

# MALIGNANT TUMOR FORMATION IN DOGS PREVIOUSLY IRRADIATED FOR ACANTHOMATOUS EPULIS

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**In this retrospective study of 57 dogs irradiated for oral acanthomatous epulis, 2 (3.5%) dogs developed a second tumor (sarcoma, osteosarcoma) in the radiation treatment field at 5.2 and 8.7 years after the end of radiation therapy. As opposed to previous reports, no second epithelial tumors developed in the radiation treatment field. There is a risk of radiation-induced carcinogenesis, but it appears that it is a relatively low risk and an event that occurs years after radiation therapy. Radiation-induced tumors are of more concern in younger dogs that undergo radiation therapy for tumors that are radioresponsive, such as acanthomatous epulis, where long-term survival is expected. The only statistically significant variable in the survival analysis was age, with dogs less than 8.3 years old having a significantly longer median overall survival (2322 days) than dogs older than 8.3 years (1106 days;  $P < 0.0001$ ). *Veterinary Radiology & Ultrasound*, Vol. 45, No. 4, 2004, pp 357–361.**

**Key words:** acanthomatous epulis, canine, carcinogenesis, radiation therapy.

## Introduction

IN PRIOR DESCRIPTIONS of the response of acanthomatous epulides to irradiation, subsequent observation of a malignant tumor of a different histologic type at the site of the epulis has been reported.<sup>1–3</sup> The incidence of malignant tumor formation at the radiation site in dogs with an acanthomatous epulis has been reported to range from 2.6% to 18%.<sup>2,3</sup> Regrettably, one of us (DT) used the term “malignant transformation” to describe this phenomenon, and it was also suggested that it was a specific complication related to irradiation of acanthomatous epulides.<sup>2</sup> Malignant transformation implies that irradiation altered the biology of the tumor with emergence of an altered, perhaps more aggressive, phenotype. Other equally or more viable possibilities are expression of a subpopulation of malignant epithelial cells surviving radiation therapy or radiation-induced carcinogenesis. Regardless, these observations and descriptions have led to some reluctance in recommending irradiation of acanthomatous epulides in spite of the excellent local control achieved in most patients with this tumor.

Considering that the most recent accounting of this observation was nearly 20 years ago, and that most patients in those accounts received treatment with orthovoltage X-rays and a suboptimal radiation dose, we reassessed this phenomenon in a larger patient population treated with more contemporary radiation technology and prescriptions. Thus the purpose of this paper is to review data from two veterinary medical teaching hospitals to more accurately assess the risk of second tumors developing in the radiation field in dogs irradiated therapeutically for acanthomatous epulides.

## Materials and Methods

Fifty-seven dogs that underwent a definitive course of megavoltage radiation therapy for oral acanthomatous epulis at two different institutions were evaluated retrospectively. Selection criteria included dogs (1) with biopsy-confirmed oral acanthomatous epulis, (2) treated with <sup>60</sup>Cobalt photons, and (3) receiving adequate follow-up.

The dogs were treated at North Carolina State University College of Veterinary Medicine ( $n = 19$  dogs) and at the University of California Davis School of Veterinary Medicine ( $n = 38$  dogs) between January 1981 and October 1996. The information on a subset of the dogs treated at the University of California Davis was previously reported.<sup>3</sup> Dogs were staged according to the World Health Organization staging scheme, with tumor stage defined as follows: T1 < 2 cm, T2 = 2–4 cm, T3 > 4 cm, and a = no evidence of underlying bone involvement and b = underlying bone involvement, with status of bone involvement determined radiographically.

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Age ranged from 0.7 to 15 years (median age of 8.3 years). There were 36 females (5 intact) and 21 males (13 intact). There were 16 mixed-breed dogs and 41 purebred dogs, with 22 different purebred breeds represented in this study. The most common breeds included Labrador Retriever ( $n=7$  dogs), German Shepherd ( $n=5$  dogs), and Old English Sheepdog ( $n=4$  dogs). The range in body weight was 5.6–56 kg (median body weight 28.7 kg).

Tumor location included mandible ( $n=35$ ) involving the rostral half ( $n=28$ ) or caudal half ( $n=7$ ), and maxilla ( $n=22$ ) with either a rostral ( $n=16$ ) or caudal ( $n=6$ ) location. The stages for the dogs in this study included T1a ( $n=6$ ), T1b ( $n=11$ ), T2a ( $n=1$ ), T2b ( $n=33$ ), T3b ( $n=5$ ); one dog had an unknown size tumor with evidence of underlying bone involvement.

