Area of specialization: oncology

Case Log #: 5

Signalment: Buddy (#199698): a 7 yr 11 mo old, M/N, German Shepherd Dog cross, canine.

Patient History: Buddy, a long term patient of the oncology (onco) service, presented for a routine recheck on 10/21/2021 for monitoring of a narrowly excised grade I soft tissue sarcoma (STS), removed from the region of the right (R) stifle on 04/06/2021. While the remainder of his PE was unremarkable, a 2mm nodule was noted in the R anal sac on rectal exam. Fine needle aspirate (FNA) for cytology performed on 11/25/2021 was consistent with suspicion for an apocrine gland anal sac adenocarcinoma (AGASAC), with surgical excision and histopathology to confirm the suspected diagnosis recommended. Buddy was clinically well with no symptoms associated with the anal sac mass (ASM). He was receiving pentosan polysulfate sodium SQ (100mg/ml, 3mg/kg x 46.7kg = 140.1mg/1.4ml) once monthly at his rDVM for management of elbow dysplasia but no other chronic medications.

Initial Exam Findings: Buddy presented on 12/03/2021, for a recheck with the DACVIM (Onco) (DACVIM-O) and staging of his newly diagnosed R ASM. A PE was performed. His BW was 46.7kg with a body condition score of 7/9 (overweight) on a 9-point scale. He was bright, alert, and responsive. He was eupneic with a respiratory rate of 20 rpm. His heart rate was 96 bpm with normal heart sounds and strong synchronous pulses. His mucous membranes were pink and moist with a capillary refill time of <2 seconds. His rectal temp was 38.7 °C. The surgical scar overlying the R stifle was covered by regrown hair and there was no palpable evidence of tumor recurrence. He was ambulating normally. His coat and skin appeared healthy. His abdomen palpated comfortably with no appreciable masses. No abnormalities were noted on

oral or ocular examination. His peripheral lymph nodes (LNs) palpated normally. On rectal examination, the R ASM was unchanged in size.

Problem List, Differential Diagnosis, and Prognosis: The DACVIM-O's problem list included a history of STS and a suspected AGASAC. Her differential diagnoses for the ASM were an AGASAC or an area of inflamed tissue. Buddy's prognosis was considered good as he was clinically well and the nodule could represent a benign process, but ultimately his prognosis would depend upon further diagnostics and the clinical behavior of the mass.

Initial Diagnostics and Results: AGASAC most commonly metastasizes to medial iliac, internal iliac, and/or sacral LNs, however, can also metastasize to distant sites including liver, spleen, bone, lungs, and other soft tissues.[1] Complete staging includes a biochemical profile (CHEM) to assess total calcium and ionized calcium (iCa) to assess free calcium, as hypercalcemia is a paraneoplastic syndrome that is associated with up to 53% of AGASAC cases,[2] and thoracic and abdominal imaging to screen for abdominal or thoracic metastasis. Advanced imaging such as CT or MRI is preferred for abdominal imaging, as the LNs in the pelvic canal cannot be visualized on ultrasound (US),[3] however, Buddy's owners declined this testing and opted for an abdominal US (AUS) due to cost constraints. 5ml of blood was aseptically collected from the left (L) jugular vein and submitted for a CBC, CHEM, and iCa. All values were within the reference range (RR). Buddy was sedated with butorphanol IV (10mg/ml, 0.1mg/kg x 46.7kg = 4.67mg/0.47ml) and dexmedetomidine IV (0.5mg/kg, 0.005mg/kg x 46.7kg = 0.23mg/0.46ml) which provided adequate sedation for his procedures. His vital signs were monitored throughout the procedure with no concerns noted. Three-view thoracic radiographs (3VCXR) were obtained by the technician and reviewed by a board-certified radiologist (DACVR). The AUS was performed and interpreted by the DACVR. The 3VCXR were

