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# Metastatic Balloon Cell Melanoma in a Dog

Melinda J. Wilkerson, Karen Dolce, Brad M. DeBey, Heather Heeb, Harriet Davidson

**Background:** Balloon cell melanoma is a rare variant of amelanotic melanoma that is difficult to differentiate from sebaceous cell carcinoma, liposarcoma, and other clear cell neoplasms without immunohistochemistry or ultrastructural evidence of melanin or melanosomes. **Objective:** The purpose of this report was to describe the clinical, cytologic, histologic, immunohistochemical, and ultrastructural findings in a dog with metastatic balloon cell melanoma. **Methods:** A 6-year-old female Golden Retriever was evaluated for a white, flocculent infiltrate in the anterior chamber of the left eye and an enlarged left prescapular lymph node. Cytologic evaluation of the eye and lymph node were performed following aqueocentesis and fine-needle aspiration, respectively. The affected lymph node was examined histologically and stained for cytokeratin, vimentin, S-100, and Melan A. Following euthanasia a necropsy was performed and samples of the affected lymph node were examined by electron microscopy. **Results:** Cytologic examination of the lymph node and aqueocentesis sample revealed round neoplastic cells that had abundant clear vacuolated cytoplasm. A tentative diagnosis of metastatic sebaceous cell carcinoma or clear cell neoplasm was made. Histologically, the affected lymph node had similar polygonal clear cells arranged in sheets and packets divided by delicate fibrovascular stroma. Immunohistochemical staining of the cells was negative for cytokeratin but positive for vimentin, weakly positive for S-100, and strongly positive for Melan A. At necropsy, metastatic lesions were identified in the diaphragm, heart, lung, kidneys, left eye, prescapular and sublumbar lymph nodes, and multiple skin sites. Ultrastructural examination of neoplastic lymph nodes revealed many membrane-bound vacuoles, myelinlike figures, and rare melanosomes. **Conclusion:** Immunohistochemical staining and ultrastructural features of the neoplastic cells supported a diagnosis of metastatic balloon cell melanoma. (*Vet Clin Pathol.* 2003;32:31-36)  
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**Key Words:** Balloon cell melanoma, dog, fine needle aspirate, histopathology, Melan A, ultrastructure

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A 6-year-old spayed female Golden Retriever was referred to the Veterinary Teaching Hospital at Kansas State University (KSU) with a 2-week history of bilateral uveitis; the left eye was worse than the right. Physical examination was unremarkable except for an enlarged left prescapular lymph node and uveitis. The left eye had a marked white, flocculent infiltrate free in the anterior chamber and attached to the anterior surface of the iris. The pupil was miotic, and the iris was swollen, inflamed, and irregular. Both eyes had low intraocular pressure (7 mm Hg OD and 5 mm Hg OS). The fundus was normal in the right eye but could not be clearly visualized in the left.

Upon presentation, a CBC (Cell-Dyn 3700, Abbott Laboratories, Abbott Park, Ill, USA), chemistry panel (Hitachi 911, Boehringer Mannheim, Indianapolis, In, USA), and fine-needle aspiration of the left prescapular lymph node were performed, and aqueocentesis of the left eye was done later in the day. Lymph node aspirates were stained with Protocol Hema 3 (Fisher Scientific, Pittsburgh, PA, USA), a quick stain. A cytocentrifuged preparation (800 rpm, 5 min, Cytospin 2, Thermo Shandon Inc, Pittsburgh, Pa, USA) of the aqueocentesis sample was made, and also was stained with Protocol

Hema 3.

