Development of a Model System to Evaluate Local Recurrence in Osteosarcoma and Assessment of the Effects of Bone Morphogenetic Protein-2

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Abstract
Purpose: It is increasingly relevant to better define what constitutes an adequate surgical margin in an effort to improve reconstructive longevity and functional outcomes following osteosarcoma surgery. In addition, nonunion remains a challenging problem in some patients following allograft reconstruction. Bone morphogenetic protein-2 (BMP-2) could enhance osseous union, but has been historically avoided due to concerns that it may promote tumor recurrence.

Experimental Design: An orthotopic xenograft murine model was utilized to describe the natural temporal course of osteosarcoma growth. Tumors were treated either with surgery alone, surgery and single-agent chemotherapy, or surgery and dual-agent chemotherapy to assess the relationship between surgical margin and local recurrence. The effect of BMP-2 on local recurrence was similarly assessed.

Results: Osteosarcoma tumor growth was categorized into reproducible phases. Margins greater than 997 μm resulted in local control following surgery alone. Margins greater than 36 μm resulted in local control following surgery and single-agent chemotherapy. Margins greater than 12 μm resulted in local control following surgery and dual-agent chemotherapy. The application of exogenous BMP-2 does not confer an increased risk of local recurrence.

Conclusions: This model reliably reproduces the clinical, radiographic, and surgical conditions encountered in human osteosarcoma. It successfully incorporates relevant chemotherapy, further paralleling the human experience. Surgical margins required to achieve local control in osteosarcoma can be reduced using single-agent chemotherapy and further decreased using dual-agent chemotherapy. The application of BMP-2 does not increase local recurrence in this model. Clin Cancer Res; 21(13): 3003–12. ©2014 AACR.

See related commentary by Weiss, p. 2889

Introduction
Osteosarcoma is the most common primary bone malignancy in both pediatric and young adult patients (3, 4) and the second most common nonhematologic primary bone malignancy in older adults (5). Tremendous improvements in overall survival have followed the adoption of modern multiagent chemotheraphy. Despite these favorable results, chemotherapy alone is insufficient to achieve cure and surgery still remains an essential component of care (6).

Although extremity osteosarcomas were historically treated with ablative surgeries such as amputations and disarticulations, limb-salvage surgery has yielded comparable overall survival outcomes (7, 8). It is currently the mainstay of surgical management and is utilized in approximately 90% of patients with extremity osteosarcomas (9). It is achieved by resecting the primary tumor via a wide excision, which is by definition, through normal surrounding tissue. However, the quantitative definition of a wide excision remains unclear and controversy exists over how to manage close or microscopically positive margins. Furthermore, although it is widely recognized that distant microscopic disease is often successfully managed using adjuvant therapy, the similar management of residual local disease remains debatable.

There remains an ongoing need to improve functional outcomes and reconstructive longevity for patients with osteosarcoma. Although oncologic cure remains of paramount importance, there is a growing demand to realize more ambitious surgical and reconstructive goals. One such goal includes sparing as much native normal tissue as possible, which in some instances permits for maintenance of a joint surface, a growth plate, or other anatomic regions of critical importance. The advent of navigation-guided surgery underscores surgical interest in this approach;
Translational Relevance

Osteosarcoma represents an extremely rare primary bone tumor, making the development and optimization of novel treatments inherently challenging. This osteosarcoma study offers a relevant and reproducible model with which to evaluate the effect of various treatments and treatment combinations on local recurrence in the extremity. By describing and validating this orthotopic xenograft model, both current and future strategies affecting local healing and local recurrence can be developed and optimized before their introduction into clinical trials. Given osteosarcoma’s rarity, this model offers tremendous translational import in formulating and realizing incremental improvements in treatment strategies. Bone morphogenetic proteins are commercially available, have accepted application to nononcologic orthopedic surgery, and have increasingly been shown to augment allograft-host bone healing in preclinical models (1, 2). Their use in allograft reconstruction following osteosarcoma resection may offer a very feasible opportunity that could be implemented in the near future.

Materials and Methods

Toward this end, it is becoming increasingly relevant to better define what constitutes an adequate surgical margin in the setting of osteosarcoma.

