1 **CANINE NODAL MARGINAL ZONE LYMPHOMA: DESCRIPTIVE INSIGHT INTO THE BIOLOGICAL BEHAVIOUR**

2

3 **Abstract**

4

5 Canine nodal Marginal Zone Lymphoma (nMZL) is classified as an indolent lymphoma. Such lymphomas are
6 typified by low mitotic rate and slow clinical progression. While the clinical behavior of canine splenic MZL
7 has been described, characterized by an indolent course and a good prognosis following splenectomy, there
8 are no studies specifically describing nMZL. The aim of this study was to describe the clinical features of and
9 outcome for canine nMZL. Dogs with histologically-confirmed nMZL undergoing a complete staging work-up
10 (including blood analysis, flow cytometry (FC) on lymph node (LN), peripheral blood and bone marrow,
11 imaging, histology and immunohistochemistry on a surgically-removed peripheral LN) were retrospectively
12 enrolled. Treatment consisted of chemotherapy or chemo-immunotherapy. Endpoints were response rate
13 (RR), time to progression (TTP) and lymphoma-specific survival (LSS).
14 A total of 35 cases were enrolled. At diagnosis, all dogs showed generalized lymphadenopathy. One-third
15 were systemically unwell. All dogs had stage V disease; one-third also had extranodal involvement. The LN
16 population was mainly composed of medium-sized CD21+ cells with scant resident normal lymphocytes.
17 Histology revealed diffuse LN involvement, referring to "late-stage" MZL. Median TTP and LSS were 149 and
18 259 days, respectively. Increased LDH activity and substage b were significantly associated with a shorter LSS.
19 Dogs with nMZL may show generalized lymphadenopathy and an advanced disease stage. Overall, the
20 outcome is poor, despite the "indolent" designation. The best treatment option still needs to be defined.

21
22 **Keywords:** lymphoma, indolent lymphoma, clinical presentation, outcome, MZL, dog

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24

INTRODUCTION

Canine Marginal Zone Lymphoma (MZL) is an indolent Non-Hodgkin lymphoma (NHL) that originates from the marginal zone of B-cell follicles. It has been described as an indolent disease, having a low mitotic index and a slow clinical progression.[1]

In human medicine, the MZL group is divided into 3 subtypes according to the World Health Organization (WHO) classification[2]: Mucosal Associated Lymphoid Tissue Lymphoma (MALT), splenic Marginal Zone Lymphoma (sMZL) and nodal Marginal Zone Lymphoma (nMZL). These three entities are described as separate diseases in terms of biology, clinical presentation and behavior. The major diagnostic criterion is the site of presentation.[3] MALT is relatively common, encompassing 5–8% of all NHLs and it most frequently involves the gastrointestinal tract (66% of all MALT cases), occurring in patients with a history of autoimmune disorders and chronic inflammation.[4,5] sMZL and nMZL are quite rare, each comprising less than 1% of NHL. sMZL is a symptomatic disease which at onset usually involves spleen, bone marrow (BM) and peripheral blood (PB), and is generally associated with splenomegaly and hematological alterations. Many patients with nMZL show regional (head and neck) lymphadenopathy, but more than 70% present with stage III/IV disease (according to the Ann Arbor staging scheme).[6,7]The prognosis is reported to be less favorable for nMZL than for MALT and sMZL.[8]

The WHO classification of hematopoietic and lymphoid tumors of domestic animals also divides canine MZL into the same three entities. In dogs, MALT lymphoma has not been well described. It is rare, and predominantly involves the respiratory and intestinal tracts, but other locations have been occasionally reported, such as the salivary gland and eyelid.[1,9] Canine sMZL has been described in terms of presentation and outcome. Unlike human sMZL, the majority of canine sMZL represents an incidental finding during physical examination and abdominal ultrasound. Canine sMZL has an indolent clinical course and splenectomy, with or without systemic chemotherapy, is usually curative.[10-12]

Canine nMZL is generally classified as an indolent lymphoma. However, in spite of the putative indolent nature, some dogs with nMZL may experience an aggressive disease course.[13-17] Specific studies focused on clinical presentation and behavior of canine nMZL are lacking.

52 The aim of the present study is to describe the clinico-pathological features and outcome in a cohort of dogs

53 with histologically- confirmed MZL with a primary nodal presentation, thus better characterize this tumor as

54 a single disease entity.

