

1 **Prognostic significance of clinical presentation, induction and rescue treatment**
2 **in 42 cases of canine centroblastic diffuse large B-cell multicentric lymphoma in**
3 **the UK**

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26

27 **Abstract :**

28 Canine lymphoma is a heterogeneous group of diseases and many previous studies have
29 evaluated the response of a mixed population of lymphoma cases to one specific treatment
30 protocol. The aim of this retrospective study was to describe the outcome and prognostic
31 factors in 42 cases of multicentric centroblastic diffuse large B-cell lymphoma treated with
32 either a COP-type (35%) or CHOP (64%) induction chemotherapy. The objective response
33 rate to induction therapy was 94%; entire dogs had a greater rate of complete versus partial
34 remissions than neutered dogs ($P=0.017$). Median progression free survival for the first
35 remission (PFS1) was 182 days; absence of anaemia at diagnosis ($P=0.002$) and pre-treatment
36 neutrophil:lymphocyte ratio (NLR) below 9.44 ($P=0.015$) were independently predictive of
37 longer PFS1. Fifty-eight percent of dogs received rescue protocols with an objective response
38 rate of 81%; 31% of dogs received further rescue protocols (up to a total of 5) and the median
39 number of protocols administered was 2. Median overall survival (OS) was 322 days, the 1-
40 year survival rate was 38% and the 2-year survival rate was 9%. Lymphocyte:monocyte ratio
41 (LMR) above 1.43 ($P=0.031$), NLR below 11.44 ($P=0.009$), the combination of induction
42 and rescue therapy ($P=0.030$) and the total number of doxorubicin doses used ($P=0.002$)
43 were independently predictive of longer OS. Use of a COP-type protocol induction compared
44 to CHOP did not undermine OS providing doxorubicin was used as rescue therapy.

45

46 **Keywords :** COP, CHOP, neutrophil:lymphocyte ratio, lymphocyte:monocyte ratio,
47 doxorubicin, complete remission, partial remission.

48

49

50 **Abbreviations:**

51 CL = Canine lymphoma

52 NHL = Non-Hodgkin lymphoma

53 WHO = World Health Organization

54 DLBCL = Diffuse large B cell lymphoma

55 mDLBCL-CB = Multicentric, centroblastic diffuse large B cell lymphoma

56 CR = Complete Remission, PR = Partial Remission, SD = Stable Disease, PD = Progressive
57 Disease.

58 PFS1 = Progression-free survival of the first remission

59 PFS2 = Progression-free survival of second remission

60 PFS3 = Progression-free survival of third remission

61 PFS4 = Progression-free survival of fourth remission

62 OS = Overall survival

63 AMC = Absolute monocyte concentration

64 ALC = Absolute lymphocyte concentration

65 ANC = Absolute neutrophil concentration

66 NLR = Neutrophil:Lymphocyte ratio

67 LMR = Lymphocyte:Monocyte ratio

68 ROC = Receiver operating characteristic.

69 AUC = Area under the curve.

70

71 **Introduction**

72

73 Canine lymphoma (CL) is one of the most prevalent cancers in dogs and the most prevalent
74 haematopoietic cancer.¹ B-cell immunophenotype and multicentric distribution of disease
75 are the most common presentations.²⁻⁴ CL is considered to be an analogue of the human,
76 Non-Hodgkin's lymphoma (NHL),^{5,6} and both the WHO and updated Kiel morphological
77 classifications of NHL have been shown to apply to CL.^{7,8} Since the common morphological
78 subtypes of CL involve diffuse effacement of lymph node architecture with a monotypic
79 population of lymphocytes, cytological review of fine needle aspirate samples is considered a
80 sensitive and specific means of diagnosis,^{7,9,10} and is the most commonly-used method of
81 diagnosis.¹¹ Furthermore, cytology has been shown to be a reliable means of applying both
82 the updated Kiel system of morphological classification^{8,10,12,13} and the WHO classification.¹⁴⁻
83 ¹⁶ The most prevalent cytomorphological subtype of CL (WHO classification) is diffuse large
84 B-cell lymphoma (DLBCL), reported to represent 48% of cases in dogs,⁷ and DLBCL can be
85 subdivided in accordance with the Kiel classification into the centroblastic subtype
86 (representing approximately 77% of cases), and the immunoblastic subtype (representing
87 approximately 23% of cases).⁷

88

89 Traditionally, outcome for CL has been described in the light of treatment with different
90 chemotherapy protocols. When treated with COP-type protocols, CL cases have been
91 reported to have a median first remission duration of 3-6 months and a 19% one-year survival
92 rate,^{17,18} whereas those treated with a CHOP protocol have been reported to have a first
93 remission duration of 8.4-11 months and a one-year survival rate of 50%.¹⁹⁻²² Nevertheless,
94 these studies have been based on populations of heterogeneous cytomorphological subtypes
95 of CL, and recent studies have shown that heterogeneity in these populations may be a
96 significant confounding factor where prognosis is concerned. Ponce et.al. (2004)
97 demonstrated significant differences in survival between small groups of dogs with different
98 cytomorphological subtypes of CL (using the Kiel classification) and Valli et. al. (2013) later

99 repeated and expanded these observations using the WHO classification of histological
100 samples.²³ Thus, the response of dogs with specific cytomorphological subtypes of CL to one
101 of the chemotherapy protocols consistently used in the literature is difficult to predict.
102
103 Aside from treatment protocol and cytomorphological subtype, many consistent prognostic
104 factors for CL have been reported, for example WHO clinical substage,^{19,24,25}
105 immunophenotype,^{26–30} histopathological grade,^{24,29,31} anatomical location of disease^{32–35} and
106 presence of anaemia at diagnosis.^{29,36} Other factors such as WHO stage of disease have
107 shown prognostic significance in some studies,^{17,37} but not others.³⁸ Many other features
108 associated with a dog's presentation may also be of prognostic value, but have been less well-
109 evaluated. In human medicine, the pre-treatment absolute monocyte concentration (AMC),³⁹
110 absolute lymphocyte concentration (ALC)⁴⁰ and the neutrophil:lymphocyte ratio (NLR)⁴¹
111 have been found to be highly predictive of prognosis in diffuse large B cell lymphoma. Other
112 parameters for example the pre-treatment absolute neutrophil concentration and
113 lymphocyte:monocyte ratio (LMR) have found prognostic significance in other solid
114 tumours.^{42,43} In veterinary medicine, AMC⁴⁴ and LMR⁴⁵ have been found to be of prognostic
115 value in lymphoma, whereas the NLR has not been found to affect prognosis.⁴⁶ Pre-treatment
116 absolute neutrophil concentration (ANC) has been found to be prognostic in acute
117 leukaemia,⁴⁷ and both AMC and ALC have been found to be prognostic in canine
118 osteosarcoma.⁴⁸ The diagnostic utility of NLR in differentiating soft tissue sarcomas from
119 benign neoplasms,⁴⁹ and identifying bone marrow involvement in canine mast cell disease has
120 been suggested.^{50,51}
121
122 The aim of this study was to describe the clinical presentation, response to treatment,
123 progression-free survival (PFS) and overall survival (OS) of a homogenous group of dogs
124 with multicentric, centroblastic diffuse large B-cell lymphoma (mDLBCL-CB) treated with
125 standardised induction and rescue chemotherapy protocols, and to identify prognostic factors
126 associated with this cytomorphological type of CL. Since heterogeneous groups of CL

127 contain cases with established negative prognostic factors (for example T cell
128 immunophenotype, cutaneous and hepatosplenic presentations), the hypothesis was that a
129 homogenous group of mDLBCL-CB cases will have a longer PFS and OS than a
130 heterogeneous population of CL cases.

