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Osteosarcoma update: Prognostic Factors & Promising New Therapeutics

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Background

Osteosarcoma accounts for 85% of all bone tumors in dogs, with ~10,000 cases diagnosed each year[1]. This disease is most common in large and giant breed dogs with a predilection for the appendicular skeleton, similar to the tendency of taller adolescents to be afflicted[1]. Middle aged to older dogs are most commonly affected, but there does appear to be a bimodal age distribution with a peak at 18-24 months and a second peak around 7 years[1]. Human osteosarcoma also shows a bimodal age distribution with the larger peak at adolescent age[1].

Despite aggressive control of local disease, without chemotherapy, 90% dogs develop metastatic disease within 1 year[1]. While chemotherapy improves survival, we have reached a therapeutic ceiling with no improvement in long term survival using traditional therapies despite attempts at dose intensification[2], combination therapies[2,3,4] and maintenance therapy with metronomic chemotherapy or Palladia upon completion of standard treatment[5].

A variety of traditional chemotherapy drugs and schedules have been evaluated for adjuvant therapy of canine osteosarcoma. These protocols are typically either doxorubicin based or platinum-based regimens. While cisplatin has shown a slight survival advantage in early studies, its high toxicity profile limits its use, making carboplatin an attractive alternative. Many studies have compared single agent doxorubicin, single-agent carboplatin, and combination/alternating carboplatin-doxorubicin therapy with no significant difference in disease free interval (DFI) or survival time (ST)[2,3]. Single agent carboplatin is associated with the lowest percentage of adverse events (AE). A recent prospective, randomized, phase III trial found that six doses of carboplatin had a significantly improved DFI compared to an alternating carboplatin and doxorubicin protocol totaling six treatments[6]. Single agent carboplatin protocols typically range between four to six total doses. Future prospective studies are needed to determine if there is an advantage to completing the full six doses or to verify that 4 doses has the same result.

Diagnostics

Staging includes radiographs of the limb, three-view thoracic radiographs, or CT scan of the lungs, +/- abdominal ultrasound. While abdominal metastasis is rare, 5% of dogs who had an abdominal ultrasound performed as part of their staging were found to have concurrent neoplasia[15]. This is a bias population as many of these dogs had a palpable abdominal mass or other biochemical abnormalities which warranted an

abdominal ultrasound,[15] but abdominal ultrasound is warranted in this subset of patients.

Sensitivity of three-view thoracic radiographs compared to CT is 81% (range 71-95%) specificity (range 67-92%). The dogs most likely to have nodules missed on radiography were all large and giant breeds[16]. CT scan may be a more appropriate screening tool for large and giant breed dogs prior to definitive local therapy.

Cytology is becoming more common place due to the ease of obtaining a sample and lower risk of a biopsy induced pathologic fracture. ALP staining can be used on cytologic specimens to confirm bone origin with a sensitivity of 100% and specificity of 89%. ALP is present in multiple tissues including liver, kidney, intestine, placenta, and bone. Other tumors that may stain positive for both vimentin and ALP include multi lobular tumors of bone, melanoma, and chondrosarcomas[14].

Prognostic factors

Osteosarcoma originates from osteoid producing mesenchymal cells of either stem cell or osteoblast origin. Grade III tumors have been associated with shorter disease free interval (DFI), and in one study, 75% of canine osteosarcoma and all metastatic lesions were assigned a grade III[13].

Other well known prognostic factors include tumor location, elevation of serum alkaline phosphatase prior to surgery, and the presence of gross metastatic disease.

Location

The scapula and proximal humerus are both locations associated with a shorter DFI[18] and ST[22]. Maxillary and mandibular osteosarcoma are associated with lower metastatic rates (35-46%) so may have a better prognosis if local control can be achieved. However, 54-80% of the dogs are euthanized due to local recurrence of the tumor[19]. Rib osteosarcoma occurs at a younger age 4.4-5.4 years and has a high metastatic rate with 27% of dogs presenting with pulmonary metastatic disease[19].

Serum ALP

Every 100% increase in ALP from normal increases the hazard for death by 1.7 in extracranial bones of the axial skeleton[18]. In appendicular OSA, median ST for dogs with normal total ALP or increased total ALP before treatment were 12.5 months and 5.5 months respectively[23]. When bone ALP was evaluated specifically, dogs with normal and elevated BALP prior to treatment had MST of 16.6 and 9.5 months respectively[23].

Stage

Dogs with Stage III osteosarcoma (visible metastatic disease at diagnosis) have a median survival time of 76 days. Palliative radiation and chemotherapy prolonged survival time to 130 days[20]. Metastasis to the regional lymph node is rare (<5%), but it has a negative impact on median survival time, 48 days compared to 238 days for dogs without positive lymph node[24].

Newer prognostic indicators

Monocyte and lymphocyte count[7].