55

56 **MATERIALS AND METHODS**

57 **Inclusion criteria**

58 Medical records of dogs with lymphoma referred to the Centro Oncologico Veterinario between 2012 and

59 2016 were retrospectively reviewed for cases with a histopathological diagnosis of nMZL.[1,18]

60 To be eligible for enrolment, dogs were required to undergo a complete staging work-up, including complete

61 blood count (CBC), serum biochemistry (including Lactate Dehydrogenase-LDH-activity and Ionized Calcium

62 concentration), cytology and flow cytometric immunophenotyping on a lymph node (LN) aspirate, thoracic

63 radiographs, abdominal ultrasound, fine-needle aspiration of liver and spleen regardless of their sonographic

64 appearance, and PB and BM infiltration degree assessed by flow cytometry (FC). The abovementioned work-

65 up is standard of care in the Centro Oncologico Veterinario. Previous lymphoma-directed therapy (including

66 steroids) was not permitted.

67

68 **Flow Cytometry**

69 FC was performed on fresh samples of LN aspirates, collected into RPMI 1640 (Sigma Aldrich, St Louis, MO,

70 USA) and processed as previously described.[19] Cells were investigated with a multicolor approach by

71 FACScalibur cytometer (Becton Dickinson, San Jose-California USA), using antibodies against the following

72 markers: CD45 (panleukocytes, clone YKIX716.13, Serotec, Oxford, UK), CD3 (T cells, clone CA17.2A12,

73 Serotec, Oxford, UK), CD5 (T cells, clone YKIX322.3, Serotec, Oxford, UK), CD4 (T helper cells, clone YKIX302.9,

74 Serotec, Oxford, UK), CD8 (T cytotoxic cells, clone YCATE55.9, Serotec, Oxford, UK), CD21 (mature B cells,

75 clone CA21D6, Serotec, Oxford, UK), CD79a (B cells, clone MCA1298F, Serotec, Oxford, UK), CD34 (precursor

76 cells, clone 1H6, Pharmigen, BD, Bioscience, USA) and MHC-II (antigen presenting cells, clone G46-6,

77 Pharmigen, BD, Bioscience, USA). Cell viability was evaluated using Propidium Iodide (PI) and considered

78   adequate if >50% cells were PI-negative. Data analysis was performed with Cell Quest Pro software (Becton

79   Dickinson, San Jose, California).

80   PB and BM involvement were defined as the presence of cells of B-lineage (CD21 positive) of medium to large

81   size. Although specific cut-off values for defining tumor infiltration in PB and BM have not been defined for

82   canine MZL, these were set at 0.56% for PB and 2.45% for BM, respectively, out of the total CD45 positive

83   cells. These values were derived from a recent study on the analytical and diagnostic performances of FC to

84   detect PB and BM neoplastic infiltration of large B-cell lymphoma cells in dogs.[20]

85   Cytological smears of LN, PB and BM aspirates were evaluated in parallel with FC in order to confirm cell

86   morphology, evaluate mitotic figures and detect neoplastic infiltration.[21]

87

88   **Histology**

89   A peripheral enlarged LN was surgically removed, formalin-fixed and paraffin embedded, stained with

90   haematoxylin and eosin, and examined by a veterinary pathologist (LA). For immunohistochemistry,

91   antibodies against CD3 (clone F7.2.38; Dako), CD5 (clone CD5/54/F6; Dako), CD79a (clone HM57; Dako) and

92   CD20 (clone RB-9013-P, Thermo Fisher Scientific) were used on paraffin-embedded sections. The diagnosis

93   of nMZL was confirmed according to the WHO classification.[22]

94

95   **Treatment and outcome**

96   Dogs were treated with a 20-week combination induction chemotherapy, consisting of L-Asparaginase (week

97   1), vincristine (week 2, 3, 4, 13), cyclophosphamide (week 2, 13), doxorubicin (week 7, 16), lomustine (week

98   10, 19), and prednisone (week 1 through 20), as previously described.[17] Dogs whose owners wished to pursue

99   immunotherapy, also received an intradermal injection of 0.5 ml autologous vaccine on weeks 4, 5, 6, 7, 12,

100   16, 20, and 24. The vaccines consisted of tumor-derived heat shock protein-peptide complex coupled with

101   hydroxyapatite ceramic powder.[17,23] Response to treatment was classified as complete remission (CR), partial

102   remission (PR), stable disease (SD) or progressive disease (PD)[24]. Response was evaluated at each therapeutic

103   session and was required to last for at least 28 days.