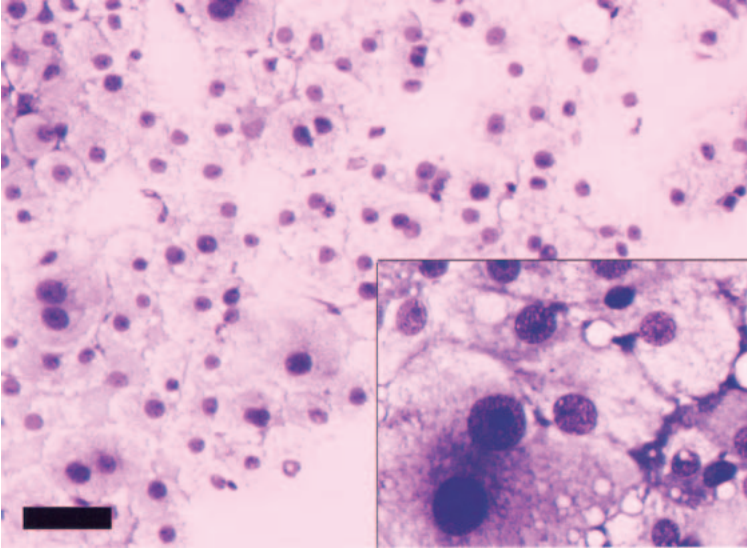
Results of the CBC included lymphopenia (830 cells/ $\mu$ L, reference interval 1500-5000 cells/ $\mu$ L). Mild increases were noted in alkaline phosphatase (ALP) (162 IU/L, reference interval 12-122 IU/L), alanine transaminase (ALT) (325 IU/L, reference interval 13-79 IU/L), and creatine kinase (CK) (264 IU/L, reference interval 58-241 IU/L) activities. Increased ALP activity and lymphopenia were attributed to systemic steroid therapy (prednisone) given to the dog by the referring veterinarian. The increase in ALT activity indicated hepatocellular damage, whereas the mild increase in CK activity suggested muscle damage or may have been insignificant.

A key historical event was described by the owner upon further inquiry. Eight months prior to presentation, a digit on the left forelimb was amputated because of a chronic lesion that was nonresponsive to antimicrobial treatment. The lesion was not submitted for histopathologic examination. In light of this new information, thoracic radiographs were obtained, and an excisional biopsy of the prescapular lymph node was performed. Thoracic radiographs revealed several 5-mm opaque nodules consistent with metastatic neoplasia within the pulmonary parenchyma.

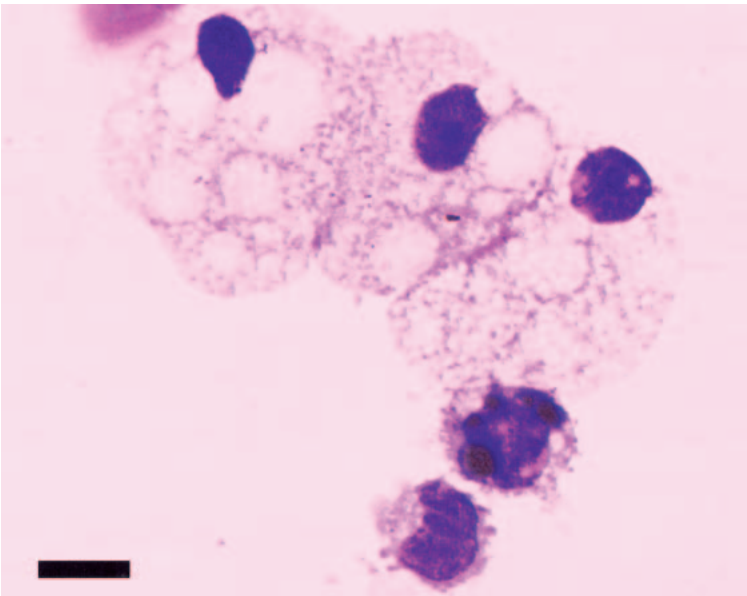
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From the Departments of Diagnostic Medicine/Pathobiology (Wilkerson, Dolce, DeBey) and Clinical Sciences (Heeb, Davidson), College of Veterinary Medicine, Kansas State University, Manhattan, KS. Corresponding author: Melinda J. Wilkerson, Rm 340 Coles Hall, 1800 Denison Ave, Manhattan, KS 66506 (wilkern@vet.ksu.edu).