Finally, bulk allograft bone is frequently used for the skeletal reconstruction following tumor resection. Although it is a well-accepted reconstructive method, it has recognized complications, including infection, fracture, and nonunion (10). Nonunion can occur in more than a quarter of patients undergoing chemotherapy (11), carrying with it substantial morbidity and cost. Although the use of bone morphogenetic proteins (BMP) has been utilized to foster bone healing and union rates in a number of specific clinical scenarios, there has been reluctance within the surgical community to use it in the setting of osteosarcoma. This stems largely from the known expression of BMP receptors by osteosarcoma (12, 13) and the concern that, if residual microscopic tumor remained following surgery, exogenous BMP could theoretically stimulate it. However, this is based upon preclinical laboratory findings, with results being controversial. To date, there has been no clinical evidence to support the concern that the addition of exogenous BMP results in osteosarcoma growth.

The purpose of this study was to develop a model in which factors that influence local control could be assessed. An orthotopic xenograft osteosarcoma murine model was utilized to evaluate the effects of surgery alone, surgery and single-agent chemotherapy, and surgery with dual-agent chemotherapy. In addition, the effects of locally applied exogenous BMP-2 were characterized, following both surgery as well as following surgery and dual-agent chemotherapy administration.

Cell lines

OS17 is a well-characterized pediatric preclinical testing program patient-derived osteosarcoma, obtained from a primary femoral tumor and grown continuously as a xenograft in SCID mice obtained with Montefiore Medical Center Institutional Review Board and Material Transfer Agreement approval as previously described (14, 15). OS187 was established from a high-grade osteosarcoma tumor collected between November 1997 and June 2001 at Memorial Sloan-Kettering Cancer Center (16). Collection was similarly performed in accordance with institutional review board regulations after obtaining an informed and written consent. Since its collection, DNA fingerprint analysis has raised the possibility that the cell line is a colon cancer cell line, HCT 15, rather than osteosarcoma (17). Conversely another group has obtained a different SNP profile for this line and it has been demonstrated to have the ability to undergo osteoid differentiation, a characteristic colorectal cancer does not possess. Recognizing the controversy surrounding this model’s selection, experiments were additionally performed using SaOS-2 to augment the data with OS17 and OS187. SaOS-2 was purchased from the American Type Culture Collection. Tumor authentication was recently performed on all lines morphologically and via differentiation markers. Cell lines were grown in Eagle’s Minimum Essential Medium and supplemented with 10% FBS and a combination of 100 U penicillin with 0.1 mg/mL streptomycin (P/S). Cells were grown in a humidified condition of 95% air and 5% CO2 at 37°C. Once confluent, cells were washed with PBS twice, then trypsinized and resuspended in media. Approximately 3 million cells in 150 μL of media were implanted per animal.

Animal model

Experiments were performed with the approval of the Albert Einstein College of Medicine Institutional Animal Care and Use Committee and in accordance with the institutional animal welfare policy. Six- to eight-week-old female CBI7 SCID mice weighing approximately 20 g were obtained (Taconic Farms) and housed in a pathogen-free barrier facility at all times. Orthotopic tumor implantation was performed under general anesthesia using isoflurane administered via an anesthesia machine. A cortical defect was created in the proximal metaphyseal region of the animal’s right tibia using a 2.6-guage needle. Tumor cells were deposited directly into the proximal tibia and allowed to grow. Once harvested, tumor was treated in 4% paraformaldehyde, decalcified, and embedded in paraffin blocks. Slides were stained with hematoxylin and eosin and evaluated microscopically. Margins were measured using histomorphometry (Nikon D52L). At the time of sacrifice, the lungs and liver were assessed for metastatic dissemination.

Temporal evaluation of tumor growth

Osteosarcoma’s natural growth and development within an orthotopic xenograft murine model was evaluated. Tumor (OS187 and OS17) was cultured and implanted into female SCID mice as previously described. Thirty mice per cell line were utilized. Following tumor implantation, five animals were euthanized at weekly intervals or once tumors reached 1.5 cm in diameter. Thereafter, amputations were performed and plain radiographs were obtained using a digital specimen radiography system (Biopics pXarray100; Biopics). The specimens were then submitted for histologic evaluation as previously described. The lungs and liver were assessed for lung and liver metastatic dissemination.

Evaluation of surgical margins with surgery

Tumor lines (OS187, OS17, and SaOS-2) were cultured and implanted into female SCID mice as previously described. Once tumors were palpable, mice were placed under general anesthesia...
as previously described. The right hind leg was cleaned with 70% ethanol and amputations were performed and ultimately classified as either being intralesional, marginal, or wide following histologic evaluation. The wound was closed using 4-0 nylon sutures with the addition of vetbond and 0.01 to 0.02 mg/kg of buprenorphine was administered subcutaneously for pain control. Animals were monitored for signs of local or distance disease for 6 weeks, after which they were euthanized and evaluated for histologic evidence of local recurrence and/or distant disease.