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132

133

134 **Materials & Methods:**

135

136 Study population: Clinical records of the Royal Veterinary College (RVC) between 2008 and
137 2016, and North Downs Specialist Referrals (NDSR) between 2010 and 2013, were reviewed
138 retrospectively for multicentric CL cases. Cases were included if they fulfilled the following
139 criteria: a cytological or histological diagnosis of lymphoma from a peripheral lymph node,⁴
140 confirmed B-cell immunophenotype (on flow cytometry, immunohistochemistry or PARR
141 testing), a categorical diagnosis of DLBCL-CB on histopathology or review of cytological
142 findings by a Board-certified clinical pathologist, induction chemotherapy treatment with a
143 COP-type or CHOP protocol, naïve to cytotoxic treatment at the time of mDLBCL-CB
144 diagnosis and where follow-up data to the end of the first remission were available. Cases
145 where therapy was commenced with l-asparaginase or glucocorticoid therapy within 10 days
146 of initiation of cytotoxic treatment were included. Cases of lymphoma that primarily
147 involved extranodal tissue (or the categorization was equivocal), or in which treatment or
148 survival were compromised by a comorbidity (for example end-stage renal failure or
149 advanced congestive heart failure), were excluded. Follow-up information was collected
150 from clinical records of the institutions concerned or obtained from the records of the
151 referring practices.

152

153 Data collected: For each case the following data was recorded: age; breed; sex; neuter status;
154 bodyweight; clinical examination findings and concurrent disease status at diagnosis;
155 cytology or histology; immunophenotype; haematology; biochemistry and urinalysis results,
156 and diagnostic imaging findings. Haematology reports were interrogated and the following
157 white blood cell ratios were calculated: neutrophil:monocyte ratio (NMR);
158 neutrophil:lymphocyte ratio (NLR) and lymphocyte:monocyte ratio (LMR). Cases that had
159 concurrent disease which could potentially confound the interpretation of elevated leukocytes
160 (for example evidence of non-cancer-related inflammation detected on physical examination)
161 were excluded from the analysis of the leukocyte concentrations and ratios. Induction

162 protocols and their associated responses, dates of relapse, details of all rescue protocols used
163 with their associated responses and time of subsequent relapse and the date and reason for
164 death or euthanasia were also recorded.

165

166 All available cytological specimens were reviewed by a Board-certified clinical pathologist
167 (BS), to assure that the cytological features were consistent with the diagnosis of DLBCL-CB
168 according to the WHO classification scheme.^{14,16} Histopathological classification was also
169 performed where possible by Board-certified anatomical pathologists at the reference
170 laboratories concerned, using the WHO classification. Cases considered not to be DLBCL-
171 CB upon review were excluded. The methods used for immunophenotyping were recorded
172 and immunophenotypic results obtained with flow cytometry were classified as normal or
173 aberrant after Gelain et. al.⁵²

174

175 Haematology, serum biochemistry and urinalysis results at diagnosis were classified as
176 normal or abnormal according to the reference intervals of the diagnostic laboratories
177 concerned. Haematology results from the chemotherapy treatments during the induction
178 protocols were scrutinized and the incidence and severity of neutropenic episodes (as defined
179 by VCOG CTCAE 1.1)⁵³ were recorded; a composite “neutropenia score” was then formed by
180 assigning points to each neutropenic event equivalent to the VCOG grade of neutropenia and
181 adding up all points acquired throughout the induction protocol.

182

183 Diagnostic imaging findings (computed tomography, radiography, ultrasonography or a
184 combination thereof) at diagnosis were recorded. Using this information the assigned stage
185 and substage according to WHO criteria⁴ were reviewed. Bone marrow aspirate or biopsy
186 was not routinely performed and so cases were categorised as presumptive stage V by
187 identification of circulating neoplastic cells in peripheral blood, on review of the blood smear
188 by a board-certified clinical pathologist.

189

190 Details of induction and rescue chemotherapy protocols were recorded. Induction protocols
191 were classified as or CHOP (cyclophosphamide, vincristine, prednisolone and doxorubicin),
192 **Supplementary Table 1**, or COP-type (an 8 week induction protocol involving
193 cyclophosphamide, vincristine and prednisolone with or without one subcutaneous dose of
194 cytarabine on the first day of treatment¹⁸), **Supplementary Table 2**. The CHOP category was
195 comprised of both 19- and 25-week Madison-Wisconsin protocols. Cases needed a minimum
196 of 28 days on a treatment protocol to be classed as either COP-type or CHOP; those who
197 died, were euthanased, or whose treatment was changed less than 28 days after starting a
198 treatment protocol, were classified as having progressive disease and excluded from statistical
199 comparison of treatment protocols. Dogs who achieved remission with COP-type protocols
200 were subsequently treated with the LMP oral maintenance protocol (involving chlorambucil,
201 methotrexate and prednisolone) until disease relapsed; dogs who received the CHOP
202 protocols did not receive maintenance chemotherapy.

203

204 Rescue protocols were categorised as DMAC (involving dexamethasone, melphalan,
205 actinomycin-D and cytarabine), **Supplementary Table 3**, COP, doxorubicin-based or
206 lomustine-based, **Supplementary Table 4**. Categories describing a dog's total combination
207 of chemotherapy protocols were defined as: COP-type with no rescue, CHOP with no rescue,
208 COP-type with doxorubicin-based rescue, CHOP with doxorubicin-based rescue, COP-type
209 with non-doxorubicin-based rescue and CHOP with non-doxorubicin-based rescue. The total
210 number of protocols a dog received, and the number of doxorubicin doses each dog received
211 through the entirety of its treatment were recorded. Dogs that received 4 doses of
212 doxorubicin throughout induction and rescue treatment (equivalent to one CHOP protocol)
213 were compared with those that received 1-3 doses (less than a CHOP protocol) and those who
214 received 5-8 doses (equivalent with induction and rescue with CHOP-based therapy).

215 Chlorambucil was substituted for cyclophosphamide in dogs who developed sterile
216 haemorrhagic cystitis. Dogs received echocardiography to monitor their systolic function at

217 the start of doxorubicin therapy; this was repeated at the sixth dose of doxorubicin if the
218 planned cumulative total dose of doxorubicin exceeded 6 doses (180mg/m²).
219
220 Response to induction and rescue treatments were evaluated at each visit using VCOG
221 criteria⁵⁴ based on physical examination and additional tests at clinician discretion. Cases
222 were classed as complete remission (CR) if lymph nodes had returned to normal size; partial
223 remission (PR) when lymph nodes remained enlarged but had reduced in size by at least 50%
224 and no new lesions were recognized; progressive disease (PD) was used for occurrence of
225 new lesions or increase in size of enlarged lymph nodes by at least 25%; and stable disease
226 (SD) as a change in size of lymph nodes which was not sufficient to be classified as PD or PR
227 with no occurrence of new lesions. The objective response rate was defined as the sum of
228 complete and partial remission rates. A response to treatment must have been maintained for
229 at least 28 days from the date it was first recorded to be classified as CR or PR. Progression-
230 free survival (PFS) was defined as the time from the administration of the first lymphoma
231 treatment (either cytotoxic drug, glucocorticoids or l-asparaginase), to disease relapse,
232 progression or death from any cause; PFS1 was used to denote the PFS for the first remission,
233 PFS2 for the second, and so forth. Dogs that had treatment changed for reasons other than
234 disease progression (for example due to patient's demeanour) were censored from PFS
235 analysis at the point the treatment was altered. Overall survival (OS) was defined as the time
236 between the administration of the first lymphoma treatment and death or euthanasia.⁵⁴ Dogs
237 that remained alive at the end of the follow-up period or who were lost to follow-up were
238 censored from OS analysis.
239
240 Statistical analysis: Data were described and analysed using IBM SPSS Version 22 and
241 Graphpad Prism™ (version 6). Data were assessed for normality by visual plotting and use of
242 the Shapiro-Wilk test. Univariable analysis of response to treatment was performed using
243 binary logistic regression; Log-rank and Cox proportional hazard regression were used to
244 analyse the effect of multiple variables on PFS and OS. Where analyses of different

245 treatment protocols were performed, the CHOP or doxorubicin-based groups were used as the
246 comparator, given the acceptance of doxorubicin-based protocols as the standard of care for
247 CL therapy.²⁰ The alpha level was set at $P=0.05$ for significance. ROC curve analysis was
248 performed to assign cut-off values to continuous variables used for the prediction of a PFS or
249 OS exceeding a certain time, usually its median value; a minimum area under the curve of 0.7
250 was required for the ROC model to be considered. Comparison of groups with different
251 survival times was performed using Fisher's exact test for categorical variables. Student's
252 (two-tailed) t-test and Mann-Whitney U test for parametric and non-parametric continuous
253 variables respectively.