considered normal. The AUS revealed markedly enlarged and hypoechoic R medial iliac and R hypogastric LNs, raising concern for metastasis from the R sided ASM. No other abnormalities were identified. US-guided FNA were obtained from the R medial iliac LN and were submitted to a reference laboratory for assessment by a pathologist. One of the slides was stained using a three-step Romanowsky type stain and examined under the microscope. The slide was abundantly cellular consisting of a population of lymphocytes on a background of RBCs with occasional neutrophils present. 50% of the lymphocytes were small, with the remaining 50% consisting of a homogenous population of large lymphocytes (approximately (~) 1.5 times the size of a neutrophil) with basophilic cytoplasm, and one to three prominent nucleoli per nucleus. There was no evidence of metastatic carcinoma cells on the slide. This finding was consistent with an emerging lymphoma (LSA) versus inflammatory response, with a diagnosis of LSA considered likely when the population of homogenous large lymphocytes comprises greater than 80% of the population within a sample.[4] Buddy was reversed with atipamezole IM (5mg/ml, 0.05mg/kg x 46.7kg = 2.33mg/0.46ml) and recovered without complications. He was discharged that afternoon and was prescribed (RX'd) amoxicillin clavulanic acid (16.06mg/kg x 46.7kg = 750mg, dispensed (DSP) thirty 500mg tablets (tab): give one and half tab (750mg) PO BID with food for ten d) as a treatment (tx) for a possible inflammatory cause of the lymphadenopathy. Buddy returned on 12/17/2021 for a focal US of his caudal abdominal LNs and repeat sampling by FNA. The R medial iliac and R hypogastric LNs were further enlarged compared to the previous study with newly recognized lymphadenopathy with the L internal iliac and L hypogastric LN now markedly enlarged and irregular in appearance. US-guided FNA were obtained from the L and R hypogastric LNs and submitted to a pathologist for assessment. The results were now consistent with a large cell lymphoblastic LSA, with greater than 80% of the

cells on the submitted slides consisting of large lymphocytes. The DACVIM-O discussed the new diagnosis with Buddy's owners and recommended switching focus to tx of his LSA, as the expected survival time is ~ one to three mo with a mean survival time (MST) of fifty d with palliative tx alone.[5] Appropriate testing had already been completed to stage his LSA using the World Health Organization staging system. Staging of LSA typically involves thoracic and abdominal imaging (3VCXR and AUS). For complete staging of LSA bone marrow biopsy may be considered, however, this was not recommended in this case as clinical suspicion for bone marrow involvement was low as there was no evidence of circulating lymphoblasts or atypical lymphocytes suggestive of bone marrow involvement on Buddy's CBC. Additionally, immunophenotyping (IPH) may be performed to distinguish between a B-cell and T-cell LSA. IPH can be performed on LN aspirates (flow cytometry), or on a biopsy sample (histopathology with immunohistochemical staining). Stage I is a LSA involving only a single LN, or lymphoid tissue in a single organ. Stage II is a LSA with the involvement of multiple LNs confined to one region of the body. Stage III is a LSA with generalized lymphadenopathy, either internal or external. Stage IV is a LSA with involvement of the liver or spleen, with or without lymphadenopathy. Stage V is a LSA with any involvement of the blood or bone marrow, with or without lymphadenopathy or other organ involvement. LSA is further categorized as sub-stage A (clinically well, no systemic symptoms of disease) or sub-stage B (systemic illness at the time of diagnosis).[6] Buddy was considered to be Stage II, sub-stage A, as he had involvement of multiple LNs confined to his caudal abdomen. The recommended frontline tx protocol for LSA is the University of Madison Wisconsin chemotherapy (CHEMO) protocol, treating with vincristine, cyclophosphamide, doxorubicin (DOX), and prednisone (PRED). Tx is given once per wk for the first two cycles, then every other wk for an additional two cycles. This tx protocol takes ~ six mo