**Evaluation of surgical margins with surgery and chemotherapy**

Tumor lines (OS187, OS17, and SaOS-2) were cultured and implanted into female SCID mice and once tumors were palpable, surgery was performed as previously described. Animals were given one week to recover, after which either single-agent chemotherapy or dual-agent chemotherapy was administered weekly for four consecutive weeks (Fig. 1). Single-agent chemotherapy consisted of either 2 mg/kg of doxorubicin or 7.5 mg/kg of cisplatin per week and dual-agent chemotherapy consisted of both. Doxorubicin was administered via tail vein injection and cisplatin was administered intraperitoneally. Following four weeks of chemotherapy, mice were observed for two additional weeks. They were then euthanized and evaluated for local recurrence and distant disease as previously described.

**Evaluation of surgical margins using varying concentrations of BMP**

Tumor lines (OS187, OS17, and SaOS-2) were cultured and implanted into female SCID mice and once tumors were palpable, surgery was performed as previously described. At the time of surgery, a BMP-impregnated cellulose matrix containing either 5 μg, 30 μg, 60 μg, or 300 μg of BMP-2 was implanted within the amputation site. Thereafter surgery was performed and animals were subsequently followed for six weeks. They were then euthanized and evaluated for local recurrence and distant disease as previously described.

**Evaluation of surgical margins using chemotherapy and BMP**

Tumor lines (OS187, OS17, and SaOS-2) were cultured and implanted into female SCID mice and once tumors were palpable, surgery was performed as previously described. At the time of surgery, a BMP-impregnated cellulose matrix containing 30 μg of

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**Figure 1.**
Timeline showing when the implantation, surgical resection, single-agent and dual-agent chemotherapy treatments along with concentration took place followed by sacrifice and autopsy.
BMP-2 was implanted within the amputation site. Animals were given one week to recover, after which 2 mg/kg of doxorubicin and 7.5 mg/kg of cisplatin were administered weekly. Doxorubicin was administered via tail vein injection and cisplatin was administered intraperitoneally. Following four weeks of chemotherapy, mice were observed for an additional two weeks. They were then euthanized and evaluated for local recurrence and distant disease as previously described.

Statistical methodology
Receiver operating characteristic (ROC) analyses were performed to evaluate how well the sizes of the surgical margins discriminated between the subsequent recurrences and nonrecurrences for different treatment groups. The ROC curve is a plot of the sensitivity versus (1 – specificity) for various cut-points of the surgical margins, where cases are defined as recurrences and controls as nonrecurrences. When the area under the ROC curve (AUC) is equal to 1, this indicates perfect separation of the surgical margins between recurrences and nonrecurrences, i.e., the margins that resulted in recurrences are uniformly smaller than those in the nonrecurrences. In contrast, an AUC = 0.50 corresponds to an inability to discriminate between the groups based on the margins. Differences in ROC curves between treatment groups were evaluated with the Z-statistic, and a two-sided P-value < 0.05 was defined as statistically significant (18). All analyses were conducted in SAS version 9.3.

Results
Xenograft tumor growth can be characterized as early, middle, or late
Within this xenograft murine model, osteosarcoma growth and development followed a characteristic natural course. The early phase demonstrated no relevant radiographic findings (Fig. 2A). Local tumor growth involved the adjacent bone and often extended through a single cortex. There was minimal intramedullary extension and the metaphyseal-based tumor did not traverse the physis. The middle phase was radiographically characterized by lytic changes, however periosteal reaction was sparse (Fig. 2B). Histologically, both the intra-osseous and extra-osseous tumor grew considerably larger. Distinct periosteal reaction was noted. The late phase was radiographically characterized by extensive lytic destruction together with...