104

Statistical Analysis

Time to progression (TTP) was calculated as the interval between initiation of treatment and PD or relapse, whereas lymphoma-specific survival (LSS) was measured as the interval between initiation of treatment and lymphoma-related death. Dogs lost to follow-up or dead for lymphoma-unrelated causes before PD, as well as those still in CR at the end of the study, were censored for TTP analysis. Dogs alive at the end of the study, lost to follow-up or dead due to causes other than lymphoma were censored for LSS analysis.

Response rate (RR) was defined as the sum of all dogs achieving CR and PR. Survival was analyzed according to the method of Kaplan-Meier. Differences between survival curves were evaluated with the log-rank test. Multivariate analyses were performed using a Cox stepwise proportional hazard model to identify variables that might be of independent significance influencing TTP and LSS. Variables considered were: breed (mixed or pure), sex, age (cutoff arbitrarily set at 7 years), weight (cutoff arbitrarily set at 10 kg), PCV (normal, decreased, increased), platelet count (normal, decreased, increased), serum LDH activity (normal, decreased, increased), serum Ionized Calcium concentration (normal, decreased, increased), substage (a or b), spleen involvement (yes or no), PB infiltration (yes or no), total lymphocyte count in peripheral blood (as a continuous variable), BM infiltration (yes or no) and extranodal site involvement (yes or no).

Binomial logistic regression was performed to investigate the independence of LDH activity and response to treatment with respect to the abovementioned variables.

Statistical analysis was performed via SPSS v20.0 for Windows. Significance was set at $P \leq 0.05$ for all tests.

RESULTS

Thirty-five dogs met the inclusion criteria. Among them, 29 have been included in a previous paper.[17]

Nine dogs (25.9%) were mixed breeds, while 26 (74.3%) were pure breeds (Table 1).

The median age was 7.0 years (mean 7.6±3.1 years, range 3.0-15.0 years). In particular, 15 dogs (42.9%) were younger than 7 years old, while 20 dogs (57.1%) were 7 or more years old. Median weight was 24.6 kg (mean 23.0±12.5 kg, range 3.0-44.4 kg), with 7 dogs (20%) less than 10 kg and 28 (80%) 10 kg or more. There were 21 (60%) males (3 neutered) and 14 (40%) females (5 spayed).

131 All dogs were presented with generalized lymphadenopathy and this was the reason for initial presentation.

132 Lymphadenopathy had been present for a median of 20 days (range, 2-120 days). At the time of diagnosis,

133 23 (65.7%) cases were asymptomatic, while 12 (34.3%) showed non-specific clinical symptoms. All dogs had

134 stage V disease. Splenomegaly was detected during physical examination in 20 (57.1%) dogs. However, the

135 percentage of cases with splenic involvement rose up to 97.1% (34 dogs) based on abdominal ultrasound and

136 cytological evaluation. In these dogs, abdominal ultrasound revealed splenomegaly; the spleen showed

137 abnormal echogenicity and echo-structure, with a diffusely heterogeneous parenchyma. Splenomegaly was

138 considered moderate in 30% and severe in 70% of the cases. Focal lesions were often observed (70% of the

139 dogs), represented by 1-2 cm large hypoechoic nodules or multiple small hypoechoic nodules, with a

140 consequent spotted appearance of the parenchyma ("honey-comb appearance"). The sonographic findings

141 suggested parenchymal infiltration, confirmed by cytological evaluation showing a homogeneous or highly

142 prevalent population of medium sized blast cells, often with macronucleoli. The liver was infiltrated in 27

143 (77.1%) dogs, as documented by sonographic changes and confirmative cytology. In addition, 10 (28.6%) dogs

144 had extranodal involvement, with the lung present (9 cases), while only 1 dog had more than one extranodal

145 site documented (eye and bladder). Lymphoma at extranodal sites was diagnosed by imaging and

146 confirmative cytology in all but one cases; the dog with ocular involvement had a resolution of bilateral uveitis

147 after the first chemotherapy administration, consistent with a presumptive neoplastic nature of the lesion.