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**Figure 1.** Fine needle aspirate of the left prescapular lymph node from a dog contains of a dense sheet of round cells of various sizes with foamy vacuolated cytoplasm and marked nuclear pleomorphism. Protocol Hema 3, bar = 50  $\mu$ m. **Inset:** bar = 25  $\mu$ m.



**Figure 2.** A cytocentrifuged preparation of aqueous fluid from the left eye contains several groups or clusters of large vacuolated cells, few monocytes, and a melanin-containing cell (presumptive melanophage). Protocol Hema 3, bar = 10  $\mu$ m.

### Cytologic Evaluation

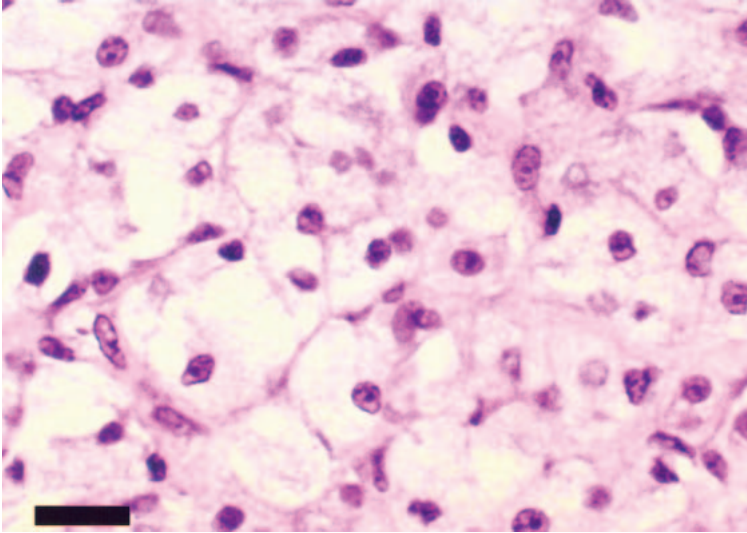
Microscopic examination of the lymph node aspirate revealed a large number of cells arranged in sheets or dense packets subdivided by pink fibrillar matrix. The cells were uniform, large, and polygonal with abundant clear vacuoles and pale basophilic cytoplasm (Figure 1). Nuclei were central to eccentric and exhibited moderate

to marked anisokaryosis, occasional karyomegaly, and coarse to ropy chromatin with prominent medium to very large nucleoli. Occasionally, binucleated and multinucleated cells were observed. The microscopic features of the cells were most suggestive of a neoplasm of epithelial cell origin; however, a mesenchymal neoplasm (such as liposarcoma) could not be excluded. Because of the cytoplasmic vacuolation, a cytologic diagnosis of sebaceous gland carcinoma or clear cell neoplasia (such as clear cell basal cell carcinoma, metastatic clear cell carcinoma of lung, clear cell sarcoma of soft parts, or balloon cell melanoma) was considered likely. Because clear cell carcinomas and sarcomas in people contain glycoproteins (glycogen), a cytologic preparation of lymph node was stained with periodic acid—Schiff (PAS).<sup>1-3</sup> The neoplastic cells were negative for PAS, however, and histopathologic examination was recommended.