254

255

256 **Results**

257

258 **Clinical Presentation**

259 Search results revealed 91 cases of canine B cell lymphoma; out of these, 65 cases of
260 multicentric CL were identified and 45 dogs were classified as mDLBCL-CB by the same
261 board-certified clinical pathologist; 35 dogs from the RVC and 10 from NDSR. Thirty-one
262 breeds were represented, and the most common were cross-breeds (8 dogs), Jack Russell
263 terriers (4), golden retrievers (2), beagles (2), West Highland white terriers (2) and flat-coated
264 retrievers (2). The mean age was 8.1 years (standard deviation 2.63 years) and the median
265 bodyweight was 28kg (range: 3.4-59.5kg). Seventeen dogs (38%) were female (16 neutered,
266 1 entire), and 28 (62%) were male (18 neutered, 10 entire). Median follow-up period was 316
267 days (range 6-1375 days); at the end of the study period, 36 dogs had died or been euthanized
268 due to lymphoma, 4 had been euthanased for another reason, 4 dogs remained alive and 1 dog
269 was lost to follow-up. Reasons for euthanasia other than lymphoma were cellulitis (1 dog)
270 and heart failure (3 dogs). The dog that developed cellulitis was euthanased 6 days after
271 starting treatment and the dogs that developed heart failure were euthanased 316 days, 790
272 days and 1297 days after starting treatment.

273

274 The key characteristics of the populations of dogs from the Royal Veterinary College and
275 North Downs Specialist Referrals were not significantly different. All dogs resided in the
276 South-East of England, and most lived in urban areas. All dogs were owned by fee-paying
277 clients and none were referred to partake in clinical trials. Both groups of dogs had
278 approximately equal gender representation (RVC 70% male versus NDSR 60% male),
279 bodyweight (RVC median bodyweight 28kg versus NDSR 27kg) and age (RVC median age
280 8yo versus NDSR 9yo). There was a similar distribution of disease stage, but a greater
281 proportion of RVC dogs presented as substage a (62%, versus 40% for NDSR). None of
282 these differences were statistically significant ($P>0.05$).

283

284 Haematology findings from before the administration of anticancer treatment (including
285 steroids or l-asparaginase) were available for 29 dogs. Eleven dogs (38%) were anaemic
286 (median PCV 33%, range 29-37%, all non-regenerative or insufficiently-regenerative), 7 dogs
287 (24%) had a neutrophilia, 12 dogs (41%) were lymphopenic, one dog (3%) had monocytosis
288 and one dog (3%) had a lymphocytosis. Median absolute leukocyte concentrations and ratios
289 are displayed in **Table 1**. Pre-treatment serum biochemistry and urinalysis were available for
290 35 dogs; 10 (29%) had biochemical abnormalities, the most common of which were
291 hyperbilirubinaemia and increased activities of serum ALT and ALP in 4 dogs (11%) and
292 elevated cholesterol in 3 dogs (9%). All dogs with hyperbilirubinaemia were stage 4 or 5.
293 Five of 35 dogs had abnormalities detected on urine dipstick; bilirubinuria was observed in
294 the 4 dogs with hyperbilirubinaemia (11%) and proteinuria (defined as at least 2+ protein on
295 dipstick analysis, in association with an unreactive urinary sediment, a urine specific gravity
296 of less than 1.030, and the absence of haematuria) was seen in 3 dogs (9%).

297

298 Immunophenotyping was performed by flow cytometry in 37 dogs (82%), by
299 immunohistochemistry in 7 dogs (16%), and by PARR in 1 dog (2%). Full flow cytometry
300 results were available for 32 dogs; for 5 dogs, only the immunophenotype was available,
301 details of the markers run and graphs were unavailable for scrutiny. A comprehensive set of
302 markers (including CD3, CD5, CD21, CD34, CD45 and CD79a) was available for 23 dogs.
303 Out of these, 12 dogs (52%) had a normal immunophenotype (consisting of positive labelling
304 with CD21, CD79a and CD45) and 11 dogs (48%) had an aberrant immunophenotype; two
305 dogs (18%) were classified as aberrant phenotypes due to the additional co-expression of T
306 cell markers (CD3 in one dog and CD5 in the other), whereas the remaining 9 dogs (82%) had
307 additional co-expression of CD34 or absent expression of CD21, CD79a, or CD45. Two dogs
308 had more than one aberration (**Table 2**). The two dogs that expressed CD34 were classed as
309 lymphoma cases rather than acute leukaemias because of the absence of both cytopaenias and
310 circulating neoplastic cells.

311

312 Stage was documented for 35 dogs (78%). Twenty-one dogs (60%) had thoracic and
313 abdominal imaging, 13 with CT and 8 with thoracic radiographs and abdominal
314 ultrasonography; 6 dogs (17%) had abdominal ultrasonography without thoracic imaging.
315 Nineteen dogs (54%) had fine needle aspiration cytology of the liver and spleen.
316 Eight dogs (23%) were categorised as stage 3, 18 (51%) as stage 4, and 9 (26%) as stage 5;
317 (four with circulating neoplastic cells and 5 with extranodal tissue involvement). Substage
318 information was available for 44 dogs; 25 (57%) were substage a and 19 (43%) were substage
319 b.

320

321 Induction Treatment and Response

322 Twenty-seven dogs (60%) were treated with CHOP induction, versus 15 (33%) COP-type; 3
323 dogs (7%) had progressive disease or death below 28 days, making them ineligible for
324 placement in COP-type or CHOP treatment groups. Nine dogs (20%) were pre-treated with l-
325 asparaginase, 3 before a COP-type protocol and 6 before a CHOP protocol (median time
326 before the protocol started was 1 day). Three dogs (7%) were pre-treated with prednisolone
327 (median time before protocol started 3 days). Haematology nadirs after chemotherapy
328 treatments in the induction protocols were available for 25 dogs; of these, 14 dogs (56%)
329 developed at least one episode of neutropenia, and the median neutropenia score for these
330 dogs was 3 (range 1-13). A greater proportion of dogs from NDSR were induced with a
331 CHOP protocol (80%) than RVC (60%), however this difference was not statistically
332 significant ($P=0.286$).

333

334 The objective response rate to induction treatment was 94%; 34 dogs (76%) obtained a CR, 8
335 dogs (18%) obtained a PR, and three dogs (6%) developed PD. Ninety percent of entire dogs
336 had complete responses to treatment whereas 55% of neutered dogs had complete responses
337 to treatment ($P=0.017$); no other variables were found to have a significant effect on response
338 to induction therapy. Thirty-seven dogs (82%) completed the induction protocol, 7 dogs
339 (16%) had progressive disease during induction treatment, and one dog's (2%) induction

340 protocol was changed while it had a partial response to treatment due to the dog's aggressive
341 behaviour.