Figure 2.
A, normal appearing plain radiograph 1-week after tumor implantation, early tumor growth on low-power micrograph (20×) demonstrating tumor with early cortical breakthrough (black arrow) adjacent to normal hematopoietic marrow (red arrow) and early tumor growth on high-power micrograph (100×) demonstrating cortical breakthrough (black arrow) with slight extraosseous tumor extension (red arrow). B, plain radiograph at 2.5 weeks after tumor implantation demonstrating proximal lytic bone changes without periosteal reaction followed by middle tumor growth on low-power micrograph (20×) demonstrating cortical breakthrough (black arrow) and larger soft-tissue extension (red arrow) and middle tumor growth on high-power micrograph (100×) demonstrating tumor extension, cortical breakthrough (black arrow), and adjacent tumor necrosis (red arrow). C, plain radiograph of tibia 5 weeks after tumor implantation demonstrating huge soft-tissue mass, extensive lytic changes, and abundant periosteal reaction followed by late tumor growth on low-power micrograph (20×) demonstrating huge tumor mass (red arrow) and extensive periosteal reaction (black arrow) and late tumor growth on high-power micrograph (100×) demonstrating tumor extension across physis and up to articular surface.
abundant periosteal reaction (Fig. 2C). Histologically, there continued to be intra-osseous and extra-osseous tumor growth. Additionally, there was extensive tumor necrosis, tumor extension across the physis, and rarely, lymphovascular invasion. Even in the late phase, the articular surface continued to serve as a reliable tumor barrier. Distant metastases were not evident even in the late phase and may represent a temporally remote phenomenon.

The surgical margins needed to achieve local tumor control were defined

Results following surgical resection without adjuvant therapy are summarized (Fig. 3A, Table 1, I). Recurrence was characterized as being either reliable, variable, or absent. By definition, tumor recurrence always occurred within the reliable region and never occurred within the absent region. These regions were separated by a variable region which contained a mixed population of recurrences and nonrecurrences. Bone margins below 235 μm and soft-tissue margins below 50 μm reliably resulted in local recurrence in all cases. Conversely, bone margins greater than 997 μm and soft-tissue margins greater than 806 μm invariably resulted in apparent local control, with no clear clinical or histologic evidence of recurrence noted within the 6-week postoperative period. Recurrence was characterized as being variable in cases where bone margins were between 235 μm and 997 μm and in cases where soft-tissue margins were between 50 μm and 806 μm. Distant lung metastases were identified in only four cases, two involving OS187 and two involving OS17.

ROC analyses of the association of margin lengths and tumor recurrence status were performed for each tumor type and treatment group. For the treatment conditions under which all the surgical margins in the recurrent cases were observed to be smaller than the margins in the nonrecurrent tumors, the ROC is not

![Figure 3.](image-url)
shown because this corresponds to perfect discrimination between groups and the AUC is equal to 1. For the surgery only group, 100% of recurrences were observed for margins < 997 μm. In the case of OS17 bone margins, 64% of nonrecurrences also meet this threshold, suggesting overlap in the margins that resulted in recurrences and nonrecurrences (Fig. 4).

Single-agent chemotherapy reduces margins needed for local tumor control

Results following surgical resection and treatment with single-agent chemotherapy are summarized (Fig. 3B, Table 1). Bone margins below 17 μm and soft-tissue margins below 16 μm reliably resulted in local recurrence in all cases. Conversely, bone margins greater than 36 μm and soft-tissue margins greater than 35 μm invariably resulted in apparent local control, with no clear clinical or histologic evidence of recurrence noted within the 6-week postoperative period. Recurrence was characterized as being variable in cases where bone margins were between 17 μm and 36 μm and in cases where soft-tissue margins were between 16 μm and 35 μm.

For the single-agent chemotherapy groups, in contrast to the surgery only group, the threshold below which 100% of recurrences were observed was much lower: 14.98 μm to 35.68 μm, but fewer nonrecurrences occurred at this threshold (range, 0%–35%), indicating improved separation in recurrences and nonrecurrences at smaller surgical margins (Supplementary Figs. S1–S6).

Evaluation of OS17 soft-tissue margins demonstrated significant differences in the AUCs between the group treated with surgery alone and the groups treated with either single-agent doxorubicin ($P = 0.002$) or single-agent cisplatin ($P = 0.002$). Evaluation of OS17 bone margins demonstrated significant differences in the AUCs between the group treated with surgery alone and the groups treated with either single-agent doxorubicin ($P = 0.004$) or single-agent cisplatin ($P = 0.005$). Evaluating OS187 margins demonstrated similar trends. In the case of soft-tissue margins, significant differences were observed in the AUCs between the group treated with surgery alone and the groups treated with single-agent doxorubicin ($P = 0.025$). SaOS-2 ROC curves failed to demonstrate significant differences between groups; however, this was likely secondary to the limited sample size and lack of power. SaOS-2 was utilized primarily as a means of validating OS187 findings, given the aforementioned controversy regarding the tumor lineage. Fewer animals were evaluated for this reason. Metastatic disease was not identified.

