3. Materials and Methods

3.1. Case Selection

The medical records of Veterinary Oncology Services (VOS), Edmonds, Washington, USA, accumulated during the years 1995-2001 were reviewed. Dogs that received external beam radiation for a confirmed histopathological diagnosis of soft-tissue sarcoma were selected for inclusion into this study. Hemangiopericytoma, fibrosarcoma, liposarcoma, infiltrative lipoma, undifferentiated sarcoma, malignant peripheral nerve sheath tumor, malignant fibrous histiocytoma, leiomyosarcoma, tendon sheath tumor, myxosarcoma, rhabdomyosarcoma, lymphangiosarcoma and spindle cell tumor of unknown origin defined the STS category, although not all tumor types are represented in the group studied. Osteosarcoma, chondrosarcoma, mast cell tumor, malignant melanoma, lymphosarcoma, hemangiosarcoma, and tumors lacking a definitive diagnosis were excluded from this study. Cases were not excluded based on previous treatment, adjuvant treatment, tumor location, bony involvement or previous irradiation. Cases that were irradiated after incomplete excision were included along with radiation therapy that was delivered in the face of macroscopic disease.

The presence of residual disease was defined as a histopathological report of an excised tumor extending to the margins of the section, a surgical report stating that complete resection was not possible or surgery undertaken at a site that precluded excision with adequate margins in conjunction with a pathological evaluation of close margins.

3.2. Diagnostics and Treatment

Current medical records were obtained from the referring veterinarian prior to consultation with the owner. All dogs underwent physical examination prior to radiation therapy. Tumor location, tumor size or a description of the surgical scar/wound resulting from excision were consistently recorded as well as any finding with possible implications for anesthesia candidacy. During initial consultation,

information concerning the number and dates of previous surgeries as well as any other previous treatment was obtained from the owner or extracted from the medical record. Multi-modality therapy involving surgery and radiation was pursued when possible. If macroscopic disease was present on initial consultation, the patient was either assessed at VOS as a candidate for resection or referred to a board certified surgeon to evaluate the feasibility of cytoreduction. Fifty-one dogs with 52 tumors underwent resection prior to being referred to VOS; 22 dogs had visible disease at the time of presentation. Of the 22 dogs with measurable disease, eight were candidates for resection and referred for cytoreduction prior to initiating radiation therapy. Fourteen dogs received radiation treatment for macroscopic sarcoma. Thirty-five tumors underwent multiple excisions before RT was delivered; 25 tumors experienced two surgeries, nine sarcomas underwent three surgeries and excision was attempted four times in one dog. Twenty-five dogs received RT after the first case of incomplete resection. The last surgery before radiation treatment was performed by the primary care veterinarian in twenty-six cases; referral surgeons conducted 34 surgeries. Margins of the last surgical procedure prior to RT were histopathologically evaluated in 58 of the 60 dogs that underwent cytoreduction. The majority of excisions were incomplete and showed residual tumor cells in more than one margin (n=42). Eight tumors were excised with microscopic residual disease confined to one margin, the deep margin (n=5) more commonly exhibited evidence of incomplete excision than the lateral margin (n=3). Ten dogs were irradiated based on histological assessment of close margins in conjunction with a surgical report consistent with a low probability of complete excision.

Radiographic examination of the thorax was conducted in all dogs before proceeding with RT to screen for the presence of metastases and to aid in the assessment of anesthesia candidacy. Radiographs were reviewed by a referral radiologist. Red and white blood counts and serum biochemistry profiles were performed in all dogs (Phoenix Central Laboratory, Everett WA, USA) to aid in the assessment of anesthesia candidacy and in screening for metastatic disease or other pathologies with possible therapeutic implications. Additional imaging studies such as computed tomography (CT) were undertaken in 19 patients that required more complex radiation planning or when exploration of the extent of macroscopic disease was necessary.

No dog was treated without a diagnosis confirmed by histopathology. All slides were reviewed by a board certified pathologist. Slides were evaluated for tumor type, completeness of excision and tumor grade was reported in 46 cases. If multiple

biopsies were taken from the irradiated field, the most current pre-radiation biopsy was used for the purpose of analysis. In 16 instances, slides were forwarded to Colorado State University (CSU) for additional review. Immunohistochemical staining was required in one case to determine tumor type. If a discrepancy was found between the original pathology report and referral review, the CSU report was used for statistical analysis.

