Abstract

Osteosarcoma is almost identical in companion animals and in people, so research in basic cancer biology and treatment is readily translational across the species. In this article the authors (one DVM and one MD) present examples of species “cross talk” based on their parallel careers in musculoskeletal oncology surgery. Dogs in particular provide a relevant osteosarcoma model that is 10 times more prevalent than the corresponding human condition and offers a unique opportunity to answer questions related to local tumor control and metastasis. Advantages of the dog model include spontaneous development of the disease; the animal’s large size, intact immune system, shared genetic aberrations, and response to traditional chemotherapies; and owners’ acceptance and compliance with clinical trials for their pets. We describe several cross-species treatment strategies for osteosarcoma—chemotherapy, limb-sparing techniques, and radiation—as well as surprising impacts of infection and immunology. We conclude with some discussion of areas for further discovery and development to advance species cross talk in support of One Health.

Key Words: bone allograft; cisplatin; comparative oncology; dog; immunology; limb sparing; One Health; osteosarcoma; stereotactic radiosurgery

Introduction

Recent Developments in Osteosarcoma Treatment

Osteosarcoma (OS) is the most common primary malignancy of bone affecting both people and pets. Until a few decades ago, it was an almost uniformly fatal disease, attended by significant local morbidity in the primary tumor site and rapid demise from metastasis. A quote from Dr. Stanford Cade in 1955 about the standard of care for children with osteosarcoma summarized the frustration of the times: “Gentlemen: If we operate they die, if we don’t operate they die. This meeting should be concluded with prayer” (Cade 1955, 111).

The advent of successful chemotherapies in the 1970s and 1980s improved survival rates so dramatically that clinicians could direct their attention to durable and functional limb-sparing strategies instead of palliation or amputation. The National Cancer Institute (NCI) accepted and funded pet animal models as relevant to human disease, and findings from research in people and dogs were readily shared across species lines for the good of all.

Advantages of the Dog Model of Osteosarcoma

Osteosarcoma in dogs and humans is strikingly similar in its clinical presentation, biology, treatment, complications, and outcomes (although human patients do better). Table 1 provides an overview of these similarities (also see Gorlick et al. 2003; Khanna et al. 2009; Mueller et al. 2007; Withrow and Khanna 2009; Withrow et al. 1991). Translational research and treatment are therefore particularly fruitful for this disease, notwithstanding the following differences between the species:

1. The incidence of osteosarcoma in dogs is significantly greater (20:1).
2. The median age of onset in dogs is 8 years (i.e., adulthood), whereas humans are most commonly affected as adolescents.
3. Issues of limb length discrepancy are not a problem for adult dogs after limb-sparing surgery as they may be in young children.
4. Closed physeal plates in dogs (versus children) limit the use of clinical OS dogs for research on “growing” prostheses.

The dog serves as an excellent OS model for basic scientists who study causation, molecular biology, and drug targets; medical oncologists who assess disease-free intervals and survival with experimental therapeutics; bioengineers who develop devices for limb sparing or prosthesis; and surgeons who use human-scale imaging devices and relevant surgical interventions (Table 2; Dernell et al. 2007).
From a surgeon’s perspective, large-breed dogs affected by osteosarcoma are much more advantageous than rodent models for two important reasons. Pre-, intra-, and postoperative interventions for dogs and humans are similar in terms of techniques, devices, allografts, clinical healing, local reactions, and complications. And current and developing imaging techniques can use the same equipment and interpretations.

In this article we draw from our experiences to describe OS-specific examples of “cross talk” from pets to people and back again. We focus on recent developments in chemotherapy delivery, the use of allografts as a limb-sparing technique, and several radiation strategies. We also discuss the unexpected impacts of infection and immunology on OS treatments. We conclude with thoughts about emerging and future directions for research in this area.

### Innovation in Chemotherapy Delivery

The intent in a preoperative (neoadjuvant) setting is to downstage the primary tumor, make the surgery safer and more feasible, and address micrometastatic disease early. Most preoperative cytotoxic chemotherapy is delivered intravenously or orally, but intravenous (IV) administration does not necessarily distribute to target tumor tissue more than normal tissue. The development of intra-arterial (IA1) and intracavitary methods makes it possible to more selectively target the primary tumor site and cells. These methods are areas of active research.