<p>| Table 1. Zones of recurrence, variable recurrence, and no recurrence by margin type for OS187, OS17 and SaOS2 in untreated SCID mice (I), and mice treated with single-agent chemotherapy (II), dual-agent chemotherapy (III), BMP-2 (IV), and BMP-2 with dual-agent chemotherapy (V) |</p>
<table>
<thead>
<tr>
<th>Recurrence (less than or equal to; μm)</th>
<th>Variable recurrence (less than; μm)</th>
<th>No recurrence (greater than or equal to; μm)</th>
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<tr>
<td>I. No chemotherapy</td>
<td></td>
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<tr>
<td>OS-187 soft-tissue</td>
<td>6.75</td>
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<td>OS-187 bone</td>
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<td>OS-17 soft-tissue</td>
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<td>997</td>
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<td>SaOS-2 soft-tissue</td>
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<td>SaOS-2 bone</td>
<td>234</td>
<td>320</td>
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<tr>
<td>II. Single chemotherapy</td>
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<td></td>
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<tr>
<td>OS-187 soft-tissue (dox)</td>
<td>14.6</td>
<td>22.1</td>
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<tr>
<td>OS-187 bone (dox)</td>
<td>14.9</td>
<td>23.9</td>
</tr>
<tr>
<td>OS-17 soft-tissue (cis)</td>
<td>13.5</td>
<td>18.9</td>
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<td>OS-17 bone (cis)</td>
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<td>21.2</td>
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<td>SaOS-2 soft-tissue (dox)</td>
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<td>34.7</td>
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<td>SaOS-2 bone (cis)</td>
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<td>25.7</td>
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<td>III. Dual chemotherapy</td>
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<td>OS-187 soft-tissue</td>
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<td>IV. BMP</td>
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<td>SaOS-2 bone</td>
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<td>137</td>
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<td>V. BMP with dual chemotherapy</td>
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<td>8.31</td>
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<tr>
<td>SaOS-2 bone</td>
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<td>8.31</td>
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Geller et al.
Dual-agent chemotherapy further reduces margins needed for local tumor control

Results following surgical resection and treatment with dual-agent chemotherapy are summarized (Fig. 3C, Table 1, III). Bone margins below 9 µm and soft-tissue margins below 8 µm reliably resulted in local recurrence in all cases. Conversely, bone margins greater than 12 µm and soft-tissue margins greater than 11 µm invariably resulted in apparent local control, with no clear clinical or histologic evidence of recurrence noted within the 6-week postoperative period. Recurrence was characterized as being variable in cases where bone margins were between 9 µm and 12 µm and in cases where soft-tissue margins were between 8 µm and 11 µm.

Evaluation of OS17 margins demonstrated significant differences in the AUCs between the group treated with surgery alone and the groups treated with dual-agent chemotherapy both with regard to soft-tissue (P = 0.002) and bone (P = 0.004). Evaluating OS187 margins demonstrated similar trends. Significant differences were observed in the AUCs between the group treated with surgery alone and the groups treated with dual-agent chemotherapy in both soft-tissue (P = 0.011) and bone margins (P = 0.027). Again, SaOS-2 ROC curves failed to demonstrate significant differences between groups. Metastatic disease was not identified.

Exogenous BMP-2 does not increase local recurrence after surgery

Results following the administration of 30 µg of exogenous BMP-2 immediately following surgical resection are summarized (Fig. 4A, Table 1, IV). Bone margins below 102 µm and soft-tissue margins below 99 µm reliably resulted in local recurrence in all cases. Conversely, bone margins greater than 304 µm and soft-tissue margins greater than 302 µm invariably resulted in apparent local control, with no clear clinical or histologic evidence of recurrence noted within the 6-week postoperative period. Recurrence was characterized as being variable in cases where bone margins were between 102 µm and 304 µm and in cases where soft-tissue margins were between 99 µm and 302 µm. Local recurrence following surgery alone was compared against local recurrence following surgery and local addition of BMP-2. In the case of OS17 and SaOS-2, no significant difference between the groups was noted (P = 0.3 and 0.24). In the case of OS187, a difference was found in favor of the BMP-2–treated group (P = 0.02). A dosage analysis of BMP-2 was carried out on the various cell lines, but no significant difference in local recurrence was noted between the varying concentrations of BMP-2 but mice treated with concentrations of 30 µg and 60 µg of BMP-2 appeared to allow for the narrowest surgical margins in comparison with those treated with 5 µg and 300 µg (Fig. 5). Metastatic disease was not identified.