All radiation treatments were conducted at human medical facilities associated with the Swedish Medical Group (Puget Sound Tumor Institute, Seattle, WA, USA; Northwest Tumor Institute; Seattle, WA, USA). Treatments were planned and conducted with animals under general anesthesia. Prior to induction, venous access was achieved with a butterfly catheter placed in the cephalic vein. Anesthesia was induced with intravenously administered propofol[1]. The dogs were then intubated and the anesthetic plane was maintained with isoflourane[2] (3%) and oxygen administered from a portable anesthetic unit. Some animals required the use of pre-medication such as diazepam[3] (n=8), butorphanol[4] (n=6), acepromazine[5] (n=3), or ketamine[6] (n=1). All animals were monitored by closed circuit video and audio to assess correct positioning and maintenance of the desired plane of anesthesia during radiation delivery. All animals were monitored until recovery and released shortly after anesthetic effects dissipated.

A Clinac-18 (Varion Medical Systems Inc., Pal Alto CA) equipped with 6 MV and 15 MV photons and 9, 12, 15, and 18 MeV electrons was used for radiation treatment. A simulator was available for radiation planning. In cases of incomplete excision, the radiation field was based on the surgical scar plus a margin of at least 2 cm in all directions of the surgical scar. In cases of macroscopic disease, the gross tumor plus a margin exceeding 2 cm comprised the radiation field. The treatment area was clipped and marked prior to treatment planning. Sixty-eight radiation plans were based upon computer assisted central axis calculations (ROCS version 5.1.6). In six instances, 3D planning was undertaken based on CT images. Fields were selected so that no portion of the planned treatment volume received more than 105% of the prescribed dose and no portion of the planned treatment volume received less that 90% of the prescribed dose.

[1] Rapinovet®, Schering
[2] Isovet® Schering
[3] Valium®, Roche
[4] Torbugesic® , Fort Dodge
[5] PromAce®, Fort Dodge
[6] Vetalar®, Fort Dodge

Proper dose distribution required the use of parallel-opposed fields in 42 cases; single fields were used in 31 treatment plans. In one instance, non-parallel fields were used. Attention was given to reproducible positioning and complex positioning was avoided whenever possible. Adhesive tape and supports were used to assure correct positioning. When fields in the distal extremity were irradiated, clothespins were used in 17 cases to retract a margin of skin outside of the radiation field and spare lymphatic drainage. Bolus material was used in 63 cases to assure accurate radiation delivery to superficial structures. Synthetic bolus material was employed on flat anatomical structures; wet gauze was conformed to irregular structures such as the digits and acted as a bolus.

Sixty fields were treated with photon radiation. Fifty-nine treatment protocols involved 6 MV radiation and one animal received 15 MV photon radiation. Electrons were used in 14 cases, ten tumors of the trunk, two tumors of the proximal extremity and two oral tumors were represented in the group of patients treated with electrons. A 12 MeV program was the most common energy selected for electron treatments (n=6), followed by 15 MeV (n=4), 9 MeV (n=3) and 18 MeV (n=1) programs. Custom blocks cut from cerrobend were applied in 14 of 14 cases that required electrons, and blocking was used in 13 of 60 cases that received photon therapy.

A definitive and a palliative protocol were used in this study. A Monday-Wednesday-Friday protocol delivered in 14-15 fractions of 350 cGy to a total dose of 4900-5250 cGy was used for definitive treatment. Sixty dogs received 15 fractions, and seven dogs with hemangiopericytoma received 14 fractions as it has been shown that HPC are more radio-responsive than other tumors of the STS category (MCCHESNEY et al., 1989b). Two animals received radiation for multiple tumors. One dog had two separate STS and one dog received radiation treatment for a mast cell tumor and a soft-tissue sarcoma. In most cases, therapy was complete in 30-35 days.

In six cases, a hypofractionated protocol consisting of three 800 cGy fraction was delivered as a sole modality over 14-21 days. A hypofractionated protocol was most commonly selected for palliation of pain or alleviation of functional deficits associated with advanced disease, not with the expectation of an increase in survival. In one case, four 800 cGy fractions were given over 28 days. In addition to six dogs that received palliative RT, two dogs received a hypofractionated protocol after failure of a definitive protocol. No animal received more than 7000 cGy at VOS. Re-irradiation with a definitive protocol was undertaken in one case that had received

radiation at another institution, although radiation fields were not identical some field overlap was present.

Animals were re-evaluated 14 days following the completion of radiation therapy for assessment and management of acute radiation effects. If warranted, acute radiation toxicity was treated with systemic antibiotics, corticosteroids or with a topical agent such as silver sulfadiazine[7]. Following resolution of acute radiation injury, recommendations to owners included physical examination every 3-4 months and thoracic radiography at six-month intervals.