All dogs were irradiated with  $^{60}\text{Cobalt}$  photons on a Monday-Wednesday-Friday alternate day schedule. The dose per fraction was 3.8 Gy ( $n=9$  dogs), or 4 Gy ( $n=48$  dogs). The total radiation dose was 40 Gy ( $n=4$  dogs), 44 Gy ( $n=10$  dogs), 45.6 Gy ( $n=9$  dogs), or 48 Gy ( $n=34$  dogs). The dogs were treated using either a single field ( $n=13$  dogs) or parallel opposed fields ( $n=44$  dogs).

Information abstracted from the medical records and via follow-up phone calls included age, gender, body weight, tumor location, tumor stage, history of previous surgery, radiation therapy protocol, response to therapy, and evidence of epulis recurrence or development of a second tumor at the radiation site.

Parameters evaluated for significance related to time to local recurrence (TLR) and overall survival (OS) included body weight ( $\leq$  or  $>$  median), site 1 (mandible vs. maxilla) and site 2 (rostral vs. caudal), age ( $\leq$  or  $>$  median as well as 3 age categories: 0–3 years, 4–10 years, or  $>10$  years), radiation dose (40 Gy, 44 Gy, 45.6 Gy, 48 Gy), normalized radiation field size (equivalent square normalized to body weight in kilograms), radiation facility, gender (male vs. female), WHO stage (T1a, T1b, T2a, T2b, and separate analysis of T1 vs. T2 and a vs. b), and surgery (at some point in time prior to radiation therapy vs. immediately prior to radiation therapy).

Data were evaluated using a first event analysis whereby tumor recurrence, metastasis or death due to any cause is considered the terminal endpoint.<sup>4</sup> Differences in time to endpoint (recurrence or death) were tested for each parameter listed above. The nonparametric 2-sample or multisample univariable survival analysis tests used were Gehan–Wilcoxon test and the logrank test. If a parameter had  $P \leq 0.05$  in either test, it was offered to the (multivariable) Cox proportional-hazards regression model for that endpoint.

Prior to the proportional-hazards modeling, parameters to be used were reduced to dichotomous variables; unless otherwise specified, the dichotomization was at the median. Each proportional-hazards model was reduced in steps by

deleting the parameter with the highest Wald's  $P$ . That the removal was reasonable was confirmed by checking for a nonsignificant change in the likelihood-ratio  $\chi^2$  test, and for what we judged to be only trivial changes in the hazards ratios of the remaining parameters. (Statistix 7, Analytical Software, Tallahassee, FL)

## Results

At the time of the review, 46 dogs were known to be dead and 11 were alive and lost to follow-up. The range of follow-up time in these 11 dogs was 3.2–103.7 months (median 55.8 months). Overall median time to first event and overall survival were 1210 and 1441 days (Fig. 1). Two of the 57 dogs (3.5%; 95% CI=0%, 9%) developed tumors of a different histologic type at the site of the irradiated epulis. One dog (6.6 years of age when irradiated) developed a sarcoma 62.2 months (5.2 years) after radiation therapy. A second dog (2 years of age when irradiated) developed an osteosarcoma 104.9 months (8.7 years) after radiation therapy.