Exogenous BMP-2 does not increase local recurrence after surgery and dual-agent chemotherapy

Results following the administration of 30 µg of exogenous BMP-2 immediately following surgical resection and administration of dual-agent chemotherapy are summarized (Fig. 4B; Table 1, V). Bone margins below 8 µm and soft-tissue margins below 9 µm reliably resulted in local recurrence in all cases. Conversely, bone margins greater than 11 µm and soft-tissue margins greater than 10 µm invariably resulted in apparent local control, with no clear clinical or histologic evidence of recurrence noted within the
6-week postoperative period. Recurrence was characterized as being variable in cases where bone margins were between 8 μm and 11 μm and in cases where soft-tissue margins were between 9 μm and 10 μm.

Evaluation of OS17 margins demonstrated significant differences in the ALICs between the group treated with surgery alone and the groups treated with BMP-2 and dual-agent chemotherapy both with regard to soft-tissue (P = 0.002) and bone (P = 0.006). Evaluating OS187 margins demonstrated significant differences observed in the ALICs between the groups treated with surgery alone and the groups treated with BMP-2 and dual-agent chemotherapy with regard to soft-tissue margins (P = 0.023). Again, SaOS-2 ROC curves failed to demonstrate significant differences between groups. Metastatic disease was not identified.

Discussion

Results demonstrate the successful development of a model system with which to evaluate local recurrence in osteosarcoma. Using an orthotopic xenograft murine model, the radiographic and histologic natural course of osteosarcoma was described in terms of early, middle, and late phases. Using this model, surgery alone universally prevented local recurrence with bone and soft-tissue margins to 12 μm, and in cases where soft-tissue margins were between 9 μm and 10 μm.

Closer examination of the literature reveals the frequent observation that local recurrence is dependent upon not only the adequacy of the surgical resection, but also the tumor’s response to chemotherapy. Grimer and colleagues reported that in patients with a poor response to chemotherapy, marginal and adequate resections yielded local recurrence rates of 36% and 4%, respectively (22). No instances of local recurrences were found in patients exhibiting a good response to chemotherapy, even if a marginal resection was performed. In a review of 1,126 patients, Bacci and colleagues (23) reported that inadequate surgery and response to chemotherapy were the only two statistically significant factors on multivariate analysis and concluded that, in the event of both inadequate margins and poor response to chemotherapy, a proximate amputation should be contemplated.

Taken together, these reports point to both surgical and chemotherapeutic requirements that are necessary to realize successful local control. Close margins are seemingly tolerated in cases of chemotherapy-sensitive tumors, whereas greater margins are required in cases of chemosensitive tumors where local recurrence, though not a foregone conclusion, is more likely. Although these studies greatly aid in conceptual management of osteosarcoma, there are currently no guidelines that consider “close” margins in a quantifiable way.

BMPs belong to the TGFβ superfamily and have been successfully used to promote osseous union in select clinical scenarios. Despite their obvious relevance to allograft non-union, their use in reconstruction has been widely avoided in the context of osteosarcoma for fear of driving growth of residual tumor. This concern stems in part from the understanding that osteosarcoma can express a wide array of BMPs and BMP receptors, theoretically implicating them in osteosarcoma tumorigenesis (12). Hayden and colleagues proposed that under normal conditions BMP-2, among other BMPs, drives osteogenic differentiation and that this effect that is lost in the case of osteosarcoma (24). Luo and colleagues similarly implicate a fault in terminal differentiation as the causative factor in osteosarcoma development (25). Conversely, other investigators have reported that BMP-2 serves as an inhibitor of osteosarcoma. Wang and colleagues reported that BMP-2–treated SCID mice failed to develop tumors following heterotopic tumor implantation and concluded that BMP-2 results in both an inhibition of tumor-inducing gene expression and an upregulation of differentiation markers (26). Geng and colleagues have shown Coleusin factor to inhibit osteosarcoma proliferation via BMP-2 upregulation and subsequent osteoblastic differentiation. The authors further demonstrated reversal of this effect with the addition of noggin, a BMP-2 inhibitor (27).