to complete and increases MST to ten to twelve mo, with ~ 20 – 25% of patients surviving twenty-four mo.[7] This prognostic information is most applicable to patients with B-cell LSA. If IPH showed a T-cell LSA the expected MST decreases to four to six mo.[8] The goal of tx is to obtain a clinical remission and to maintain an excellent quality of life (QOL). Buddy's owners declined IPH and elected to treat with a single-agent protocol using DOX rather than committing to a multi-agent protocol. Buddy presented on 12/22/2021 for his first dose of DOX. His PE was unremarkable aside from his ASM which remained stable in size. His peripheral LNs remained palpably normal. His BW was 47.0kg. 5ml of blood was aseptically collected from the L jugular vein and submitted for a CBC and CHEM. All values were within the RR. Buddy was approved for tx by the DACVIM-O. His BW was confirmed as 47.0kg. The DACVIM-O elected to treat Buddy at a lean BW (LBW) of 42.0kg. His m2 was calculated (0.101 x (LBW 42.0kg)^{⅔} = 1.220m2). The DACVIM-O's formula was double-checked prior to preparation to screen for calculation errors. Using a class II laminar flow biological safety cabinet, a closed system drug transfer device (CSTD), and wearing full personal protective equipment (PPE) (waterproof gown, two pairs of nitrile gloves, fluid-resistant cap, impermeable half-face respirator with N100 filter, and safety goggles) the calculated dose of DOX (2mg/ml, 30mg/m2 x 1.220m2 = 36.6mg/18.3ml) was prepared. The dose range of DOX is 25 - 30mg/m2 in large dogs. DOX is administered as a slow infusion over 30 min to reduce the risk of acute toxicities including hypersensitivity reactions and cardiac arrhythmias. DOX can cause both acute and cumulative cardiotoxicity with a lifetime dose limit of 180 - 240mg/m2.[9] An echocardiogram and ECG are recommended for breeds at high risk of developing DCM, however, ideally all patients should have a full cardiac work-up prior to tx with DOX. Buddy's owners declined referral to a DACVIM (Cardiology) for an echocardiogram. An ECG was performed prior to his first dose of

DOX and was consistent with a normal sinus rhythm. No heart murmur or arrhythmia was identified during his PE. DOX, an anthracycline antibiotic used for its antineoplastic properties, is a severe vesicant and must be administered through a perfectly placed IV catheter (IVC).[10] Extravasation (EV) of DOX can result in cellular damage, pain, irritation, erythema, sloughing, and tissue necrosis. In case of EV, the infusion should be immediately halted and a 10ml syringe used to aspirate as much drug as possible. A new IVC should be placed in a different vein and dexrazoxane administered IV at ten times the dose of the DOX. The first dose of dexrazoxane should be given within three to six h of EV, with an additional full dose given in 24 h, and a third half-dose given in 48 h. Cold compresses should be applied to the EV site for 15 min every six h for the first 48 h to promote vasoconstriction and limit the spread of the drug.[11] The hair over the L cephalic vein was clipped, the skin aseptically prepped with 4% chlorhexidine and 70% isopropyl alcohol scrub, and a 22-gauge IVC was placed in the vein. IVC patency was tested with 6ml of sodium chloride (NaCl) 0.9% flush, drawing back intermittently to view a flash of blood. The IVC insertion site and distal limb were carefully monitored for leaking or swelling. Maropitant citrate IV (10mg/ml, 1mg/kg x 47.0kg = 47mg/4.7ml) was given to help prevent CHEMO-induced nausea. A second member of the onco team confirmed the appropriate dose had been prepared before the DOX was administered. The prepared dose of DOX (36.6mg) was administered over 30 min using the CSTD with a Y-extension set allowing for intermittent flushing with NaCl 0.9%. Manual administration of DOX allows for the technician to judge changes in resistance that may indicate EV, as well as allowing for assessment of IVC patency by drawing back to visualize blood in the hub of the IVC. The insertion site was monitored throughout the infusion. On completion of the DOX infusion, the IVC was flushed with an additional 10ml of NaCl 0.9%. The IVC was removed and all equipment used to administer

