (p<0.05 for both). As expected, bone histomorphometric analysis of the lumbar vertebral body confirmed that trabecular bone volume per tissue volume and trabecular thickness were 89% and 92% greater in the Romo group compared with the vehicle group, respectively (p<0.05 for both). Eroded surface, an index for bone resorption, was significantly lower in the Romo group compared with the vehicle group. Romo significantly increased newly formed bone on the cortical surfaces of the vertebral body compared with vehicle.

**Discussion:** This study demonstrated that Romo in combination with DBM improved bone regeneration of critical-size ulnar defect in a non-human primate model. To our knowledge, this is the first study of this kind. These data corroborate recent case reports in human patients that evaluated Romo alone or in combination with bone graft substitute in the management of delayed union or non-union.<sup>6,7</sup> Strengths of this study include that: 1) the healing mechanism of intramembranous ossification in this model mimics the condition of stabilized fixation in humans; 2) results from non-human primates most closely resembles human bone remodeling; 3) the size of defect simulates a challenging-to-heal condition. A potential limitation was that the dose of Romo used in this study was much higher than the approved dose for osteoporosis treatment in humans. However, even with high dose of Romo, no adverse impact on bone regeneration was observed. Consistent with this, delayed fracture healing or non-union was not observed with Romo treatment in either the phase 2 fracture healing studies or a post-hoc analysis of the Romo phase 3 pivotal trial.<sup>8</sup> Taken together, these findings provide evidence that Romo could be considered for use immediately after osteoporotic fracture to increase bone strength and prevent subsequent fragility fractures.

**Significance/Clinical Relevance:** This study demonstrated that Romo in combination with DBM improved bone regeneration in a challenging-to-heal condition in non-human primates. Further studies are required to define the clinical utility of Romo in improving bone regeneration in humans.

**References:** <sup>1</sup>Saag, N Engl J Med 2017;377:1417. <sup>2</sup>Bhandari, Clin Orthop Relat Res 2016, 474:1234. <sup>3</sup>Aspenberg, J Bone & Joint Surg Am 2016;98:1868. <sup>4</sup>Schemitsch, J Bone & Joint Surg Am 2020;102:693. <sup>5</sup>Bhandari, J Bone & Joint Surg Am 2020;102:1416. <sup>6</sup>Lee, Trauma Case Reports 2022;37:100595. <sup>7</sup>Uemura, Jt Dis Relat Surg 2021;32:526. <sup>8</sup>Lane, (WCO-IOF-ESCEO 2022) Aging Clin Exp Res 2022;34 (Suppl 1):35.



3D Micro-CT images of ulnar (front and corresponding side view) at week 28 from individual cynomolgus monkeys administered vehicle (n=10) or Romo (n=11). Images are ranked by the values of the new bone area within the defect region from low (left) to high (right).