Introduction
Limb reconstruction after tumor resection continues to be the major challenge in orthopaedic surgery. Many techniques are available, but the choice of the most appropriate is dictated by local factors (size, location, etc) and patient factors (age, activity level, systemic disease, etc). The more popular techniques include autografts, segmental allografts, allograft-prostheses composites, and segmental bone and joint replacement prostheses. However, cumulative rates of complications approach 50% including wound necrosis, infection, nonunion, fracture, prosthesis loosening, and immunologic complications.

Effective methods of graft modification or bone graft alternatives can be of great help clinically. Bone morphogenetic proteins (BMPs) have been shown to enhance bone formation in animal experiments, but concurrent chemotherapy may alter their effect. Clinical evaluation of BMPs to date has been limited to the treatment of tibial nonunions, and applicability to other indications awaits further experimental and clinical research. In oncologic reconstructive surgery, most of the bone healing will occur coincident with the administration of chemotherapy agents to treat the underlying disease. For BMPs to be used in oncologic bone reconstructions, it is imperative to understand the modifying effects of chemotherapy on the bone healing induced by these proteins. The purpose of this study was to evaluate the effects of chemotherapy on the bone healing induced by rhBMP-2 in a rabbit model.

Material and Methods
Experimental Design: A unilateral two-centimeter critical-segmental bone defect was created in the radial diaphysis of 60 young adult New Zealand White rabbits. Six groups of animals were studied: Group 1: the defect will be left empty in one group (untreated controls); Group 2: defect filled with a collagen-carrier containing zero micrograms of rhBMP-2; Group 3: defect filled with a collagen-carrier containing thirty micrograms of rhBMP-2. Groups 4, 5, 6; surgically treated as groups 1, 2, and 3, but each group will receive intravenous doxorubicin and cisplatin.

Operative procedure: The surgical approach to the radius was identical in all rabbits and performed using intravenous anesthesia and aseptic techniques. A osteo-periosteal radial bone segment was removed and the defect filled with the experimental delivery system. Muscle, fascia and skin were closed. The animals were monitored closely for signs of discomfort or surgical complications post-operatively. Cephalexin (40 mg/kg), was administered prior to surgery and twice a day for 2 days postoperatively. Analgesics were administered based on observation by a veterinarian as individually needed to insure the animals’ comfort. All animals will remain individually caged.

Preparation and placement of the delivery system containing rhBMP-2: The experimental delivery system was insoluble bone collagen (Hellistat, Integra Life Sciences, Plainsboro, NJ) reconstituted with zero or 30 micrograms of recombinant human bone morphogenetic protein-2 (rhBMP-2) (Genetics Institute, Andover, MA).

Doxorubicin and cisplatin treatment: The chemotherapy groups (Groups 4, 5, and 6) received 2.5 milligrams per kilogram of body weight (of both doxorubicin and cisplatin intravenously 4 days before the index operation and again at 7 and 14 days after the procedure. Hydration during drug administration was performed to decrease nephrotoxicity. These dosages have been demonstrated to produce significant cytotoxic and myelosuppressive effects in rabbits.

Radiographic Analysis: Antero-posterior and lateral radiographs were taken at two-week intervals (up to 8 weeks) to evaluate bone healing. The radiographs were interpreted by two investigators blinded to the type of treatment. Digitized images of x-rays were used to calculate brightness and area of the healing bone using the Image software from NIH.

Histological Analysis: After the animals were euthanized, forearm specimens were stripped of surrounding soft tissues (except directly over the fracture site) and fixed in 10% neutral buffered formalin. Non-decalcified specimens were then embedded in plastic, sectioned longitudinally (30m thick) and stained with hematoxylin and eosin. Stained sections were photographed and assessed histologically.

Results
There was evidence of an increase in bone formation by using both collagen type I sponge and rhBMP as compared to controls, both radiographically (Figure 1) and histologically (data not shown).

When chemotherapy was given, there was a slight decrease (15-20%) in bone formation in the control and Hellistat groups. However, in the BMP group there was an almost 40% reduction in the bone formation. (Figure 2).

Conclusion
1. In this animal model, rhBMP2 demonstrated an increase in bone formation both radiographically and histologically compared to controls.
2. Chemotherapy demonstrated no effect on bone formation in the control group but a slight decrease in the rhBMP2 group.

References