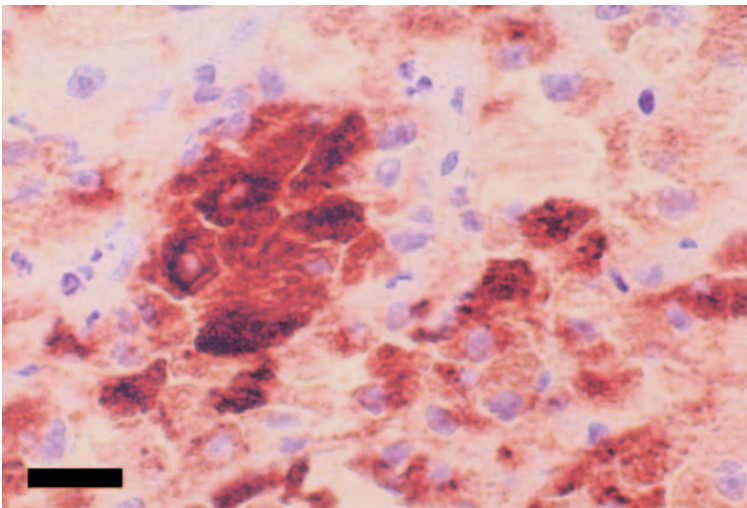
The cytocentrifuged specimen of ocular fluid contained a mixture of WBCs (5% nondegenerate neutrophils, 7% lymphocytes, 66% macrophages) and large foamy cells (22%) arranged in loose clusters. The macrophages were moderately vacuolated. Rare mononuclear cells contained large melanin granules and were presumed to be melanophages or pigmented epithelial cells. The large foamy cells had central to eccentric nuclei and lower nuclear to cytoplasmic ratios than cells in the lymph node aspirate (Figure 2). Nuclei had dense chromatin and indistinct single nucleoli. There was no evidence of fungal or other infectious agents. The cytologic interpretation was mild inflammation (uveitis) with suspected metastatic neoplasia of undetermined origin. The cells in the lymph node and aqueous chamber of the left eye were thought to be of the same cell type.

### Histology and Immunohistochemistry

Histopathologic sections of the lymph node biopsy sample were routinely prepared and stained with hematoxylin and eosin. The specimen contained large polygonal cells arranged in sheets and packets separated by fine, delicate fibrous stroma. The cells had abundant vacuolated and finely granular cytoplasm with discernible cytoplasmic membranes (Figure 3). Mild to moderate nuclear pleomorphism with rare mitoses was present. Typically, nuclei were eccentric and hyperchromatic. Other nuclei had stippled to vesicular chromatin and prominent central nucleoli. Based on the morphologic appearance of the lymph node specimen and the history of an undiag-



**Figure 3.** Histologic section of lymph node contains large polygonal cells with numerous cytoplasmic vacuoles arranged in sheets. Hematoxylin and eosin, bar = 25  $\mu$ m.



**Figure 4.** Histologic section of lymph node. Immunohistochemical stain for Melan A shows positive staining within vacuolated neoplastic cells. Immunoperoxidase, Mayer's hematoxylin counterstain, bar = 25  $\mu$ m.

nosed lesion on the toe, metastatic balloon cell melanoma was considered likely.

To support a diagnosis of an amelanotic melanoma, immunohistochemistry for vimentin, S-100 protein, and Melan A was performed on paraffin-embedded sections using antibodies to S-100 and vimentin (prediluted; Dako, Carpinteria, Calif, USA) and Melan A (clone A103; Novacastra Laboratories, Benton Lane, Newcastle upon Tyne, UK) by previously described methods.<sup>4</sup> Antibodies to high- and low-molecular-weight cytokeratins (prediluted; Dako) were used to rule out cells of epithelial origin. The neoplastic cells were negative for cytokeratin, positive for vimentin, weakly positive for S-100, and pos-

itive for Melan A (Figure 4). These findings confirmed that the neoplasm invading the prescapular lymph node was a melanoma with morphologic features consistent with a balloon cell melanoma. The primary neoplasm was initially thought to be from the toe based on the history; however, without histologic confirmation a primary ocular neoplasm could not be ruled out.