342

343 First Remission Progression-Free Survival (PFS1)

344 Median PFS for the first remission (PFS1) was 182 days (95% CI: 144-228 days). There was
345 no difference in PFS1 between the two institutions ($P=0.710$). Dogs that were not anaemic at
346 presentation had a significantly longer median PFS1 (254 days, 95% CI: 189-319 days) than
347 those that were anaemic on presentation (median 147 days, 95% CI: 22-272 days; $P=0.002$,
348 **Fig.1**). Dogs with a neutrophil:lymphocyte ratio below a cut-off value of 9.44 had longer
349 median PFS1 (216 days, 95% CI: 138-294 days) than those with NLR above 9.44 (median
350 PFS1 104 days, 95% CI: 0-213 days; $P=0.015$). The cut-off value of 9.44 had a 84%
351 sensitivity and 64% specificity of predicting the probability of PFS1 exceeding its median
352 value, 182 days (AUC 0.75). Dogs with a neutropenia score above 1.5 during induction
353 therapy had a significantly longer median PFS1 (254 days, 95% CI: 141-367 days), than dogs
354 with a score below 1.5 that had a median PFS1 (216 days, 95% CI: 38-394 days; $P=0.049$).
355 The cut-off value of 1.5 had a sensitivity of 50% and specificity of 86% for predicting the
356 probability of PFS1 exceeding its median of 182 days (area under the curve 0.7). Use of a
357 COP-type protocol provided shorter median PFS1 (147 days, 95%CI: 73-221 days) than
358 CHOP (median PFS1 251 days, 95% CI: 215-293 days; $P=0.000$, **Fig.2**) and achieving a
359 complete response was associated with longer PFS1 (246 days, 95% CI: 183-309 days) than a
360 partial response (median 105 days, range 32-178 days; $P=0.003$). No other variables were
361 found to have any significant effect on PFS1.

362

363 On multivariable analysis, only the absence of anaemia at diagnosis ($P=0.005$) and treatment
364 with a CHOP protocol over a COP-type protocol ($P=0.008$) were found to be independently
365 predictive of a longer PFS1.

366

367 Rescue Therapy and Subsequent Progression-Free Survival

368 Out of the 42 dogs (93%) that responded to induction treatment, 39 dogs (93%) relapsed, two
369 dogs (5%) remained in remission at the time of study completion, and one dog (2%) was lost
370 to follow-up. The three dogs (7%) that had progressive disease with induction therapy did not
371 receive rescue therapy, and were euthanased. For the 37 dogs (82%) that completed the
372 induction protocol, the median time between protocol completion and disease relapse was 118
373 days (range 7-309 days); 120 days (range 7-309 days) for dogs treated with CHOP protocol,
374 and 49.5 days (range 7-208) with COP-type ($P=0.01$). Twenty-five dogs (55%) received
375 rescue protocols, comprised of 14 (56%) doxorubicin-based, 6 (24%) COP-based protocols, 3
376 (12%) DMAC and 2 (8%) lomustine-based. Ten dogs (67%) that received COP-type
377 induction had rescue treatments, versus 15 (55%) that received CHOP; the distribution of
378 rescue treatments is illustrated in **Table 3**. A greater proportion of dogs from RVC received
379 rescue protocols (73% versus 40% from NDSR, $P=0.024$).

380

381 The objective response rate to the first rescue protocol was 86%; 15 dogs (68%) achieved CR,
382 4 dogs (18%) achieved PR, 3 dogs (14%) developed PD. The median PFS2 was 147 days
383 (95% CI: 104-190 days). The only variable with a significant effect on PFS2 length was
384 response to treatment; dogs who had a CR had a median PFS2 of 166 days (95% CI: 132-199
385 days), dogs that had a PR had median PFS2 56 days (95% CI: 25-87 days) and those who
386 didn't respond to treatment had a median PFS2 of 14 days (range 1-14 days; $P=0.000$).

387

388 Fifteen dogs received a second rescue protocol. Protocols used were: 6 (40%) lomustine-
389 based, 4 (27%) doxorubicin-based, 4 (27%) DMAC, and 1 (6%) COP. The objective
390 response rate was 28%; 2/14 dogs (14%) each obtained a CR, PR and SD, whereas 8 dogs
391 (58%) developed PD. Median PFS3 was 23 days (95% CI: 9-37 days). Eleven dogs received
392 a third rescue protocol; 5 (45.5%) were lomustine-based, 3 (27.5%) were DMAC, 2 (18%)
393 received a clinical trial drug and 1 (9%) was doxorubicin-based. The objective response was
394 60%; 2/10 dogs (20%) obtained CR, 4 dogs (40%) a PR, while 2 dogs each maintained SD
395 and developed PD. Median PFS4 was 32 days (95% CI: 18-46 days). Four dogs received a

396 fourth rescue protocol; one case received a clinical trial drug, one received DMAC, one
397 masitinib and the other received alternating doses of bleomycin and mitoxantrone. All
398 maintained SD and the median PFS5 was 39 days (range 28-111 days). Statistical analysis of
399 factors influencing the duration of PFS3, PFS4 and PFS5 was not performed due to small
400 group sizes.

401

402 Overall, 5 dogs (12%) received a COP-type induction protocol with no rescue, and 12 dogs
403 (29%) received a CHOP induction protocol with no rescue. Eight dogs (19%) received a
404 COP-type induction followed by rescue protocols which contained doxorubicin, 2 dogs (5%)
405 received COP-type induction followed by rescue protocols which did not contain
406 doxorubicin, 9 dogs (21%) received CHOP induction followed by rescue protocols which
407 contained doxorubicin, and 6 dogs (14%) received a CHOP induction followed by rescue
408 protocols which did not contain doxorubicin.

409

410 The median number of treatment protocols received was 2 (1-5). Thirty-seven dogs (82%)
411 received doxorubicin at some point during their overall treatment. The median number of
412 doxorubicin doses for those dogs receiving this drug at some point in induction and rescue
413 therapy was 4 (range 1-8; **Table 4**); the total number of doxorubicin doses did not differ
414 significantly between dogs from RVC and NDSR ($P=0.259$). The 3 dogs who were
415 euthanased due to heart failure had a total number of 3, 4 and 5 doses of doxorubicin. No
416 cardiotoxicity was seen in the 8 dogs that received more than 5 doses of doxorubicin (total
417 dose exceeding 150mg/m²).

418

419 Overall Survival (OS)

420 The median OS for all dogs was 322 days (95% CI: 259-385 days). No significant difference
421 in OS was found between the two institutions ($P=0.366$). OS was significantly shorter for
422 large dogs ($P=0.010$); using an ROC (AUC 0.73) a cut-off value of 31.15kg was found, with
423 82% sensitivity and 70% specificity for predicting OS of less than the median of 322 days.

424 Dogs with a lower NLR ($P=0.047$) and a higher LMR ($P=0.020$) had a longer OS; using
425 ROC analysis a LMR with a cut-off of 1.43 was found with a sensitivity of 83% and
426 specificity of 63% for predicting OS greater than the median (AUC 0.72), **Fig. 3**, and a cut-
427 off for NLR of 11.44 predicted survival less than 90 days with 80% sensitivity and 100%
428 specificity (AUC 0.87), **Fig. 4**.