Peripheral monocyte count numbers on the higher end of the normal range may have significant impact on DFI. Peripheral blood monocytes (>400/ul) and lymphocytes (>1,000/ul) prior to treatment are associated with shorter DFI in dogs with OSA[7]. However, overall ST was not significantly different in a follow-up study[8]. In dogs with monocyte counts <400/ul, the median DFI was 466 days compared to 202 days for dogs with monocyte counts >400/ul[7], median survival times was 247 days versus 337 days[8]. Further studies on the role of peripheral blood monocytes in canine osteosarcoma have found that monocyte chemokine receptors are down regulated by increased inflammatory mediators by the tumor, specifically PGE2 and TNFa. This change results in monocytes with a decreased ability to migrate to the primary tumor or metastatic sites. This is likely one of many mechanisms that OSA uses to evade the immune system[8.] This knowledge opens a window for the development of novel and effective immunotherapy strategies.

Immunotherapy

A natural model of immunotherapy is spontaneous regression of osteosarcoma which has been reported previously in dogs[21]. This is further supported by multiple reports of dogs who developed secondary infections following limb-sparing procedures which repeatedly show lower metastatic rates and better survival times[9-12]. Studies suggest that an infection restores monocyte chemotaxis[8].

A complete history of immunotherapy in canine osteosarcoma can be reviewed here https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4895426/

Listeria vaccine

Recombinant *Listeria* vaccines offer a unique pathogen based cancer vaccine due to the unique pathogenic behavior of the gram positive Listeria[25]. Listeria can enter phagocytic and non-phagocytic cells, escape from phagosomes, circumvent host

immune responses, and is able to move both intracellularly and from cell to cell[25]. Studies on mice and now canines have used a Listeria based vaccine containing fragments of the HER2/neu tyrosine kinase receptor in order to stimulate an innate and adaptive immune response. Listeria's unique pathogenic abilities allow it to access MHCI and MHCII receptors stimulating CD4 and CD8 based immunity[26]. HER2/neu is a member of the epithelial growth factor receptor family of tyrosine kinase receptors. HER2/neu overexpression contributes to numerous carcinomas, most notable breast cancer where the addition of Herceptin to HER2/neu positive breast cancer has significantly improved survival. HER2/neu over expression is found in 30-40% of canine and pediatric/adolescent osteosarcoma patients[26]. In a Phase I study evaluating safety and efficacy of this vaccine in dogs with confirmed HER2/nue positive osteosarcoma, the vaccine was safe, well tolerated, and significantly improved survival time compared to historic controls. These dogs were treated with surgery or limb sparing therapy, followed by four doses of IV carboplatin, then given a series of three Listeria based HER2/neu vaccines three weeks apart. Side effects were mild, transient, and included mild fever, increased leukocyte counts, and increased ALP in some cases. Dogs with higher elevations in leukocyte counts had longer survival times. The median disease free interval and median survival time for this group of dogs was 615 days and 956 days respectively[26].

This vaccine AT-014 is currently in the therapeutic pipeline by Aratana therapeutics.

A full copy of this Phase I trial can be reviewed at http://clincancerres.aacrjournals.org/content/22/17/4380.long

Personalized medicine study

A collaborative study between the OHSU Pediatric Cancer Biology Program and Oregon State University College of Veterinary Medicine was performed on a handful of dogs with osteosarcoma. The first patient was published in a case report,[27] and the other patients are mentioned briefly in a study specifically evaluating the role of dasatinib in canine osteosarcoma cell lines[28]. The collaborative study took individual tumors from canine patients following limb amputation and created a cell line from their tumors. The cells were then put through a drug screening process where they were exposed to eighty-six commercially available small-molecule kinase inhibitors to see which drug would have the highest impact on cell viability. Dasatinib was the most promising therapeutic option based on drug screening as very low concentrations were needed to result in high tumor cell kill. Dasatinib (Sprycel) is a tyrosine kinase inhibitor of the non-receptor tyrosine kinase Src, among other targets (c-kit, Bcr-abl, PDGFR, ephrin receptors). Immunohistochemistry was done with a Src antibody confirming

overexpression of Src and pSrc. A pharmacokinetic study was carried out to determine safe and effective dosing of dasatinib in this patient. This patient was treated with dasatinib following amputation and standard carboplatin chemotherapy as well as taurolodine. This patient took oral dasatinib for 20 weeks following standard therapy and survived 31 months following diagnosis[27]. Among the small number of patients that were able to be included in this study, dasatinib was a promising option in the majority of cases. Follow-up on other patients include most notably a Shepard mix who developed multiple small pulmonary metastases following his third dose of carboplatin. Therapy was switched to dasatinib at that time and the dog was lost to follow-up 44 months after diagnosis with stable disease at that time[28].

While personalized medicine is not available yet in the commercial setting, canine patients may benefit from IHC for Src expression on their tumor to determine if dasatinib is a rational therapy option.

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