148 Cytologically, the neoplastic cells were medium-sized and characterized by nuclei of intermediate size (1.5-

149 2x the size of a red blood cell) with fine chromatin, prominent single central nucleoli and a moderate amount

150 of weakly basophilic cytoplasm. Few residual mature lymphoid cells were also present. Sometimes a scant

151 population of larger lymphoid cells, defined as centroblasts with anisocytosis and anisokaryosis, was

152 observed. Mitotic index was low with less than 1 mitotic figure/5 HPF (40x).

153 FC confirmed the B-cell lineage of the neoplastic cells. CD21 and/or CD79a positive cells represented the

154 predominant cells in LN samples (median=82.7%, range 42.0-95.7, mean 78.1±15.6). They showed median

155 FSC of 432.8 (mean 440.5 ± 46.2, range 357.1-521.6). An admixed population of small residual lymphocytes

156 was also present, yet scarce in percentage (median=6.4%, mean 12.4±18.2%, range 3.0-22.8%). Regarding PB

157 and BM infiltration, 34 (97.1%) dogs had PB involvement, with a median percentage of neoplastic cells of

158   6.4% (mean 12.4±18.2%, range 0.7-53.5%), while BM was infiltrated in 20 (57.1%) cases, with a median

159   percentage of neoplastic cells of 8.1% (mean 12.5±11.4%, range 3.0-51.6%).

160   Histology and immunohistochemistry were performed in all cases. Histological grade was available for 31

161   cases; among them, 30 were at a late stage of development, characterized by a diffuse growth pattern and

162   loss of follicle-related architecture. The capsule was documented to be thinned and taut. The greatest

163   proportion (80-90%) of cells was medium-sized (1.5-2x red blood cell), with scant eosinophilic cytoplasm,

164   round nucleus and single prominent central nucleolus. The remaining 10-20% of the LN population was

165   represented by small mature lymphocytes. Sometimes, large cells defined as centroblasts and immunoblasts

166   were observed; mitotic activity of these cells was variably, low to moderate, and the mean mitotic index

167   ranged from 0 to 5 in 10 HPF (40x).[16] Tingible body macrophages were present. CD79a and CD20

168   immunohistochemical positivity confirmed B-cell origin.[1,22]

169   Regarding CBC at diagnosis, 3 (8.6%) dogs were anemic (PCV<37%), 31 (88.5%) had a PCV within the reference

170   interval, and 1 (2.9%) dog had an increased PCV (57%); 4 (11.4%) dogs had thrombocytopenia (platelet count

171   <200 10^3/μL confirmed by smear evaluation), while 31 (88.5%) had a normal platelet count. Thirty-four dogs

172   were normocalcemic, while one dog had a decreased ionized calcium concentration. LDH activity (< or ≥300

173   IU/L) was increased in 14 (40%) cases, while it was normal in the remaining 21 (60%) dogs. Binomial logistic

174   regression revealed no significant correlation between LDH activity and all abovementioned variables. No

175   correlation was found between PB lymphocyte count and TTP or LSS.

176

177   **Treatment and outcome**

178   Thirty-four dogs were treated with chemotherapy, and 18 (52.9%) received concurrent immunotherapy. One

179   dog received no treatment at all and was excluded from the survival analysis. TTP and LSS for this dog were

180   49 and 340 days, respectively.

181   Of the 34 dogs that were treated, 25 completed the planned treatment schedule and 9 died during treatment

182   due to PD. Among all others, 20 (80%) achieved CR (of those, 13 received concurrent immunotherapy) and 5

183   (20%) achieved PR (of those, 3 received concurrent immunotherapy).

184   Sixteen dogs having completed the planned protocol received rescue chemotherapy after documentation of

185   PD: 12 received a CHOP-based protocol, whereas 3 were treated with DMAC (dexamethasone, d-

186   actinomycin, melphalan, cytosine arabinoside). A second CR was obtained in 14 of them.

187   Binomial logistic regression revealed that platelet count was significantly associated with treatment response

188   (p=0.033). Thrombocytopenic dogs had a lower probability of responding to treatment (OR=0.071; 95% CI

189   0.006-0.810). RR was 50% for dogs with thrombocytopenia and 80% for dogs with a normal platelet count.

190   Overall median TTP was 149 days (range 1-994 days). None of the investigated variables significantly

191   influenced TTP.