429

430 Dogs that attained CR with induction therapy had a significantly longer OS (median 400 days,
431 95% CI: 291-509 days) than dogs that attained a PR (median OS 169 days, 95% CI: 57-281
432 days; $P=0.037$). Dogs that attained CR with the first rescue protocol had a significantly
433 longer OS (median OS 493 days, 95% CI: 383-603 days) than those that developed PD or SD
434 (median OS 216 days, 95% CI: 86-346 days; $P=0.009$), but there was no significant
435 difference in OS between dogs that had a CR or a PR (median OS 422 days, range 214-630
436 days; $P=0.693$). Dogs that received CHOP induction had a median OS of 401 days (95%
437 258-544 days) whereas dogs that had a COP-type induction had a median OS of 257 days
438 (95% CI: 157-357 days); this difference was not significant ($P=0.313$)

439

440 Dogs that received rescue protocols had a significantly longer OS (median 401 days, 95% CI:
441 288-514 days) than dogs that received no further treatment when lymphoma recurred (median
442 OS 227 days, 95% CI: 132-322 days; $P=0.009$). However, the total number of treatment
443 protocols a dog received had no significant effect on OS ($P=0.351$). Dogs that received COP-
444 type induction and no rescue therapy had a median OS of 192 days (95% CI: 153-231 days)
445 compared with 316 days (95% CI: 219-413 days) for dogs that received a CHOP protocol and
446 no rescue ($P=0.024$). No significant difference in OS was found between dogs that received
447 a CHOP induction protocol with no rescue therapy, and those that received COP-type
448 induction followed by doxorubicin-based rescue therapy ($P=0.213$). No significant
449 difference in OS was found between dogs that received a CHOP induction protocol with no
450 rescue therapy and dogs that received a CHOP induction followed by non-doxorubicin-based
451 rescue therapy ($P=0.925$); however, dogs that received CHOP induction followed by

452 doxorubicin-based rescue therapy had a significantly longer OS (median 706 days, 95% CI:
453 350-1062 days) compared with CHOP induction alone ($P=0.04$; **Fig. 5**).

454

455 Overall, the number of doxorubicin doses (from 0-8) was highly significantly associated with
456 longer overall survival ($P=0.000$). When dogs that were not given doxorubicin were
457 excluded from analysis, the strong association remained ($P=0.002$, **Table 5**). Dogs that
458 received 1-3 doses of doxorubicin had a median OS of 216 days (95% CI: 24-408 days),
459 which was not significantly different to dogs that had 4 doses, equivalent to a complete
460 CHOP protocol (median OS 322 days, 95% CI: 280-364 days, $P=0.777$), but significantly
461 shorter than dogs that had 5-8 doses (median OS 706 days, 95% CI: 297-1115 days, $P=0.049$,
462 **Fig. 6**).

463

464 The two dogs with expression of the T cell markers CD3 and CD5 had a subjectively shorter
465 OS of 32 and 262 days compared with the rest of the study group (median OS 422 days, 95%
466 CI: 260-584 days; $P=0.031$). No such apparent difference in OS was noted with other
467 aberrations in immunophenotype.

468

469 On multivariable analysis, the variables found to be independently predictive of OS were
470 NLR ($P=0.009$), LMR ($P=0.031$), and the combination of induction and rescue protocols
471 ($P=0.030$) and the number of doxorubicin doses (for the dogs who received doxorubicin,
472 $P=0.002$). No other variables had a significant effect on OS.

473

474 Prolonged and Short Survival Groups

475 In this study, the 6-month survival rate was 73%, the 1-year survival 38% and the 2-year
476 survival rate was 9%. Dogs living more than 1 year had lower median bodyweight (mean
477 22.5kg versus 31kg, $P=0.019$), were less likely to be anaemic on presentation (prevalence of
478 anaemia at diagnosis of 11% versus 53%, $P=0.049$), had a CR with induction therapy (100%
479 versus 64%, $P=0.007$) and when the dogs who received doxorubicin were considered, those

480 who received more doxorubicin doses were more likely to survive greater than one year
481 (median number of 5 doxorubicin in dogs living greater than one year compared with 4 doses,
482 for those that lived less than 1 year, $P=0.031$). Statistical analysis of dogs living longer than
483 2 years was not performed due to small group size.

484

485 Dogs with shorter survivals than 6 months had lower rates of CR with induction therapy (42%
486 versus 91%, $P=0.001$), were older (mean age of 9.5 years versus 7.6 years, $P=0.031$) and less
487 likely to be treated with rescue therapy (27% receiving rescue versus 69%, $P=0.031$) than
488 those who lived over 6 months.

489

490 **Discussion**

491

492 In this study, a homogenous population of mDLBCL-CB cases are described. The median
493 PFS1 and OS of the whole group (182 days and 322 days respectively), and proportions
494 surviving to 1 and 2 years (38% and 9% respectively) were lower than^{19,55,56} or similar to^{57,58}
495 previous reports of groups containing mixed subtypes of multicentric CL. The OS of dogs in
496 this study may not have been greater than that reported in mixed groups because many
497 previous studies have been designed to evaluate a specific chemotherapy protocol, whereas
498 this study evaluated a specific subtype of disease. A homogeneous population also lacks the
499 potential influence of subtypes of disease that have much longer survival times for example
500 T-zone lymphoma,⁵⁹ or shorter survival times for example lymphoblastic lymphomas.²³ The
501 level of treatment a dog received varied in this study, including dogs that were given
502 treatments which are not considered the “standard of care,” prescribed for reasons such as
503 finance or owner convenience. When specific combinations of induction and rescue therapy
504 are evaluated, greater OS times are seen, for example dogs who received CHOP induction
505 followed by doxorubicin-based rescue had a median OS of 706 days (95% CI: 350-1062
506 days).

507

508 The response to induction treatment of mDLBCL-CB (objective response rate of 94%,
509 complete response rate of 76%) was high. This rate has been similar in some studies^{18,58,60}
510 and lower in others.^{19,55,56} Since mDLBCL-CB is the most prevalent type of lymphoma in
511 dogs,⁷ a level of similarity between this study group and a random selection of CL cases
512 would be expected. Studies of mixed immunophenotype groups are likely to have included
513 dogs with less responsive variants of the disease for example indolent lymphomas, that may
514 not achieve complete responses.⁵⁹ In this study group, entire dogs had a significantly higher
515 rate of complete responses to induction therapy, however a greater proportion of entire dogs
516 were treated with CHOP induction therapy than neutered dogs (73% versus 61%) and the low
517 number of entire dogs (n=11) throws the independence of this finding into question.

518

519 The presence of anaemia at diagnosis was shown to be associated with a significantly shorter
520 PFS1 and was significantly more common in the group of dogs who survived less than one
521 year, compared with dogs that survived over 1 year. The negative prognostic value of
522 anaemia in canine lymphoma has been demonstrated in previous studies,^{36,61,62} although the
523 aetiology is unclear. Bone marrow infiltration by neoplastic lymphocytes has been
524 hypothesized. However, one study found no significant difference in occurrence of marrow
525 infiltration between groups of anaemic and non-anaemic dogs with lymphoma, and no
526 significant difference in haematocrit between dogs with marrow involvement and those
527 without.⁶² Anaemia of inflammatory disease is another potential aetiology. A previous study
528 has shown no laboratory evidence of this in a group of dogs with lymphoma, although
529 decreased response of the bone marrow to erythropoietin is still possible.⁶³ The
530 pathophysiological mechanism associating anaemia with a poor prognosis is unclear. One
531 theory suggests that the state of chronic hypoxia may induce expression of proteins which
532 enable cancer cells to deal better with stress; increased concentration of one such protein,
533 vascular endothelial growth factor has been associated with a poorer prognosis in canine
534 lymphoma,⁶⁴ and both anaemia and a poorer prognosis in humans with NHL.⁶⁵

535

536 Choice of a CHOP induction protocol over a COP-type protocol was shown as independently
537 prognostic for PFS1 in this study, in line with previous publications.¹⁸ The superior PFS1
538 associated with the CHOP protocol may be related to both the longer duration of treatment
539 and the use of another agent, doxorubicin. In agreement with other publications,^{66,67} this
540 study found an association between the development of neutropenia and PFS1. Since data on
541 the development of neutropenia were only available for fewer than half of the dogs, and the
542 prevalence of neutropenia among these dogs was low, we believe that assessment of the
543 prognostic effect of neutropenia in this study was statistically under-powered. The prevalence
544 of neutropenia may have been low because dogs induced with a COP-type protocol would