<table>
<thead>
<tr>
<th>Table 1 Comparison of canine and human osteosarcoma</th>
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<tbody>
<tr>
<td><strong>Variable</strong></td>
</tr>
<tr>
<td>Mean age</td>
</tr>
<tr>
<td>Race/breed</td>
</tr>
<tr>
<td>Body weight</td>
</tr>
<tr>
<td>Site</td>
</tr>
<tr>
<td>Etiology</td>
</tr>
<tr>
<td>% clinically confined to the limb at presentation</td>
</tr>
<tr>
<td>% histologically high grade</td>
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<tr>
<td>DNA index</td>
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<tr>
<td>Molecular and genetic alterations</td>
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<tr>
<td>Prognostic indicators</td>
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<tr>
<td>Metastatic rate without chemotherapy</td>
</tr>
<tr>
<td>Metastatic sites</td>
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<tr>
<td>Improved survival with chemotherapy</td>
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<tr>
<td>Duration of adjuvant chemotherapy</td>
</tr>
<tr>
<td>Regional lymph node metastasis</td>
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<td>Surgical repair</td>
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</table>

Intra-Arterial Chemotherapy

Intra-arterial delivery of the chemotherapy drug cisplatin (Pt) was introduced in the mid-1980s at selected human medical centers (Jaffe et al. 1985). As part of a subsequent NCI-sponsored randomized clinical trial in dogs with OS, it proved to be technically feasible, safe, and effective in treating the disease (Withrow et al. 1993). Based in part on these findings, the number of preoperative cycles of IA Pt was further refined in humans. In vivo tumor response was measured on the catheter insertion arteriogram that occurs on each cycle (Cullen et al. 2005). Based on interpretation of the serial angiography (3 to 6 cycles), most patients experienced maximal response—more than 90% necrosis. Other noninvasive measures of percent necrosis (e.g., MRI angiography, PET/CT scans) are under investigation.

A protocol based on the Colorado State University (CSU) animal work and the Denver Clinic for Extremities at Risk experience, using a combination of IA Pt and IV doxorubicin, has resulted in an improvement in both pediatric and adult disease-free and overall survival (Wilkins et al. 2005). 

Intracavitary Chemotherapy

Marginal resection of bone and soft tissue sarcomas can leave viable tumor at the edge of the wound cavity. We developed a cisplatin/polymer delivery system to provide very high Pt levels in the wound bed, low but biologically active systemic doses of Pt, and possible enhanced regional lymph node drug dosing. All of these goals are achievable in different tumor types and model systems (Dernell et al. 1997; Withrow et al. 1995, 2004).

Locally implanted slow-release Pt polymers are characterized by a very high Pt concentration in the wound bed, 30-day Pt release profile, mild local reaction, an almost doubling of the maximum tolerated dose (compared to IV Pt), increased systemic area under the curve for equivalent IV dosing, and a change from renal toxicity to bone marrow suppression. An unsuspected toxicity is direct and severe neurotoxicity if the Pt polymer is placed on or in brain tissue. This material has been used successfully on one human OS patient with “compassionate use” exemption approval from the US Food and Drug Administration, saving the patient from a hemipelvectomy and resulting in long-term survival (now over 15 years). Further development of the original product has been hampered by dissolution of the parent production company, inadvertent public disclosures of intellectual property, and unclear pathways to licensure (for the device, drug, or both).

Limb-Sparing Techniques

As preoperative downstaging with chemotherapy and improved survivals became commonplace in the 1980s, the techniques, biology, durability, and function of intraoperative measures such as limb sparing became more relevant. There are two limb-sparing camps: one endorses the use of metal endoprostheses (implanted rods or plates), which, thanks to advances