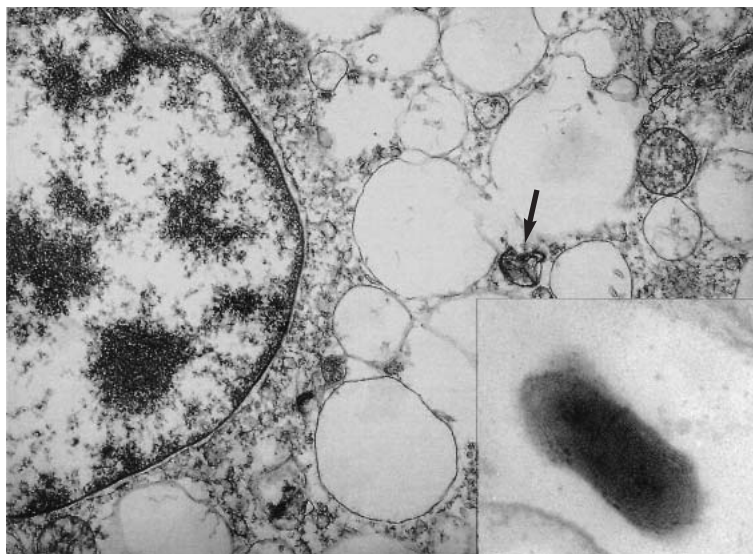
### Clinical Outcome and Necropsy Findings

Because of the poor prognosis for metastatic melanoma, the owner elected to pursue only piroxicam and cimetidine therapy. The dog was started on piroxicam at a dosage of 0.3 mg/kg once daily and cimetidine at a dosage of 15 mg/kg 3 times daily. Four months subsequent to initiating therapy, the owners elected euthanasia.

The attending veterinarian performed a cosmetic necropsy, and selected tissue samples were submitted in 10% buffered neutral formalin to the Kansas Veterinary Diagnostic Laboratory at KSU for histopathologic examination. Grossly, metastatic lesions were located in the diaphragm, heart, all lung lobes, kidneys, left eye, prescapular and sublumbar lymph nodes, and skin over the left rear medial thigh and right lateral thigh. Histopathologic evaluation of the tissue samples demonstrated balloon cells invading all of these tissues. The polygonal cells were arranged in solid sheets with minimal fibrous stroma. The cells had abundant highly vacuolated cytoplasm, round to oval nuclei with marked anisokaryosis, and occasional gigantic nucleoli. Lung and kidney sections contained balloon cells and a more sarcomatous arrangement of spindle-shaped cells with eosinophilic granular cytoplasm (not shown). Mitotic figures were frequent in the lung (6 per high-power field,  $\times 400$ ). The spindle cell form of the neoplasm also stained positively for Melan A. Two skin nodules on the thighs showed invasion of the deep dermis and subcutis by balloon cells; however, neoplastic cells were not identified in skin collected from the area of the amputated toe. In the left eye, balloon cells infiltrated the iridocorneal angle, uveal tract, and retina. Although there was no gross evidence of liver involvement, microscopic metastasis could not be ruled out and may have contributed to the increased ALT activity documented 4 months prior to euthanasia.

### Ultrastructural Examination

Samples of the neoplasm from the prescapular lymph node and lung were retrieved from 10% buffered neutral



**Figure 5.** Transmission electron micrograph. A neoplastic cell from the prescapular lymph node contains a nucleus with heterochromatin and euchromatin and coalescing electron-lucent vacuoles. A myelinlike figure (arrow) is located in the center.  $\times 14,000$ . **Inset:** An early stage melanosome is located within a vacuole.  $\times 95,000$ .

formalin followed by fixation in modified Karnovsky's fixative (2% paraformaldehyde, 2.5% glutaraldehyde, 0.2 M cacodylate, pH 7.4) for electron microscopy. Samples were rinsed several times in 0.1 M cacodylate (pH 7.4), postfixed in osmium tetroxide and 0.1 M sodium cacodylate, stained with 70% ethanol/uranyl acetate followed by acetone, and embedded in LX 112 resin. Ultrathin sections were stained with lead citrate and uranyl acetate and examined with a H300 Hitachi transmission electron microscope. Ultrastructural examination of the neoplastic cells invading the prescapular lymph node revealed numerous, often coalescent membrane-bound electron-lucent vacuoles and myelinlike figures (Figure 5). Melanosomes were rare and were found within vacuoles. Early stage melanosomes were oval to cigar shaped and contained a concentric arrangement of filaments or membranes that were moderately electron dense (Figure 5, inset).