545 have received a less immunosuppressive and shorter induction period compared to other
546 studies, and because dose reductions were often performed following neutropenic episodes.
547
548 Response to the first rescue protocol (86%) and the median PFS2 (147 days) were similar to
549 the response to induction therapy (94%) and the median PFS1 (182 days). Such findings have
550 been described before.⁶⁸ For a number of dogs in this study group, the first rescue protocol
551 can be regarded as “re-induction” since the median time between completing induction
552 therapy and relapse was 118 days, making the development of appreciable drug resistance
553 less likely, and secondly a number of dogs who were induced with a COP-type protocol
554 received a doxorubicin-based rescue therapy. The response rates and remission durations
555 associated with the second, third and fourth rescue protocols were more similar to previous
556 reports of rescue treatment for lymphoma when drug-resistance is established.^{69–72}
557
558 This study has shown the independent prognostic significance of the pre-treatment
559 neutrophil:lymphocyte ratio (NLR) in predicting progression-free survival and OS in canine
560 mDLBCL-CB. Absolute leukocyte counts and their ratios are ways of measuring different
561 aspects of systemic inflammation and in cases of cancer systemic inflammation may be
562 caused by tumour-related inflammation.⁴³ Tumour-related inflammation is associated with
563 cancer progression⁷³ and in human medicine NLR has been recognised as holding prognostic
564 significance in diffuse large B cell lymphoma,⁴¹ and many solid tumours.⁴³ In veterinary
565 medicine, NLR has been shown to be of some prognostic significance (on univariable but not
566 multivariable analysis) in canine mast cell tumours.⁷⁴ Mutz et.al recently reported no
567 prognostic significance of NLR in a study of canine multicentric lymphoma treated with a
568 CHOP protocol.⁴⁶ If the findings of our study are supported by subsequent work, the apparent
569 disagreement with the study of Mutz et.al may be due to an innate feature of mDLBCL-CB
570 and the homogenous population of mDLBCL-CB cases in this study. Conversely, increased
571 NLR is a very non-specific finding and despite screening dogs for concurrent disease, it is

572 possible that the results are confounded by dogs with sub-clinical benign conditions, for
573 example dental disease, otitis externa, or pancreatitis.

574

575 We have reported that high NLR values (above a cut-off of 9.44 for PFS1 and 11.44 for OS),
576 correspond to a poor prognosis. In human medicine, a cut-off value of 3.5 is established,
577 above which DLBCL patients have both a significantly poorer PFS and overall survival.⁴¹

578 Such high NLR cut-off values may make this test insensitive in veterinary medicine; however
579 it may also reflect that neutrophilia and lymphopenia due to stress is more common in canine
580 patients than humans, and so significant NLR levels will need to be in excess of that expected
581 from a normal stress leukogram. Previous veterinary studies have evaluated absolute
582 monocyte and lymphocyte counts in cancer; prognostic significance has been shown in canine
583 osteosarcoma,⁴⁸ and in some studies of canine lymphoma⁴⁴ but not others.⁴⁶ LMR has
584 previously been reported as prognostic in human NHL,⁷⁵ in canine mast cell disease⁷⁴ and in
585 canine lymphoma.⁷⁶ This study concurs with the previous report of the prognostic
586 significance of LMR for survival in mDLBCL-CB. However just as for the significance of
587 NLR, caution is needed in interpreting LMR values due to the non-specific aetiologies of its
588 elevation.

589

590 This study has demonstrated that use of a rescue protocol, particularly rescue therapy
591 involving doxorubicin, and the total number of doxorubicin doses received to be a strong
592 prognostic factor in mDLBCL-CB cases. These observations should be interpreted with
593 caution as many dogs who received a higher number of doses of doxorubicin are likely to be
594 owned by the most committed owners, and subsequent doxorubicin treatments were unlikely
595 to be offered unless the dog had responded to the drug previously. Thus, dogs who received
596 the most doxorubicin may have been selected by lack of drug resistance relative to the other
597 dogs. This could highlight an innate tractability in a subset of dogs with mDLBCL-CB which
598 has yet to be identified. The lack of significantly different OS between dogs that received a
599 COP-type induction followed by doxorubicin-based rescue therapy and dogs that received a

600 CHOP induction protocol (with or without non-doxorubicin-based rescue therapy) suggest
601 that the temporal placement of doxorubicin in either a dog's induction or rescue treatment
602 does not seem to be of prognostic value. Being able to give a COP-type protocol for
603 induction therapy without the concern of undermining prognosis may offer flexibility in
604 choice of induction protocols to veterinarians and their clients.

605

606 Aberrant immunophenotypes of CL have been described based on flow cytometry,^{52,77}
607 although their prognostic significance is largely uncertain. Possession of an aberrant
608 immunophenotype overall held no prognostic value in this study, however statistical analysis
609 of the particular aberrations present was not possible due to small group sizes. The poorer OS
610 experienced by the two cases that expressed T cell markers is interesting; further work is
611 necessary to investigate different prognoses conferred by different aberrations of
612 immunophenotype.

613

614 In this study group, the majority of dogs presented in clinical stage 4 or 5, and almost half of
615 them in substage b. These proportions may be normal for mDLBCL-CB, or the apparent bias
616 towards more advanced disease may be a result of thorough application of staging tests and
617 the phenomenon of stage migration³⁸ or selection bias since all cases were recruited at
618 specialist referral centres. Stage, substage, gender, and bodyweight have been previously
619 reported as prognostic in canine lymphoma^{17,19,36,37,61,67} and a previous publication has
620 reported a different behaviour of stage 5 cases with extranodal involvement compared with
621 those with haematological involvement.⁵⁵ None of these findings were supported by this
622 study. Prognostic significance of clinical stage in previous, heterogeneous groups of
623 lymphoma cases may have been confounded by the inherent variability of different CL
624 subtypes. Equally, the prevalence of certain factors was too low in the current study for
625 meaningful inclusion in statistical analysis.

626

627 Different factors were found to have prognostic significance for PFS1 and OS. Although the
628 application of some variables to PFS1 would be meaningless, the discrepancy between the
629 prognostic significance of LMR, response to induction therapy and the presence of anaemia at
630 diagnosis for PFS1 versus OS is harder to explain. These findings may have arisen due to the
631 population size and the effect of censoring some cases from OS analysis which were included
632 in PFS1 analysis. Nevertheless if these findings hold true in larger populations one
633 explanation could be that features such as some absolute cell concentration or ratios reflect
634 the susceptibility of the disease to treatment at that point in time, thus they may be prognostic
635 for length of the subsequent remission only, rather than for OS.

636

637 In this study, the authors feel justified in combining two different hospital populations since
638 they are not significantly different in any key feature of clinical presentation or induction
639 therapy, and their cytology has been reviewed by the same board-certified clinical
640 pathologist. The only significant difference between the two groups is that a higher
641 proportion of cases from RVC received a rescue protocol; we feel that this doesn't make the
642 combination of populations any less valid since the choice of whether to use rescue therapy or
643 not is dependent on the owners' wishes and finances, and it is not possible for these variables
644 to be well controlled in retrospective studies. The finding of this study with regard to rescue
645 therapy was that a response to rescue therapy is positively prognostic, and this is corroborated
646 by other studies.⁶⁸

647

648 This study was limited by its retrospective nature and relatively small population size;
649 resultantly the study is most likely underpowered and significant findings may have been
650 missed. A large number of variables have also been statistically evaluated, giving the
651 possibility that false positive associations have arisen by chance. Larger multi-institution
652 studies are clearly needed to clarify this study's findings. The recruitment of cases from
653 speciality referral centres might have also biased the caseload to those which had more
654 complicated presentations, more likely to be of substage b, and with more committed owners.