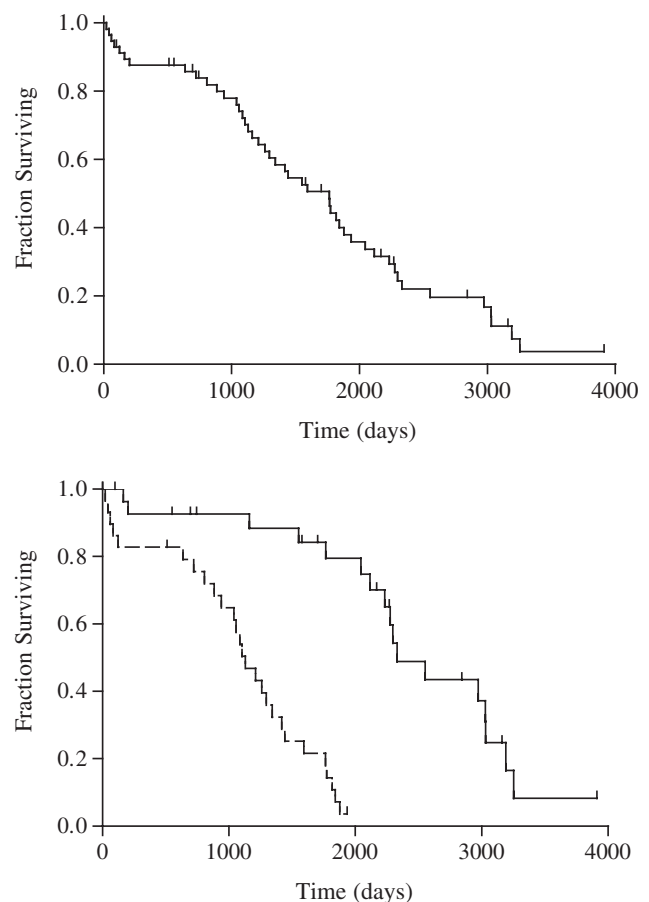


FIG. 1. Overall survival of dogs irradiated for acanthomatous epulis (top). Overall survival of dogs irradiated for acanthomatous epulis as a function of age ( $P<0.0001$ ) (bottom). Dogs older than the median (8.3 years) are indicated by the broken line ( $n=29$ ). Dogs younger than the median are identified by the solid line ( $n=28$ ).

TABLE 1. Parameters Significantly Related to Time to First Event as Determined by Univariate Analysis

Variable	Median Survival (days)	Gehan-Wilcoxon ( <i>P</i> value)	Logrank ( <i>P</i> value)
Age			
<3 years†		0.008	0.02
≥3 years	2922		
Normalized radiation field size*			
<0.23	>2453‡	0.01	0.02
≥0.23	2432		
Total dose (Gy)			
40	143	0.005	0.01
>40	2994		

\*Equivalent square normalized by body weight in kilograms. †Not possible to calculate; the young dog with recurrence had recurrence at 158 days; the other two were followed 2044 or 3189 days without recurrence. ‡Median not reached; 2453 days = 75th percentile.

For time to first event (Table 1), age, normalized field size, dose, and stage were of interest for the hazard model. Stage had the lowest univariable *P* of 0.06, but there was interest in it a priori. Age dichotomized at the median had  $P \geq 0.26$  in the univariable tests, but age categorization was highly significant in overall survival modeling. So, age was dichotomized at <3 years and  $\geq 3$  years. Dose was dichotomized (for a priori reasons) at 40 Gy vs. higher total doses, and tumor stage at 1 vs. higher stage. In the multivariable analysis, only lower total dose significantly increased hazard of time to first event (hastened recurrence: 143 days if dose 40 Gy, but 2,994 days with higher doses). However, normalized radiation field ( $P=0.11$ ) was retained in the model because its removal caused the hazard ratio for dose to increase to 16.1 from 10.2 (suggesting confounding by normalized radiation field size).

The parameters significantly related to survival as determined by univariate analysis included age (dichotomized at the 8.3 year median), normalized radiation field size, and site (rostral or caudal) (Table 2). These parameters were offered to the multivariable model of survival. Only age

TABLE 2. Parameters Significantly Related to Survival as Determined by Univariate Analysis

Variable	Median Survival (days)	Gehan-Wilcoxon ( <i>P</i> value)	Logrank ( <i>P</i> value)
Age			
<8.3 years	2322	<0.0001	<0.0001
≥8.3 years	1106		
Normalized radiation field size*			
<0.23	1864	0.03	0.05
≥0.23	1383		
Tumor site			
Rostral	1844	0.03	0.03
Caudal	1088		

\*Equivalent square normalized by body weight in kilograms.

was retained; dogs <8.3 years old at the time of radiation treatment had longer median survival (2322 days) than those  $\geq 8.3$  years old (1106 days;  $P<0.0001$ ; Fig. 1).

## Discussion

The only statistically significant variable in the survival analysis was age, with dogs less than 8.3 years old having a significantly longer median survival than dogs older than 8.3 years. This finding is consistent with the expectation of longer survival in younger dogs when irradiating a tumor that responds well to radiation therapy with long-term local control.

We observed two differences regarding development of malignant tumors at epulis irradiation sites compared to prior reports. First, the overall incidence seems lower at 3.5% (2/57) in this study compared to as much as 18% (7/39) reported previously.<sup>2</sup> Second, we observed no malignant epithelial tumors in our patients compared to a prior report where 5 of 7 tumors developing after epulis irradiation were of epithelial origin.<sup>2</sup>