192   Overall median LSS was 259 days (range 5-1605 days). Four dogs were alive at data analysis closure after 601,

193   613, 1016 and 1605 days. Three dogs died of tumor-unrelated causes after 93, 181 and 238 days. Cause of

194   death was due to lymphoma in the remaining 28 dogs.

195   LDH activity (p=0.025) and substage (p=0.008) significantly influenced LSS. In particular, median LSS was 385

196   days (range 111-1605 days, n=20) for dogs with a normal LDH serum level, and 211 days (range 5-601 days,

197   n=14) for dogs with increased LDH; asymptomatic dogs (substage a) had a median LSS of 399 days (range 93-

198   1605, n=22), compared to 125 days (range 5-613, n=12) for symptomatic dogs (substage b). Multivariate

199   Cox's proportional hazard regression analysis showed the influence of platelet count (p=0.01) on LSS.

200

201   **DISCUSSION**

202   This study describes the clinical presentation and outcome of 35 dogs with histologically-confirmed nMZL.

203   Despite the retrospective nature of the design, data concerning initial staging, treatment and follow-up were

204   available for all dogs, thereby providing robust information. Canine nMZL is considered an indolent disease,

205   occurring in adult dogs that usually retain normal appetite and activity, and could be characterized by the

206   enlargement of a single lymph node, typically in the submandibular or cervical lymph node chain,[22] or by a

207   generalized lymphadenopathy.[16]

208   However, the published studies describing the clinical and morphological features of indolent lymphomas

209   suggest that a subset of nMZL cases may display a more aggressive clinical course.[13-17]

210 Indeed, based on our results, the indolent designation may not always be appropriate, as all dogs had

211 generalized lymphadenopathy and one third of them were symptomatic at initial presentation. A complete

212 staging work-up for all cases showed that most dogs had an advanced disease stage at time of initial

213 presentation.

214 In contrast with previous reports, suggesting that nMZL typically does not cause any systemic involvement,[1]

215 all dogs but one had PB involvement and 57.1% had BM involvement, while one third of them had extranodal

216 involvement. The cause for the discrepancy between PB and BM infiltration is unclear, but an overspill of

217 neoplastic cells from affected nodes in the absence of true BM invasion could be a possible explanation,

218 similar to what has been described for T-zone lymphoma.[26] Alternatively, it may be due to the different cut-

219 offs used to define positive PB and BM samples and to the use of FC for staging, which is a very sensitive tool

220 to detect BM and PB infiltration compared with standard light microscopy.[21]

221 Although splenic involvement was detected in 97% of cases, a primary splenic MZL was considered unlikely,

222 based on the integration of clinical and pathological data. Indeed, in canine primary sMZL, the spleen is

223 usually the only site involved, and the diagnosis is frequently incidental.[10,11,13] In the present study,

224 ultrasonographic findings including splenomegaly, diffuse heterogeneity and hypoechoic nodular lesions

225 suggested diffuse secondary infiltration of the parenchyma. Conversely, primary splenic MZL is characterized

226 by a solitary focal hypoechoic mass without any changes of the surrounding tissue.[10,11,22]

227 In humans, sMZL usually also involves liver, BM and PB, and is complicated by anemia and thrombocytopenia.

228 Peripheral lymphadenopathy is infrequent (15–25% of cases), but splenic hilar LNs may be involved (35–65%

229 of cases);[27] nMZL is defined by the WHO classification as "a primary nodal B-cell neoplasm that

230 morphologically resembles LNs involved by MZL of extranodal or splenic types, but without evidence of

231 extranodal or splenic disease". This implies that also in humans the diagnosis of nMZL is mainly based on the

232 pattern of dissemination of the disease, essentially based on the fact that sMZL involves the spleen without

233 concomitant peripheral lymphadenopathy, while nMZL does not have a clinical evidence of extranodal or

234 splenic disease.[2,28]

235 In spite of these considerations, we cannot definitely rule out a primary splenic origin of the tumor with a

236 secondary late dissemination to peripheral nodes. In human medicine, progress in the field of molecular

biology has aided differentiation between nMZL, sMZL and MALT. A molecular and cytogenetic variability between the three subtypes has emerged, but to date no unique alterations have been documented.[29] In particular, among human B-cell neoplasms, nMZL is still lacking specific genetic lesions, although recently the occurrence of a gene deletion involving the receptor-type tyrosine-protein phosphatase delta (PTPRD) was found in a cohort of nMZL cases.[30]