### Discussion

This case was a rare variant of amelanotic melanoma initially identified from a fine-needle aspirate. The neoplasm resembled others reported in dogs<sup>5,6</sup> and shared morphologic features with amelanotic melanomas found in cats and humans.<sup>7-10</sup> In a recent retrospective study in cats, 5 cell types of melanoma were described: epithelioid, spindle, mixed, signet, and balloon cell.<sup>10</sup> The latter two cell types were amelanotic and were thought to be variants of the epithelioid cell type. In humans and cats, most balloon cell type melanomas were cutaneous and

localized to the head.<sup>8-10</sup> Cells in balloon cell melanomas have abundant vacuolated cytoplasm with delicate, focally disrupted cytoplasmic membranes, and sometimes are referred to as pseudolipoblastic cells.<sup>7</sup> Glycogen- and lipid-rich balloon cell melanomas have been described based on ultrastructural features and special staining characteristics.<sup>1-3,11</sup> Additionally, the PAS reaction has been used to identify the glycoprotein-rich enzyme tyrosinase, which is abundant in melanocytes and is responsible for the formation of melanin.<sup>3</sup> However, the cells in this dog were PAS negative, corresponding with the lack of cytoplasmic forms of glycogen noted in the samples processed for electron microscopy. Although glycogen-rich (PAS positive) balloon cell melanomas have been reported in humans (principally metastatic and primary invasive tumors), balloon cells can have accumulation of substances other than glycogen. Moreover, PAS positivity is not a reliable differentiating feature because non-melanocytic tumors as well as melanocytic tumors can contain glycogen.<sup>3</sup>

Ultrastructurally, the vacuoles in balloon cell melanomas represent enlarged coalescent melanosomes. The origin of the balloon cells in balloon cell melanoma is controversial, and recent hypotheses suggest the vacuolar changes occur as a result of a metabolically active process rather than a degenerative process.<sup>3,8</sup> Ultrastructural findings in this case included membrane-bound electron-lucent vacuoles, myelinlike figures, and oval structures that contained spiral arrangements of filaments suggestive of early stage melanosomes (presumptive stage II or stage III). This internal pattern resembled that previously reported in melanomas and was consistent with early melanosomes.<sup>12,13</sup> The internal structure of a melanosome provides a scaffold for the oxidation of tyrosine by tyrosinase to pigmented melanins. Altered or abnormal melanosomes, as seen in this dog, are not uncommon in malignant tumors.<sup>12</sup> The normal compact banded structure of the melanosome is replaced by loosely arranged filaments with patchy or incomplete melanin deposition.

Balloon cell melanoma is uncommon in humans and constitutes only 0.15% of the cutaneous cases<sup>8</sup> but 10% of choroidal melanomas.<sup>14</sup> Although the primary site of the neoplasm in this case was hypothesized to be the toe mass removed 8 months prior to presentation, we could not rule out the possibility that the eye may have been the site of the primary tumor. Rare cases of ocular melanoma with balloon cell morphology that metastasize to distant sites (ie, liver) have been described in humans.<sup>11</sup> Although there is one report of a xanthomatous lesion resembling balloon cell melanoma in the iris and ciliary

body of a dog, the behavior of this tumor was benign.<sup>15</sup> Moreover, metastasis of ocular melanoma in the dog via the lymphatics is rare compared with that derived from the oral cavity, lip, and digit.<sup>16</sup> The balloon cell type of melanoma appears to predominate in locally recurring or metastatic lesions.<sup>5</sup> However, as described here, spindle-shaped cells also may be found in sites of metastasis.