DOX was discarded in a biohazard bucket approved for disposal of CHEMO waste. At discharge, CHEMO safety was discussed with Buddy's owner. DOX is metabolized by the liver and primarily excreted in bile and feces. The elimination half-life of DOX is triphasic (phase one: 0.6 h, phase two: 3.3 h, phase three: 17 – 32 h).[12] Drug residues may be present in urine or feces for several d. Buddy's owner was instructed to wear gloves while handling any bodily fluids for a minimum of 72 h and up to seven d following administration of DOX. Dogs receiving CHEMO should be walked in a low-traffic area with feces removed from the environment, double bagged, and discarded in the garbage. Soiled bedding should be washed separately from other laundry with detergent and bleach. Bodily fluids in the environment should be absorbed with paper towels, then cleaned with soapy water followed by a disinfectant. Thorough hand washing is recommended after handling patient waste. Persons who are pregnant, immune-compromised, or under the age of 18 yr should not handle the waste of CHEMO patients. The nadir of neutropenia occurs seven to ten d following tx,[13] with a CBC performed seven d following tx. Buddy's owner was counseled to monitor for exercise intolerance or collapse that could indicate cardiac dysfunction and to monitor the infusion site for pain and erythema. DOX can cause gastrointestinal (GI) side effects, with vomiting and nausea occurring most commonly in the first 72 h and diarrhea occurring four to five d following tx.[14] Buddy was RX'd maropitant citrate (1.91 x 47.0kg = 90mg, DSP six 60mg tab: give one and a half tab (90mg) PO SID for four d) and metronidazole (10.64mg/kg x 47.0kg = 500mg, DSP forty 250mg tab: give two tab (500mg) PO BID PRN for soft stool/diarrhea, give for up to ten d or until two d past clinical resolution of symptoms) for management of GI side effects. Buddy was also RX'd PRED (1.06mg/kg/d x 47.0kg = 50mg, DSP eight 50mg tab: give half a tab (25mg) PO BID for three d, decrease to 0.8mg/kg/d x 47.0kg = 37.5mg: give half a tab (25mg) PO in the morning

and one quarter of a tab (12.5mg) PO in the evening for three d, decrease to 0.53mg/kg/d x 47.0kg = 25mg: give half of a tab (25mg) PO SID in the morning for three d, decrease to 0.266mg/kg/d x 47.0kg = 12.5mg: give one quarter of a tab (12.5mg) PO SID in the morning for three d then STOP. Give with food). Possible PRED side effects were reviewed with Buddy's owners including polyuria (PU) and polydipsia (PD), polyphagia, anxiety, excessive panting, and GI upset or ulceration. They were cautioned to never give NSAID drugs in combination with PRED due to increased risk of GI ulceration. PRED must be given with food in order to reduce the risk of GI side effects. They were counseled to seek advice if his PRED side effects were unmanageable or they noticed GI symptoms such as vomiting, melena, or hematemesis. Buddy was RX'd famotidine (0.43mg/kg x 47.0kg = 20mg, DSP eighteen 20mg tab: give one tab (20mg) PO BID for nine d) to be given while receiving high doses of PRED. Buddy presented on 12/29/2021 for a CBC. His owners reported PU/PD secondary to his PRED and diarrhea which had begun four d following tx and was responding well to tx with metronidazole as RX'd. Otherwise, his QOL was considered good and his appetite remained excellent. There were no abnormalities on his CBC. Buddy received a total of five doses of DOX given three weeks apart (12/22/2021, 01/12/2022, 02/02/2022, 02/23/2022, and 03/16/2022) with no dose adjustments required. An AUS was repeated at the time of his third dose (02/23/2022) with complete resolution of his caudal abdominal lymphadenopathy, indicating an excellent response and clinical remission of his LSA. The DACVIM-O recommended monthly rechecks to include a PE, CBC/CHEM/UA, and AUS to monitor for recurrence of Buddy's LSA, however, Buddy's owners declined close monitoring. A recheck was scheduled on 07/28/2022 for increased lethargy, exercise intolerance, and a dry hacking cough. On PE Buddy had marked bilateral enlargement of his mandibular and prescapular LNs. FNA were collected from the affected LNs

