Inhibition of SDF-1/CXCR4 Signaling Impairs Fracture Repair

INTRODUCTION
Skeletal fractures pose a significant social and economic burden to the United States health system. The discovery of treatments that can speed and/or enhance fracture healing will be of great benefit. A promising strategy is to supplement a healing fracture with either autologous or allogenic stem cells (SCs). Previous studies have shown that systemic injection of adult progenitor cells promotes bone tissue regeneration and healing in critical defect and fracture models (1,2). The SDF-1(CXCL12)/CXCR4 ligand receptor interaction plays a role in SC homing to injured tissue. SCs, which express the SDF-1 receptor CXCR4, home to SDF-1 released by injured, usually hypoxic tissues and promote tissue regeneration (3). The role of SDF-1/CXCR4 homing in skeletal fracture healing is unknown. This study assessed the effects of AMD3100, a CXCR4 antagonist, on the healing of a closed murine femoral fracture.

METHODS
Animals, Surgery, Fracture Creation, Drug Administration. All procedures were approved by the IACUC UC Davis. Transverse fractures were created in the right femur of 13-14 week old male C57BL/6 mice (Jackson Labs, ME) using the method of Bonnaren and Einhorn (4). Mice were injected with AMD3100 (1.25mg/kg subcutaneously) every 12 hours until euthanasia, starting the morning before fracture (Drug). Other mice were injected with an equal volume of saline as a control (Carrier). Mice were euthanized 3, 7, 14, and 42 days after surgery.

Micro-Computed Tomography, Histology, and Immunohistochemistry. Prior to euthanasia, mice were injected with 60 mg/kg pimonidazole hydrochloride (HypoxyprobeTM) which forms protein adducts in cells with a pO2<1.4% O2. Mice were then euthanized and right (fractured) and left (intact) femurs were extracted, fixed, and evaluated by μCT. Femurs were then decalcified, paraffin embedded, serially sectioned and stained with either H&E and Alcian Blue, or immunohistochemically for Hypoxia probeTM. Stereological assessments of whole callus volume and hyaline cartilage volume were determined using an established point counting technique.

Real-time QT-PCR. RNA was extracted from both the experimental and the contralateral limbs. Quantitative PCR was performed for the genes Collagen1a 1(Col1a1), Collagen2a 1(Col2a1), Collagen10a1 (Col10a1), Sdf-1 (Cxc12), Integrin-binding bone sialoprotein (Ibsp), Vascular endothelial growth factor (Vegf), and Annexin A5(AnxA5). Results are expressed as 2ΔΔCt relative to the contralateral limb.

RESULTS
Regions of the fracture callus are hypoxic. Positive staining for HypoxygenTM adducts was generally found in immature hyaline cartilage adjacent to pre-existing cortical bone. One out of four samples were found to be hypoxic at day 3, whilst all day 7 samples had hypoxic regions in both carrier and drug groups (n=3). Two out of four Carrier samples at day 14 were hypoxic, while one out of three Drug samples were hypoxic. No samples had detectable hypoxia by day 42.

Expression of key genes associated with endochondral ossification was reduced in mice receiving AMD3100 compared to vehicle control. Seven days after fracture, the gene expression of Colla1, Col2a1, Vegf and AnxA5 were significantly lower in the Drug groups when compared to the Carrier groups, Figure 1. This was also true of Col10a1 and Ibsp at day 7, though changes were not statistically significant. There were no observable differences between Drug and Carrier mice in Sdf-1 expression at any of our time points.

Total callus volume and percentage of hyaline cartilage was smaller in AMD3100 treated groups. By day 42, AMD3100 treated mice had significantly smaller total callus volume (TV) and mineralized callus volume (BV) than control (Carrier) mice, Figure 2A. BV/TV and bone mineral density (BMD) were not statistically different. At day 14, the volume percentage of cartilage in the callus was significantly lower in AMD3100 treated mice than vehicle control (Carrier), Figure 2B.

DISCUSSION
A significant decrease in the expression of genes associated with endochondral ossification, coupled with reductions in callus, bone, and cartilage volume suggest that AMD3100, a CXCR4 antagonist, impairs fracture healing. We also provide data that show that hypoxic regions exist in the fracture callus using HypoxypenTM staining. In conclusion, we propose that AMD3100 exerts its negative effects on fracture healing by inhibiting the mechanism by which SCs home to the fracture site. Furthermore this homing mechanism may require the presence of hypoxic regions in the callus that locally provide SDF-1 for homing. Future experiments will be focused on localizing the expression of SDF-1 in the callus and manipulation of the SDF-1/CXCR4 pathway to enhance healing.

REFERENCES