Table 2 Molecular and genetic factors associated with osteosarcoma (OS) in dogs

<table>
<thead>
<tr>
<th>Factor</th>
<th>Comments</th>
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<tbody>
<tr>
<td>p53</td>
<td>Mutated and/or overexpressed in several investigations</td>
</tr>
<tr>
<td>IGF-1/IGF-1R</td>
<td>May contribute to the malignant phenotype</td>
</tr>
<tr>
<td>HGF/c-Met</td>
<td>May contribute to the malignant phenotype</td>
</tr>
<tr>
<td>erbB-2/HER-2</td>
<td>Overexpressed in several canine OS cell lines</td>
</tr>
<tr>
<td>PTEN</td>
<td>Mutated or downregulated in high percentage of canine OS cell lines</td>
</tr>
<tr>
<td>sis/PDGF</td>
<td>Overexpressed in some canine cell lines</td>
</tr>
<tr>
<td>Matrix metalloproteinases</td>
<td>Overexpressed in canine OS cell lines</td>
</tr>
<tr>
<td>Ezrin</td>
<td>A membrane-cytoskeleton linker associated with the metastatic phenotype in canine OS</td>
</tr>
<tr>
<td>COX-2</td>
<td>Expression upregulated in some canine OS; prognostic in some investigations, not in others</td>
</tr>
<tr>
<td>Angiogenic factors</td>
<td>VEGF measurable in plasma of dogs with OS; angiotatin present in urine of dogs with OS</td>
</tr>
<tr>
<td>Telomerase reverse transcriptase gene</td>
<td>Upregulated in some canine OS</td>
</tr>
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in design, feature good durability, function, and longevity, are capable of rapid weight bearing, and require little host integration to be functional; the other camp prefers a more “biologic” solution with the use of allograft tissue reconstructions. We focus on the latter approach.

Large allografts may be used as weight-bearing diaphyseal grafts, osteoarticular replacements, or as part of metal/allograft hybrid reconstruction. Dog studies have shown that bone cement placed in the marrow of massive allografts can reduce implant loosening and allograft fracture and enhance durable allograft healing (Straw et al. 1992). Dogs were also models for early research demonstrating that bone allografts were not immunologically “inert” and that varying degrees of antigenicity can affect bone healing (Stevenson et al. 1996). The ideal limb reconstruction technology is the subject of ongoing research.

Infection, fractures (allograft or metal implant), and local tumor recurrence are the most common complications of limb-sparing surgery. Local recurrence rates after limb sparing vary between species, with the human rate around 5% and the canine rate between 10% and 20%. Differences may be related to the preoperative chemotherapy regimens routinely used in people, as they facilitate adequate surgical resection (wide resections are more feasible in humans than in dogs).

**Radiation Strategies for Osteosarcoma**

Osteosarcoma has long been thought to be radiation “resistant,” but studies of canine OS have shown a dose response for fractionated external beam radiation and indicated that cisplatin was at least additive if not synergistic with radiation on local tumor cell kill (Withrow et al. 1993). When combined with cisplatin, the radiation doses required to induce over 80% local necrosis can be reduced below those that cause host bone necrosis. Routine use of radiation for OS has not become widespread because of the high rate of “success” of preoperative chemotherapy alone, perceived and actual late effects on normal tissue, especially in pediatric patients, and possible reduced osteointegration in metal implants or allografts.

Novel techniques for radiation therapies continue to be developed, including intraoperative radiation, radionuclides, and stereotactic radiosurgery.

**Intraoperative Radiation**

Intraoperative radiation involves the surgical isolation of the affected bone from radiation-sensitive adjacent skin, muscle, and neurovascular bundles. One large dose of radiation is then delivered ex vivo or in vivo (in the operating room), and the now-dead host bone and tumor bone are reconstructed, not unlike an allograft (Liptak et al. 2004).

Local tumor control has been good at single intraoperative doses exceeding 50 gray (Gy; unit of absorbed radiation). Poor healing of the host bone interface and structural collapse of the tumor-weakened bone have hampered the widespread acceptance of these techniques.

**Radionuclides**

Bone-seeking radionuclides are an attractive option for both whole body and local tumor control. Samarium has received attention in both the human and canine OS literature (Anderson et al. 2002; Ehrhart et al. 2006), but doses required to achieve durable and clinically meaningful local control often overlap with those that cause serious and long-lasting bone marrow suppression, which may delay the start of necessary adjuvant chemotherapy. To avoid such suppression, a dog OS study used the maximally tolerated dose of systemically administered samarium isolated to the affected leg with a heart/lung bypass machine. The procedure was well tolerated by normal tissues and resulted in a modest increase in percent necrosis in the perfused tumor (Ehrhart et al. 2006).

Tumor heterogeneity due to inherent tumor necrosis and variable vascular patterns may limit homogeneous dose distribution. The technique may be better suited for combination treatment rather than as a standalone neoadjuvant treatment.