Other investigators have reported that rBMP both mitigates tumor growth and increases tumor predilection for apoptosis (28, 29). Taken together, these studies support the idea that BMP-2 acts to inhibit tumor growth and in fact, may either drive differentiation, apoptosis, or both.

The current study seeks to address the relevant clinical question of whether the addition of exogenous BMP-2 in the surgical bed following either adequate or inadequate surgery results in an increase in local recurrence. In addition, it applies BMP-2 both with and without systemic, effective and currently utilized chemotherapy, further reenacting to the extent possible the clinical human scenario. Our results demonstrate no evidence of enhanced tumor growth or increased local recurrence rates.

![Figure 5](image-url)
following exogenous BMP-2 administration and in one case it appears to provide a protective benefit, supporting the notion that it may in fact act as a tumor inhibitor. Numerous limitations are recognized. The study utilizes an immune-deficient animal model, which, at best, serves as an artificial recapitulation of the human condition and as such, is subject to all the limitations inherent in such a design. In addition, it is recognized that the tumor lines evaluated are themselves chemotherapy sensitive and for this reason, the effects of chemotherapy could be predicted. Although chemotherapy-resistant lines were not used, it is expected that local recurrence would occur more frequently or, put otherwise, that larger margins would be needed to affect the same rate of local control. However, local recurrence would likely be similar to or slightly less than that observed in the group treated with surgery alone. The lack of distant disease is likely a function of time. It is recognized that a longer period of observation would probably offer further insight into the development of metastatic disease, but given the project’s focus on local control, this was not pursued. It is difficult to know whether BMP-2, a member of the TGFβ superfamily, will behave in an immunodeficient system as expected in an immunocompetent system. In addition, the systemic effect of BMP-2, as it pertains to metastatic disease, was beyond the scope of this project and was not investigated. Lastly, it is also difficult to know whether conclusions about quantitative linear margins can be transposed from a mouse model, whose tumor is volumetrically much smaller, to a human model, whose tumor is many-fold volumetrically larger. It could be imagined that local recurrence secondary to residual microscopic disease likely occurs in a single location, independent of tumor presence elsewhere. If so, the exact size of the tumor would have little import; however, this argument is admittedly speculative and clearly has not been proven.

In conclusion, this investigation has sought to outline the natural temporal course of osteosarcoma growth and differentiation. Using a xenograft orthotopic murine model, the natural course was radiographically and histologically shown to recapitulate the human condition. Using this model, it was shown that the surgical margins required to achieve local control are similar to historically acceptable margins when adjusted for human scale. In addition, it has been shown within this model that margins can be progressively narrowed using single- and dual-agent chemotherapy. Using a standardized mouse-to-human scaling factor of 20-fold, the bone margin needed without chemotherapy translates to 2.0 cm. Similarly, when adjusted for human scale, the bone margin required for local control using dual-agent chemotherapy decreases to 0.28 mm, a finding that falls well below conventionally acceptable margins. These results quantitatively frame the surgical margins needed in chemoresponsive tumors and presuppose that chemotherapy-insensitive tumors likely require much greater margins. The exogenous application of BMP-2 to the surgical site following tumor excision does not increase the risk of local recurrence in groups treated with either surgery alone or in groups treated with surgery and chemotherapy. These findings strongly support a number of previously published findings, which argue that BMP-2 can be utilized safely following osteosarcoma resection and reconstruction. Admittedly, the effect of exogenous BMP-2 on both the development and growth of metastatic disease remains to be characterized. Use of BMP-2 in the clinical setting could only proceed if no increase in the rate or growth of metastatic disease could be clearly demonstrated. Additional preclinical work is needed to define the effect of systemic BMP on the development and progression of metastatic disease and to prove its safety. If realized, these findings together with current results could serve as the preclinical foundation upon which a randomized prospective clinical trial could be considered. This would ultimately provide a more conclusive understanding of BMP-2’s effects and limitations in the treatment of osteosarcoma. Given the real and costly impact of nonunion, allograft fracture, and implant failure, the ability to both spare host bone and augment tumor reconstructions offers an incremental but real promise of improvement for these patients. Lastly, this model system will be expanded to evaluate other locally active agents that may serve to augment local surgical control, narrow surgical margins, or improve subsequent reconstruction.

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No potential conflicts of interest were disclosed.

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