For the cases included in the present study, we were able to evaluate the neoplastic population by means of three different techniques, namely cytology, histopathology and FC. The different techniques gave concordant and overlapping information: samples mainly comprised medium-sized cells, but sometimes were accompanied by a population of centroblasts/immunoblasts and scant resident small lymphocytes. These features also correspond to those previously described in the literature for humans, where the presence of sheets of centroblasts appears to be related to disease progression and tumor transformation into large B-cell lymphoma.[31] Indeed, histology revealed that all cases but one were at a late stage of development, characterized by a diffuse growth pattern and loss of the follicle-related architecture. The only dog with the classical histologic marginal presentation and a slight PB and BM infiltration experienced long LSS (680 days). More cases are needed to define if the histological architecture pattern may be independently associated with a differing clinical behavior.

Based on the above, it may be hypothesized that late-stage nMZL behaves clinically like high-grade lymphomas, with a tendency to spread systemically. Accordingly, Richards et al. found molecular similarities between nMZL and diffuse large B-cell lymphoma (DLBCL), suggesting that these conditions might represent a continuous spectrum of the same disease.[32] Although tumor transformation into a more aggressive disease has only been rarely reported in veterinary oncology,[33] an evolution from nMZL into DLBCL may be hypothesized from the available data.

Approximately one third of dogs died due to lymphomas within 6 months despite treatment, thereby exhibiting a poor outcome that contrasts with the "indolent" tumor designation. This discrepancy is likely due to the different inclusion criteria among studies. Equally, the case selection of the current study might be biased as dogs with generalized lymphadenopathy are more likely to be referred to a referral center and undergo a full staging work-up. Indeed, this is the first case series focused exclusively on nMZL, with strict

264 staging criteria. Most of the studies published in the veterinary literature include many different lymphoma

265 subtypes or are limited to small case series with incomplete staging and follow-up data.[13-15]

266 Overall TTP and LSS were disappointingly low, suggesting that the CHOP-based protocol used in the current

267 series of dogs may not be the best option. This may be due to the relatively low dose-intensity of the adopted

268 protocol, and it remains to be elucidated whether a different chemotherapy dosing intensity may improve

269 clinical outcome. Alternatively, the incorporation of different alkylating drugs may better target MZL cells.

270 Regarding prognostic factors, platelet count significantly influenced RR. Thrombocytopenic dogs had a

271 significantly lower RR and shorter LSS. Thrombocytopenia is reported in 10-13% of cancer-bearing dogs,[34,35]

272 and is generally considered to be a poor prognostic factor.[1,36,37] Substage b was an additional independent

273 risk factor, in agreement with previous studies, showing a correlation with a poor outcome.[38,39]

274 Increased LDH serum level was also significantly associated with a shorter LSS. An increased LDH level at

275 diagnosis has been associated with a shorter survival in people with indolent lymphoma.[40,41] It may be

276 possible that the same holds true in dogs.

277 Surprisingly, PB and BM involvement were not significantly associated with outcome. This is in contrast with

278 what has been described for DLBCL.[42] The cut-off values may have influenced the definition of stage. Indeed,

279 in the present series all dogs had stage V disease when the currently defined cut-off values were applied, and

280 a significant difference may not have emerged. It must be acknowledged that the cut-off values used in the

281 current study and extrapolated from previously published data may be inappropriate for MZL, due to the

282 smaller size of neoplastic cells that impede discrimination between neoplastic and reactive B-lymphocytes.

283 A specific validation study is needed to define the correct FC approach for staging MZL.[20]

284 The main limitation of the present study is the absence of cases with nodular presentation and earlier disease

285 stages (I to IV). This prevents extrapolation about the clinical course of nMZL and the clinical significance of

286 PB and BM involvement. As all dogs enrolled in the present study had generalized lymphadenopathy, it is

287 possible that dogs were initially asymptomatic for a long time and during that time regional

288 lymphadenopathy may have gone unnoticed.

289   In conclusion, dogs with nMZL may present at an advanced stage of disease, with an overall poor prognosis

290   despite the indolent designation. Due to the significant clinical interest, the issue of dose-intensity should be

291   further explored in dogs with nMZL.

292

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