**Stereotactic Radiosurgery**

New real-time image-guided radiation strategies, such as stereotactic radiosurgery (SRS) and image-guided radiation therapy, are evolving to deliver biologically relevant doses to in situ osteosarcoma while sparing normal tissue.

In a recent CSU study, 25 dogs with osteosarcoma were treated with limb-sparing curative intent SRS (mean dose of 36 Gy in one, two, or three fractions). Results have been encouraging, with no local recurrences and 100% tumor necrosis in eight retrieved specimens. The biggest problem has been 10 postradiation fractures that required amputation or repair (Ryan et al. 2009). Stereotactic surgery has been reserved in humans for patients with unresectable metastatic disease. While early anecdotal reports show promise, much more information is needed before accurate conclusions can be made.

Ongoing studies with advanced imaging are designed to create a predictive model for fracture, which will guide case selection and possibly preemptive stabilization. The dogs in the study are large enough for imaging strategies that can be used in people.

**Infection and Immunology**

An unexpected benefit from infection at a limb-sparing surgical site appears to be improved survival (Lascelles et al. 2005). First recognized in dogs, this phenomenon was later found in people with osteosarcoma (Jeys et al. 2007). The survival advantage seems to be independent of bacteria type(s), severity (most are low grade), or duration (most are chronic). The presumed mechanism is a nonspecific immunologic stimulation, but the specific mechanism remains...
elusive; other proposed mechanisms include low-grade fever, antiangiogenic aspects of certain antibiotics, or even host-versus-graft immune response to the allograft.

An understanding of the immune system in terms of immunosurveillance (causation/prevention) or immunotherapy has been the subject of research for decades. Canine OS trials first showed that an immunomodulator (muramyl tripeptide phosphatidyl ethanolamine, MTP-PE) could improve survival in dogs after amputation and chemotherapy (Kurzmann et al. 1995). A recently completed trial in children (based on dog results) also demonstrated a survival advantage for patients that received MTP-PE (Meyers et al. 2008).

Future Directions

The entire field of oncology has seen a meteoric increase in molecular and genetic approaches to cancer diagnosis, prognosis, and treatment. Osteosarcoma is no exception and one translational example involves the identification of ezrin as a protein that helps drive the m-TOR pathway of tumor cell growth and is a negative prognostic indicator in both dogs and people (Khanna 2008; Khanna et al. 2004). In the spirit of comparative research, several clinical trials in dogs with osteosarcoma are ongoing to assess the feasibility of blocking ezrin function with rapamycin. The trials were endorsed by the Children’s Oncology Group (www.childrensoncologgygroup.org), funded by the Morris Animal Foundation, and run by the NCI-managed Comparative Oncology Trials Consortium (14 veterinary schools running clinical trials with agents likely to improve human drug development strategies).

The release of the human and canine genome into the public domain has opened new opportunities for targeted therapies. Molecular and genetic pathways for OS development and progression are strikingly similar. Emerging areas of research in limb sparing include enhanced allograft healing, new methods/materials/metals to integrate into host tissues, targeted pre- and postoperative chemotherapy or radiation, and development of biologically inert osseous and soft tissue–integrated prostheses.

Conclusion

Osteosarcoma is a classic example of the One Health integration of disciplines such as general surgery, orthopedic surgery, chemistry, pharmacology, basic cancer biology, imaging, radiation, pathology, rehabilitative medicine, and even social work to fight a common enemy across species lines. The veterinary/human medical collaboration has resulted in improved oncological outcomes for both dogs and people. Thanks to advances in cytotoxic methods, treatments now poison the patient just a little bit less than the tumor. The models that have been established through the One Health cooperative venture should be instrumental in further improving cancer care. For scientists’ continuing efforts to explore and develop these frontiers, Yogi Berra said it best: “The future ain’t what it used to be.”

About the Authors

We have approached our work and this article from the “One Health” philosophy of medicine. As a veterinarian and a physician, we treat the same diseases in our respective species. We both received advanced training in musculoskeletal oncology at the Mayo Clinic (in Rochester, Minnesota); were elected into the Musculoskeletal Tumor Society in the same year (1987); serve on the board of the Limb Preservation Foundation in Denver, Colorado; and have shared clinical and translational research programs treating both people and pets for almost 30 years. In addition, one of us (RMW) served as an advisor for several decades of NCI-funded research at CSU to study comparative aspects of canine OS biology and limb-sparing research.

References