Adjuvant doxorubicin[8] therapy was offered in cases of high-grade sarcoma and in other selected instances. Nine owners pursued treatment with doxorubicin, six were oral sarcomas and three were non-oral sarcoma. The three non-oral sarcomas receiving doxorubicin were all high-grade, while the oral group contained Grade I (n=1), Grade II (n=1), ungraded (n=1) and Grade III (n=3) sarcomas. Doxorubicin dosed at 25-30 μg/m^2 for 3-5 treatments was administered at three week intervals during the peri- or post- RT period. Serial monitoring of hematological parameters was conducted during the course of chemotherapy. One dog with oral sarcoma received low-dose (10 μg/m^2) doxorubicin every seven days as a radiosenstizer.

3.3. Follow-up

Current information concerning patients included into this study was obtained prior to analysis. If recent examination had not been conducted at VOS, referring veterinarians were contacted and patient information was relayed per telephone or copies of the medical records were forwarded. In some instances telephone interviews were conducted directly with the owner. It was determined if the patient was alive or dead and the date and cause of death when appropriate. Tumor progression or recurrence since completion of radiation therapy was also a subject of inquiry. If progression or recurrence were noted, the date of recurrence was recorded along with accompanying information such as relationship of the recurrent tumor to the treatment field (i.e. inside radiation field vs. out of field recurrence) and histological data of the recurrent tumor (if available). Of the seventy-three patients included into this study, current information was obtained on 70 patients; three dogs

[7] Silvadene®, Marion
[8] Adriamycin®, Pharmacia

were lost to follow-up and censored at last contact. During follow-up contact inquiries were made to major toxicities; less severe reactions such as permanent alopecia, pigment change and fibrosis of the skin were not studied due to inconsistent reporting. Necropsies were not performed.

3.4. Analysis and Statistics

The commercial software packages *SPSS 10.0.7* (SPSS Inc., Chicago IL, USA), and *Excel 2000* (Microsoft, Redmond WA, USA) were used to conduct statistical analysis. The descriptors mean, median, maximum, minimum and standard deviation were calculated with *Excel 2000*, other statistical analyses and graphical depictions were conducted with *SPSS 10.0.7*.

In selected instances, Box-and-Whiskers plots were used to graphically summarize distribution of a data set. The upper and lower quartiles were defined with vertical lines, with the “box” encompassing the central 50% of the data points. The median was marked by a horizontal line within the box. The “whiskers” represent values not exceeding 1.5x the box length. Cases with values between 1.5 and 3 times the box length from the upper or lower edge of the box were considered outlier values and depicted with an open circle. Values farther than three times the length of the box from the edges of the box were considered extreme values and denoted with an asterisk (*)(PETRIE and WATSON, 1999c).

Assumptions of distribution and variance were formally examined prior to conducting parametric tests. Distribution was formally examined with a Shapiro-Wilk Test and Levene’s test was used to examine variance (PETRIE A and WATSON P, 1999f). If test assumptions were fulfilled, parametric tests were employed. Departure from test assumptions justified the use of non-parametric tests. Comparison of means was examined with an independent sample T-Test, the hypothesis of which is that the two groups examined have similar means. A low P-value is considered as evidence for rejecting the test hypothesis. Failure to satisfy test assumptions of normal distribution or equal variance resulted in the use of the nonparametric Mann-Whitney test (PETRIE A and WATSON P, 1999d; PETRIE A and WATSON P, 1999e)

For the purposes of analysis, groups were constructed based on tumor type, tumor grade, tumor location and the presence of measurable disease. Tumors were placed into groups based upon the most recent pathology review prior to RT. HPC, FSA, MPNST represented tumor type groups large enough to analyze;

remaining tumors that were infrequent were collectively analyzed as the Misc group. Tumors were assigned a grade based on the most recent pathology report that preceded radiation therapy. Four groups were created: Grade I (low-grade), Grade II (intermediate-grade), and Grade III (high-grade) – tumors that did not receive a grade or tumors without a definitive grade (e.g. low to intermediate) were collectively analyzed as the Ungraded Group. The following groups were constructed for the purpose of analysis based on anatomic location of the primary tumor: proximal extremity, distal extremity, trunk, head/neck or oral cavity. Tumors distal to the elbow or stifle regions were considered tumors of the distal extremity. Sarcoma located between the shoulder and elbow or hip and stifle were included in the proximal extremity group. Sarcoma of the thoracic or abdominal regions were analyzed as trunk tumors. The head/neck group encompassed all tumors of the head/neck not invading the oral structures. Oral tumors included all tumors involving any structure, regardless if bony or soft-tissue, of the oral cavity. In instances where non-oral tumors covered more than one region, the region that contained the majority of tumor tissue was selected for the purposes of analysis. Macroscopic disease was defined as palpable or visible tumor tissue.