and submitted for cytology, with a diagnosis of relapsed LSA reported. His BW was 42.9kg, a decrease of 10% from his baseline BW at the start of tx. The remainder of his PE on admission to the hospital was unremarkable. 3VCXR were obtained and showed marked tracheobronchial lymphadenopathy and moderate mediastinal lymphadenopathy. Buddy was given dexamethasone SQ (5mg/ml, 0.2mg/kg x 42.9kg = 8.58mg/1.7ml) with plans to DSP PRED for palliative tx of his LSA. Prior to discharge Buddy developed severe tachycardia of 260 - 280 bpm with poor pulse quality. ECG showed severe sinus tachycardia. A focal US of his thorax did not reveal pleural or pericardial effusion. While his heart appeared normal in size and shape on his 3VCXR the concern was for a DOX induced diffuse cardiomyopathy. Buddy's owners declined emergency referral to a DACVIM (Cardiology) and elected to trial tx with oral diltiazem (1.4mg/kg x 42.9kg = 60mg, DSP forty-two 60mg tab; give one tab (60mg) PO TID until otherwise instructed). He was RX'd PRED (1.16mg/kg/d x 42.9kg = 50mg, DSP forty-five 50mg tab: give half a tab (25mg) PO BID for five d, decrease to 0.58mg/kg/d x 42.9kg = 25mg: give one quarter of a tab (12.5mg) PO BID for five d, decrease to 0.29mg/kg/d x 42.9kg = 12.5mg: give one quarter of a tab (12.5mg) PO SID ongoing.) He presented for a recheck on 08/03/2022. His BW was 42.9kg and his vital signs including heart rate (90 bpm) were normal. His affected LNs had partially responded to tx with PRED and had decreased in size by 25 - 50%. His QOL was considered fair to good. Buddy's owners were instructed to continue tx with his previously DSP medications and to contact us if his QOL declined or they wished to schedule further recheck appointments.

Final Outcome: Buddy was euthanized on 08/25/2022 for progressive lymphadenopathy, and declining QOL (lethargy and poor appetite). A necropsy was not performed.

Discussion of Case: Buddy's case is an excellent example of the importance of complete clinical staging for any suspected cancer, and the sampling of LNs or other tissues suspected to be affected by metastatic disease. If surgery had been pursued for his ASM and suspected metastatic LNs without sampling, the diagnosis of LSA would have been made later and tx would have been delayed. Buddy's owners had significant cost constraints, so undergoing surgery for the ASM would have used up funds that ultimately were directed towards tx for the LSA. Tx of LSA with a multi-agent protocol is the standard of care and is expected to provide longer and more durable remissions over single-agent protocols, however, tx with single-agent protocols offer a significant survival advantage over palliative tx with PRED alone.[15] Single-agent tx with DOX is expected to provide an MST of six to eight mo.[16] Buddy lived nine mo past his diagnosis of LSA, therefore just exceeding the MST. Most importantly Buddy maintained an excellent QOL during his tx with minimal side-effects that were easily managed with PO medications at home. Buddy may have experienced the cumulative cardiotoxicity associated with DOX administration, however, it is unclear whether his cardiac event reflected a DOX induced cardiomyopathy as his owners could not afford an echocardiogram and declined a necropsy. Buddy received a total of 150mg/m2 of DOX which is below the lifetime dose limit of 180 - 240mg/m2, however, cardiomyopathy can be seen with cumulative doses as low as 90mg/m2.[17] Prophylactic tx with dexrazoxane may provide a cardioprotective benefit for patients receiving DOX, however, high cost and limited availability of this drug make this impractical in most cases.[18] Ultimately, Buddy was euthanized due to progressive relapsed LSA and not due to congestive heart failure or other cardiovascular concerns. Rescue CHEMO with novel tx protocols can be considered for patients with relapsed LSA,[19] however, Buddy's owners were not financially able to commit to additional tx and were happy to have received an additional nine mo with good QOL.

References

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