Metastatic balloon cell melanoma is difficult to differentiate from sebaceous cell carcinoma, liposarcoma, and other clear cell variants without immunohistochemistry or ultrastructural evidence of melanin or melanosomes. The lack of cytokeratin staining in the current case ruled out clear cell carcinoma. Although detection of S-100 and melanoma-specific antigens (HMB-45) are required to establish a diagnosis in human cases, antibodies against human melanoma-specific antigens such as HMB-45 (against glycoprotein 100 of premelanosomes)<sup>17</sup> and NKI/C3 (against premelanosome vesicles)<sup>18</sup> have limited reactivity in feline and canine melanomas.<sup>10,19</sup> In this dog, S-100 staining was weak and Melan A staining was strong. In humans and cats, balloon cell melanomas demonstrate reactivity for S-100, vimentin, and neuron-specific enolase (NSE). However, S-100 staining may be absent or weak in balloon cell melanomas.<sup>7,10</sup> Vimentin, NSE, S-100, and NKI/C3 are very useful screening markers but lack specificity because they react with other cell types.

Melan A expression in the tumor cells of this dog supported the diagnosis of a variant of amelanotic melanoma. Melan A and MART-1 (an immunodominant melanoma antigen recognized by cytotoxic T cells) stain both benign and malignant melanomas. The MART-1 antibody recognizes a different epitope of the same antigen as is recognized by the Melan A antibody described here.<sup>20</sup> Both Melan A and MART-1 antibodies are preferred over HMB-45 for the diagnosis of metastatic malignant melanoma in fine-needle aspirate and biopsy material.<sup>17,20</sup> The reactivity of the Melan A antibody was previously investigated in cell lines established from dogs with canine melanoma and in clinical specimens from 29 dogs with melanoma.<sup>21</sup> In that study, the cell

lines were less positive than the clinical samples, and Melan A was detected in 62% of the clinical samples but not in amelanotic tumors.<sup>21</sup> Recently, 3 cases of clear cell variants were described out of 338 cases of oral melanoma, and cells in 2 of these cases were strongly positive for Melan A.<sup>4</sup> Moreover, antibody specific for Melan A was a better diagnostic tool for canine amelanotic melanomas (balloon cell and signet ring) than was an antibody to tryosinase.<sup>6</sup> Expression of Melan A in canine melanomas was correlated with better biologic behavior than were those neoplasms that did not express the antigen.<sup>4</sup> Several human patients with balloon cell melanoma were reported to have longer survival despite metastases.<sup>22</sup> This observation may have therapeutic significance if Melan A/MART expression can be used as a target for immunotherapy in dogs as is currently done for humans.<sup>17,23</sup>

Identification by fine-needle aspirate of this unique case of balloon cell melanoma stresses the importance of obtaining a good clinical history and of employing other methods to confirm the diagnosis. ◊

**Addendum:** Subsequent to acceptance of this paper, immunohistochemical staining for chromogranin A performed in our diagnostic laboratory demonstrated that the metastatic tumors present in the prescapular lymph node (balloon cell variant) and lung sections (spindle cell variant) were negative for chromogranin A, a 49 kD peptide that is the major constituent of secretory granules of the adrenal medulla and other endocrine cell types.<sup>24</sup> These results further support the diagnosis of a melanocytic tumor in this case and rule out the possibility of a tumor of adrenal gland or other endocrine gland origin. This is an important distinction to make since melanosomes have been demonstrated in some neuroendocrine neoplasms (ie, medullary thyroid carcinomas),<sup>25</sup> and the antibody to Melan A (clone A103) may show immunoreactivity in adrenocortical neoplasms and Leydig tumors of the ovary and testes.<sup>4,20</sup>

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