INTRODUCTION
Fracture repair involves a specialized healing process in which bone is regenerated through endochondral or intramembranous bone formation. Previous studies demonstrated that sclerostin inhibition by a monoclonal antibody (Scl-Ab) enhanced fracture healing in several rodent models of fracture repair and in a fibular osteotomy model in nonhuman primates [1-3]. Endochondral bone repair appeared to be the major healing mechanism in these models. The objective of this study was to examine the effects of Scl-Ab on the bone healing response in an ovariectomized rat cortical defect model [4] in which intramembranous bone formation is the primary mechanism for bone repair.

METHODS
This study was approved by Amgen’s Institution Animal Care and Use Committee. Six-month-old virgin female Sprague Dawley rats (n=28) were ovariectomized and then left untreated for 2 months, allowing for the development of osteopenia. At the age of 8 months, a full-thickness defect (2 mm in diameter) was created in the tibial shaft (0.5 mm below the tibial crest). The rats were injected subcutaneously with vehicle (n=14) or Scl-Ab (n=14) at 25 mg/kg twice per week for 5 weeks.

In vivo microCT (Viva 40, Scanco Medical) was used to monitor the bone healing response on a weekly basis. The X-ray source settings were 70 kVp and 114 mA with an integration time of 350 ms. Quantitative 3D analyses of whole and central (0.21 mm) defect regions were performed using a Viva 40 (Scanco Medical) at a voxel size of 21 μm. Utilizing the Scanco evaluation software, the bone volume per total volume (BV/TV) of the whole (Figure 1) and central (Figure 3) defect regions were quantified.

The bridging of the whole defect was evaluated qualitatively from reconstrcuted microCT images in a blinded manner by 3 individual observers. Each observer evaluated the percentage of defects in each group that were fully bridged, as identified by the absence of an external defect from images thresholded to 240 mg-HA/cm3. The percent of baseline defect area was calculated for each individual rat and the mean was reported.

The contralateral femur was scanned ex vivo using a Pixium II (GE Lunar) for determination of bone mineral density (BMD). Data are presented as mean±SEM. The two-tailed unpaired student t-test was used for the statistical comparison of BMD. Two-way ANOVA was used for statistical analysis of the data sets with multiple time points.

RESULTS
There was no significant difference in body weights between Scl-Ab-treated O VX rats and vehicle controls at weeks 1-5. Ex vivo DXA analysis demonstrated that the contralateral femoral BMD was 16% greater in the Scl-Ab-treated group compared with vehicle controls after 5 weeks of treatment (p<0.001).

Figure 1 shows the in vivo microCT images of the whole defect region. Scl-Ab-treated rats appeared to heal faster than vehicle controls. Qualitative bridging analysis revealed that none of the vehicle-treated rats had a fully bridged defect at days 7, 14, or 21. However, 26% and 78% of rats had fully bridged defects at days 28 and 35, respectively, indicating the self-healing potential of rat bone. In contrast, 38%, 84% and 90% of Scl-Ab-treated rats had fully-bridged defects at days 21, 28, and 35, respectively, indicating the acceleration in the bone healing process in Scl-Ab-treated rats.

The cortical defect area analysis demonstrated that the defect area decreased over time in vehicle-treated rats (Figure 2). The cortical defect area was significantly smaller in Scl-Ab-treated rats compared with vehicle controls at weeks 2, 3, 4, and 5, indicating an accelerated healing process in the Scl-Ab-treated group.

MicroCT analysis of the whole defect region revealed that BV/TV was 8%, 11%, 13%, and 13% greater in the Scl-Ab-treated group compared with the vehicle control group at weeks 2, 3, 4, and 5, respectively (p<0.05 for all time points). Linear regression analysis showed a significant negative correlation (r² =0.52-0.66) between BV/TV and percent of baseline defect area across all groups at these time points.

Figure 3 shows the in vivo microCT images of the central defect region. The newly formed bone appeared to be thicker in the Scl-Ab- treated group compared with vehicle controls. The cortical area of the entire central defect region was significantly increased in the Scl-Ab-treated group compared with vehicle controls at weeks 3, 4, and 5 (data not shown). Cortical thickness was significantly increased in the Scl-Ab-treated group compared with vehicle controls at weeks 4 and 5 (data not shown).

DISCUSSION
Mature ovariectomized rats with a cortical defect were used in an effort to model bone repair in an osteopenic setting. These results demonstrated that systemic treatment with Scl-Ab accelerated the intramembranous-based bone healing process in the cortical defect model in ovariectomized rats. Analysis of contralateral femurs confirmed that Scl-Ab significantly increased BMD at a non-fractured site. These results suggest that sclerostin inhibition may have potential to accelerate fracture healing as well as increase bone mass at non-fractured sites in patients with osteoporotic fracture.

REFERENCES

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