Analysis was undertaken based on field size; the area eliminated by blocks used during radiation delivery was not eliminated for the purposes of analysis. Field size is reported in units of cm^2, not in equivalent squares. Due to demonstrable difference in field size between electrons and photons, factors examined were based on photon field sizes. Mean field size was examined according to surgical history, and the presence of macroscopic disease.

All reporting of signalment data, such as age and weight, was based upon the status of the patient at the time the first fraction of radiation was delivered.

Disease free interval (DFI), survival and overall survival curves were generated using the Kaplan-Meier Product Limit Method (PETRIE and WATSON, 1999a). Kaplan-Meier analysis offers the advantage of incorporating censored data and producing a non-parametric estimation the interval examined. Median interval is estimated as the point where an event has occurred in 50% of the animals. All incomplete observations were censored. Censored data are not eliminated from calculations, but instead are not considered as an event at the last point observed (PETRIE and WATSON, 1999a).

The disease free interval was calculated as the interval from the last radiation fraction delivered to the time when recurrence was noted in or near the radiation field. The event of recurrence was restricted to tumors that underwent

surgical reduction to microscopic disease prior to radiation. For the purposes of DFI analysis, the event examined was local recurrence the unit examined was the radiation field and the time interval was days. All animals that died without local tumor recurrence, were alive without recurrence at the end of the study or lost to follow-up were censored. Recurrence was limited to one event per treatment field.

The overall survival interval was calculated from the delivery of the last radiation fraction until death regardless of cause. All animals that were alive at the end of the study were censored along with those lost to follow up.

The survival interval was calculated from the last radiation fraction delivered until the survival event, death due to a tumor related cause, occurred. Tumor related causes included metastasis, tumor progression, tumor recurrence, radiation toxicity or euthanasia related to one the previously listed causes. All animals that died of non-tumor related causes, were alive at the end of the study or lost to follow up were censored at last contact.

Survival and DFI were compared according to several prognostic factors using Log-rank tests, if more than two independent groups were formed based on a factor, groups were compared in a pair-wise fashion. The Log-rank test is a non-parametric test that compares survival (or other interval of interest) curves. The test hypothesis is that the two groups examined have equal survival and the test hypothesis approaches rejection with decreasing P-value (PETRIE and WATSON, 1999a).

The development of recurrence was examined as a survival factor in all dogs that underwent resection prior to irradiation, as was oral vs. non-oral location and resected vs. macroscopic disease. Dogs were placed into one of four groups for the purposes of examination of additional prognostic factors and more exact clinical characterization; no patient was placed in more than one group. Group I consisted of animals that were treated with a definitive radiation protocol for incompletely resected sarcoma of a location other than the oral or nasal structures. Group II consisted of animals that were treated with a definitive radiation protocol for sarcoma of the oral cavity regardless if macroscopic or microscopic disease was present. Group III consisted of animals that were treated for macroscopic of non-oral sarcomas with a definitive radiation protocol. Group IV consisted of animals that received only hypofractionated radiation as radiotherapy.

Survival intervals and DFI were compared among dogs according to tumor type, tumor grade and tumor location. DFI was examined according to the number of surgeries performed (first surgery or multiple surgeries), field-size (field size above 75

cm^2 vs. field size below 75 cm^2), and interval between surgery and radiation therapy. The surgery to radiation therapy interval (Sx-RT) was calculated as the interval between the last surgery to delivery of the first fraction of radiation. Kaplan-Meier analysis was undertaken based on dogs with an Sx-RT interval over 45 days vs. Sx-RT interval below 45 days.

The results of all statistical tests are reported as P-values. Decreasing P-values are assumed to represent superior documentation of departure from the test hypothesis. P-values are dependent upon the degree of difference and the sample size; a statistical difference does not necessarily imply a biologically or clinically important difference. Failure to demonstrate a difference (i.e. low P-value) does not justify the premise that a difference does not exist; the tests employed are not designed to demonstrate similarity-especially in cases where sample sizes are small (PETRIE A and WATSON P, 1999b). A level of significance was not set in this study as all statistical tests were conducted with descriptive and explorative intent (PETRIE A and WATSON P, 1999b). All references to differences according to prognostics factors are not based solely upon the results of hypothesis testing. The term “difference” is to be considered as a subjective interpretation of the author based upon the results of hypothesis testing and descriptive statistics. A p-value below 0.05 is commonly considered to represent a difference in veterinary medicine (PETRIE A and WATSON P, 1999b).