655 Although the study describes the response to different treatments, the authors caution that a
656 retrospective study represents weak evidence to guide future therapeutic decisions. We hope
657 that this study provides grounds for prospective controlled trials in this area. Investigation of
658 the genetic differences within this group of dogs may help to predict the dogs that will
659 develop drug resistance sooner versus those will demonstrate prolonged response to therapy.⁷⁸

660

661 In conclusion, this study has shown the behaviour of disease and response to certain drugs in
662 a population of dogs with mDLBCL-CB. Absence of anaemia at diagnosis and a pre-
663 treatment NLR below 9.44 were associated with longer PFS1 while LMR above 1.43, and
664 NLR below 11.44 were associated with longer OS. The use of rescue therapy and the number
665 of doxorubicin doses received were strongly associated with longer OS. The choice of
666 induction protocol did not influence survival, providing doxorubicin was later used as rescue
667 therapy. Further, prospective studies are warranted to further assess the importance of these
668 findings.

669

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673

674

675 **References**

- 676 1. Merlo DF, Rossi L, Pellegrino C, et al. Cancer Incidence in Pet Dogs: Findings of the
677 Animal Tumor Registry of Genoa, Italy. *J Vet Intern Med.* 2008;22:976-984.
- 678 2. Sueiro FAR, Alessi AC, Vassallo J. Canine lymphomas: a morphological and
679 immunohistochemical study of 55 cases, with observations on p53 immunoexpression.
680 *J Comp Pathol.* 2004;131(2-3):207-213. doi:10.1016/j.jcpa.2004.04.002.
- 681 3. Vezzali E, Parodi AL, Marcato PS, Bettini G. Histopathologic classification of 171
682 cases of canine and feline non-Hodgkin lymphoma according to the WHO. *Vet Comp*
683 *Oncol.* 2010;8(1):38-49. doi:10.1111/j.1476-5829.2009.00201.x.
- 684 4. Madewell BR, Thesen GH. Hematopoietic neoplasms, sarcomas and related
685 conditions. In: *Veterinary Cancer Medicine; Ed. Theilen GH & Madewell BR.* 2nd ed.
686 Lea & Febiger; 1987.
- 687 5. Modiano JF, Breen M, Burnett RC, et al. Distinct B-Cell and T-Cell
688 Lymphoproliferative Disease Prevalence among Dog Breeds Indicates Heritable Risk.
689 *Cancer Res.* 2005;65:5654-5661.
- 690 6. Richards KL, Suter SE. Man's best friend: what can pet dogs teach us about non-
691 Hodgkin's lymphoma? *Immunol Rev.* 2015;263(1):173-191. doi:10.1111/imr.12238.
- 692 7. Valli VE, San Myint M, Barthel a, et al. Classification of Canine Malignant
693 Lymphomas According to the World Health Organization Criteria. *Vet Pathol.*
694 2011;48(1):198-211. doi:10.1177/0300985810379428.
- 695 8. Fournel-Fleury C, Magnol JP, Bricaire P, et al. Cytohistological and Immunological
696 Classification of Canine Malignant Lymphomas; Comparison with Human Non-
697 Hodgkin's Lymphomas. *J Comp Path.* 1997;117:35-39.
- 698 9. Rout ED, Avery PR. Lymphoid Neoplasia: Correlations Between Morphology and
699 Flow Cytometry. *Vet Clin North Am Small Anim.* 2016:292-300. doi:10.1016/B0-72-
700 160422-6/50029-2.
- 701 10. Ponce F, Marchal T, Magnol JP, et al. A morphological study of 608 cases of canine
702 malignant lymphoma in France with a focus on comparative similarities between
703 canine and human lymphoma morphology. *Vet Pathol.* 2010;47(3):414-433.
704 doi:10.1177/0300985810363902.
- 705 11. Villiers EJ, Berlato D. Plenary Lecture: Clinical research in oncology: the win-win
706 challenge between clinical pathologists and oncologists. In: *ESVONC Congress 2016.*
707 ; 2016.

- 708 12. Teske E, van Heerde P. Diagnostic value and reproducibility of fine-needle aspiration
709 cytology in canine malignant lymphoma. *Vet Q.* 1996;18(3):112-115.
710 doi:10.1080/01652176.1996.9694630.
- 711 13. Ponce F, Magnol JP, Ledieu D, et al. Prognostic significance of morphological
712 subtypes in canine malignant lymphomas during chemotherapy. *Vet J.*
713 2004;167(2):158-166. doi:10.1016/j.tvjl.2003.10.009.
- 714 14. Raskin RE. Hemolymphatic System. In: Raskin RE, Meyer D, eds. *Canine and Feline*
715 *Cytology; a Color Atlas and Interpretation Guide*; . 3rd ed. Elsevier; 2016:91-137.
- 716 15. Raskin RE, Fox LE. Clinical relevance of the World Health Organization
717 classification of lymphoid neoplasma in dogs. *Vet Clin Pathol.* 2003;32:151.
- 718 16. Vernau W. Cytology of Lymphoma and Useful Adjunctive Diagnostics. In: *13th*
719 *ESVCP Congress*. Dublin, Ireland; 2011.
- 720 17. Cotter S, Goldstein M. Treatment of lymphoma and leukemia with cyclophosphamide,
721 vincristine and prednisone I: Treatment of dog. *J Am Anim Hosp Assoc.* 1983;19:159-
722 165.
- 723 18. Hosoya K, Kisseberth WC, Lord LK, et al. Comparison of COAP and UW-19
724 protocols for dogs with multicentric lymphoma. *J Vet Intern Med.* 2007;21(6):1355-
725 1363. doi:10.1892/06-284.1.
- 726 19. Keller ET, MacEwen EG, Rosenthal RC, Helfand SC, Fox LE. Evaluation of
727 prognostic factors and sequential combination chemotherapy with doxorubicin for
728 canine lymphoma. *J Vet Intern Med.* 1993;7(5):289-295. doi:10.1111/j.1939-
729 1676.1993.tb01021.x.
- 730 20. Stone M, Goldstein M, Cotter SM. Comparison of two protocols for induction of
731 remission in dogs with lymphoma. *JAAHA.* 1991;27:315-321.
- 732 21. Graham JC, Myers RK. The prognostic significance of angiogenesis in canine
733 mammary tumors. *J Vet Intern Med.* 1999;13(5):416-418. doi:10.1111/j.1939-
734 1676.1999.tb01456.x.
- 735 22. Myers NC, Moore a S, Rand WM, Gliatto J, Cotter SM. Evaluation of a multidrug
736 chemotherapy protocol (ACOPA II) in dogs with lymphoma. *J Vet Intern Med.*
737 1997;11(6):333-339.
- 738 23. Valli VE, Kass PH, Myint MS, Scott F. Canine Lymphomas: Association of
739 Classification Type, Disease Stage, Tumor Subtype, Mitotic Rate, and Treatment With
740 Survival. *Vet Pathol.* 2013;50(5):738-748. doi:10.1177/0300985813478210.