The exact reason for these differences is unknown, but there are possible explanations. With regard to tumor type, the lack of secondary epithelial tumors in our population could have resulted from the different radiation technology. In previous reports of results of radiation therapy in dogs with acanthomatous epulis, dogs were treated with orthovoltage radiation, which is relatively ineffective for treatment of tumors with bone involvement, such as acanthomatous epulis. The low photon energy of orthovoltage X-rays results in overtreatment of superficial bone and undertreatment of deeper bone. Malignant epithelial tumor cell subpopulations in undertreated bone could lead to recurrence of an epithelial tumor following irradiation. Another consideration is the possible presence of an inherently more radiation-resistant population of cells within a heterogeneous tumor matrix. Additionally, there has been an ongoing debate as to the appropriate histopathological classification of epulides in dogs.<sup>5-7</sup> Similarities in morphology and variability in nomenclature have resulted in difficult interpretations between epulides, squamous cell carcinoma, adamantinoma, and ameloblastoma. This raises the concern that the recurrent epithelial tumors in the original report might have represented recurrence of a primary squamous cell carcinoma rather than emergence of a resistant population of malignant epithelial cells.<sup>2</sup> It is much less likely that the recurrent epithelial tumors were bona fide radiation-induced tumors as most radiation-induced tumors that develop in a heavily irradiated treatment field are of mesenchymal origin rather than epithelial origin.<sup>8-10</sup>

Radiation carcinogenesis is the most logical explanation for the observation of mesenchymal-origin tumors following irradiation of the epulis. Ionizing radiation is a well-known potential carcinogen. The first documented death due to

development of a radiation-induced tumor following exposure to X-rays was in 1904.<sup>11</sup> Since then radiation-associated tumors have been extensively documented in humans.<sup>12-17</sup> Both carcinomas and sarcomas are potential radiation-associated malignancies, but the risk for sarcoma development in an irradiated field is significantly greater.<sup>8-10</sup> The risk of a secondary sarcoma within the irradiated field has been shown to be dose-dependent, with a higher risk in patients that receive greater than 60 Gy total radiation dose.<sup>8</sup> Radiation-induced solid tumors in humans have an average latency period on the order of 5-15 years.<sup>9,13</sup>

The criteria for diagnosis of a radiation-induced second tumor have been described and include (1) history of irradiation and tumor development within the irradiated field, (2) latent period of adequate duration after irradiation before development of the second tumor, (3) for bone tumors (i.e., osteosarcoma that develops at the irradiated site), documentation that the bone previously was normal, and (4) biopsy and histopathologic confirmation of a malignant tumor at the irradiated site.<sup>12</sup>

Radiation carcinogenesis has been observed in dogs after therapeutic irradiation<sup>1,18-21</sup> and experimental studies.<sup>21-25</sup> A higher risk for development of a radiation-induced osteosarcoma has been seen with high dose per fraction and higher total dose of radiation.<sup>21</sup> Seven of 27 dogs (26%) treated with a combination of a single intraoperative dose of radiation (25-47.5 Gy) and fractionated external beam radiation therapy developed an osteosarcoma in the irradiated field (50 Gy).<sup>21</sup> Of a total of 87 dogs with soft tissue sarcomas that were irradiated, 3 (3.4%) developed osteosarcoma in the irradiated field, although only 25% of the 87 dogs lived 20+ months to a time where a radiation-induced tumor could arise. In effect 13.6% of the 22 dogs that lived

longer than 20 months after radiation developed a radiation-induced tumor.<sup>21</sup> A relatively high fractional dose of radiation was used ranging from 3.5 to 5 Gy per fraction on an alternate-day basis for a total of 10 fractions.<sup>21</sup>

It is difficult to assess the absolute risk of a second tumor in canine radiation therapy patients. There has been discussion of local inflammation as a predisposing factor which may be present in dogs with bone involvement that undergo irradiation.<sup>26</sup> There typically is underlying bone involvement with acanthomatous epulides, which has also been considered a risk factor.<sup>7,21</sup> At the same time, it also has been documented that bone sarcomas can arise secondary to irradiation in previously normal bone. It is also difficult to predict which patients may be at greater risk for development of a second tumor.

In summary, in 57 dogs with an acanthomatous epulis treated with megavoltage radiation therapy, we observed 2 patients (3.5%) where a malignant mesenchymal tumor developed at the irradiated site. This incidence is lower than in prior reports and there was also an absence of secondary epithelial tumors in contrast to what has been previously reported. The developing mesenchymal tumors are most likely due to radiation carcinogenesis; all defining criteria were met. The lack of developing epithelial tumors in our patients is more difficult to explain but could be due to the more effective therapy of the primary tumor through use of megavoltage photons, which may have contained a subpopulation of malignant epithelial cells. Regardless, the concept of "malignant transformation" of acanthomatous epulides should be discarded, especially considering the overall excellent response of these tumors to irradiation and the low overall incidence of radiation carcinogenesis being similar to other tumor types.

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