- 741 24. Teske E, van Heerde P, Rutteman G, Kurzman I, Moore P, MacEwen E. Prognostic
742 factors for treatment of malignant lymphoma in dogs. *JAVMA*. 1994;2015(12):1722-
743 1728.
- 744 25. Jagielski D, Lechowski R, Winiarczyk S. A Retrospective Study of the Incidence and
745 Prognostic Factors of Multicentric Lymphoma in Dogs (1998 – 2000). 2002;424:419-
746 424.
- 747 26. Rebhun RB, Kent MS, Borroffka SAE, Frazier S, Skorupski K, Rodriguez CO.
748 CHOP chemotherapy for the treatment of canine multicentric T-cell lymphoma. *Vet*
749 *Comp Oncol*. 2011;9(1):38-44. doi:10.1111/j.1476-5829.2010.00230.x.
- 750 27. Seelig DM, Avery P, Webb T, et al. Canine T-zone lymphoma: unique
751 immunophenotypic features, outcome, and population characteristics. *J Vet Intern*
752 *Med*. 2014;28(3):878-886. doi:10.1111/jvim.12343.
- 753 28. Beaver LM, Strottner G, Klein MK. Response rate after administration of a single dose
754 of doxorubicin in dogs with B-cell or T-cell lymphoma: 41 cases (2006-2008). *J Am*
755 *Vet Med Assoc*. 2010;237(9):1052-1055. doi:10.2460/javma.237.9.1052.
- 756 29. Marconato L, Stefanello D, D P, et al. Predictors of long-term survival in dogs with
757 high-grade multicentric lymphoma. *JAVMA*. 2011;238(4):2-7.
- 758 30. Williams MJ, Avery AC, Lana SE, Hillers KR, Bachand AM, Avery PR. Canine
759 Lymphoproliferative Disease Characterized by Lymphocytosis: Immunophenotypic
760 Markers of Prognosis. *J Vet Intern Med*. 2008;(22):596-601.
- 761 31. Flood-Knapik KE, Durham AC, Gregor TP, Sánchez MD, Durney ME, Sorenmo KU.
762 Clinical, histopathological and immunohistochemical characterization of canine
763 indolent lymphoma. *Vet Comp Oncol*. 2013;11(4):272-286. doi:10.1111/j.1476-
764 5829.2011.00317.x.
- 765 32. Rassnick KM, Moore a S, Collister KE, et al. Efficacy of combination chemotherapy
766 for treatment of gastrointestinal lymphoma in dogs. *J Vet Intern Med*. 2009;23(2):317-
767 322. doi:10.1111/j.1939-1676.2008.0270.x.
- 768 33. Williams LE, Rassnick KM, Power HT, et al. CCNU in the treatment of canine
769 epitheliotropic lymphoma. *J Vet Intern Med*. 2006;20(1):136-143. doi:10.1892/0891-
770 6640(2006)20[136:CITTOC]2.0.CO;2.
- 771 34. Fry MM, Vernau W, Pesavento P a, Brömel C, Moore PF. Hepatosplenic lymphoma in
772 a dog. *Vet Pathol*. 2003;40(5):556-562. doi:10.1354/vp.40-5-556.
- 773 35. Keller S, Vernau W, Hodges J, Vilches-Moure J, McElliot V, Moore P. Hepatosplenic
774 and Hepatocytotropic T-Cell Lymphoma – Two Distinct Types of T-Cell Lymphoma

- 775 in Dogs. *J Comp Pathol*. 2012;146(1):56. doi:10.1016/j.jcpa.2011.11.046.
- 776 36. Miller AG, Morley PS, Rao S, Avery AC, Lana SE, Olver CS. Anemia is associated
777 with decreased survival time in dogs with lymphoma. *J Vet Intern Med*.
778 2009;23(1):116-122. doi:10.1111/j.1939-1676.2008.0210.x.
- 779 37. Carter R, Harris C, Withrow SJ. Chemotherapy of canine lymphoma with
780 histopathological correlation: Doxorubicin alone compared to COP as first treatment
781 regimen. *J Am Anim Hosp Assoc*. 1987;23:587-596.
- 782 38. Flory AB, Rassnick KM, Stokol T, Scrivani P V., Erb HN. Stage migration in dogs
783 with lymphoma. *J Vet Intern Med*. 2007;21(5):1041-1047. doi:10.1892/0891-
784 6640(2007)21[1041:SMIDWL]2.0.CO;2.
- 785 39. Tadmor T, Bari A, Sacchi S, et al. Monocyte count at diagnosis is a prognostic
786 parameter in diffuse large B-cell lymphoma: Results from a large multicenter study
787 involving 1191 patients in the pre- and post-rituximab era. *Haematologica*.
788 2014;99(1):125-130. doi:10.3324/haematol.2013.088161.
- 789 40. Ray-Coquard I, Cropet C, Van Glabbeke M, et al. Lymphopenia as a prognostic factor
790 for overall survival in advanced carcinomas, sarcomas, and lymphomas. *Cancer Res*.
791 2009;69(13):5383-5391. doi:10.1158/0008-5472.CAN-08-3845.
- 792 41. Porrata L, Ristow K, Habermann T, Inwards D, Micallef I, Markovic S. Predicting
793 survival for diffuse large B-cell lymphoma patients using baseline
794 neutrophil/lymphocyte ratio. *Am J Hematol*. 2010;85(11):896-899.
- 795 42. Huang CY, Yang YC, Wang KL, et al. Possible surrogate marker for an effective
796 dose-dense chemotherapy in treating ovarian cancer. *Taiwan J Obstet Gynecol*.
797 2016;55(3):405-409. doi:10.1016/j.tjog.2016.04.017.
- 798 43. Templeton AJ, McNamara MG, Šeruga B, et al. Prognostic role of neutrophil-to-
799 lymphocyte ratio in solid tumors: A systematic review and meta-analysis. *J Natl*
800 *Cancer Inst*. 2014;106(6). doi:10.1093/jnci/dju124.
- 801 44. Perry JA, Thamm DH, Eickhoff J, Avery AC, Dow SW. Increased monocyte
802 chemotactic protein-1 concentration and monocyte count independently associate with
803 a poor prognosis in dogs with lymphoma. *Vet Comp Oncol*. 2011;9(1):55-64.
804 doi:10.1111/j.1476-5829.2010.00235.x.
- 805 45. Marconato L, Martini V, Stefanello D, et al. Peripheral blood lymphocyte/monocyte
806 ratio as a useful prognostic factor in dogs with diffuse large B-cell lymphoma
807 receiving chemoimmunotherapy. *Vet J*. 2015;206(2):226-230.
808 doi:10.1016/j.tvjl.2015.07.009.

- 809 46. Mutz M, Boudreaux B, Kearney M, Stroda K, Gaunt S, Shiomitsu K. Prognostic value
810 of baseline absolute lymphocyte concentration and neutrophil/lymphocyte ratio in
811 dogs with newly diagnosed multi-centric lymphoma. *Vet Comp Oncol.*
812 2015;13(4):337-347. doi:10.1111/vco.12045.
- 813 47. Novacco M, Comazzi S, Marconato L, et al. Prognostic factors in canine acute
814 leukaemias: a retrospective study. *Vet Comp Oncol.* 2015;4:409-416.
815 doi:10.1111/vco.12136.
- 816 48. Sottnik JL, Rao S, Lafferty MH, et al. Association of Blood Monocyte and
817 Lymphocyte Count and Disease-Free Interval in Dogs with Osteosarcoma. *J Vet Intern*
818 *Med.* 2010;(2):1439-1444.
- 819 49. Macfarlane L, Morris J, Pratschke K, et al. Diagnostic value of neutrophil-lymphocyte
820 and albumin-globulin ratios in canine soft tissue sarcoma. *J Small Anim Pract.*
821 2016;57(3):135-141. doi:10.1111/jsap.12435.
- 822 50. Marconato L, Bettini G, Giacoboni C, et al. Clinicopathological Features and Outcome
823 for Dogs with Mast Cell Tumors and Bone Marrow Involvement. *J Vet Intern Med.*
824 2008;22:1001-1007.
- 825 51. Endicott MM, Charney SC, McKnight JA, Loar AS, Barger AM, Bergman PJ.
826 Clinicopathological findings and results of bone marrow aspiration in dogs with
827 cutaneous mast cell tumours: 157 cases (1999-2002). *Vet Comp Oncol.* 2007;5(1):31-
828 37. doi:10.1111/j.1476-5829.2006.00115.x.
- 829 52. Gelain ME, Mazzilli M, Riondato F, Marconato L, Comazzi S. Aberrant phenotypes
830 and quantitative antigen expression in different subtypes of canine lymphoma by flow
831 cytometry. *Vet Immunol Immunopathol.* 2008;121(3-4):179-188.
832 doi:10.1016/j.vetimm.2007.09.018.
- 833 53. Vail DM. Veterinary cooperative oncology group - common terminology criteria for
834 adverse events (VCOG-CTCAE) following chemotherapy or biological antineoplastic
835 therapy in dogs and cats v1.1. *Vet Comp Oncol.* 2011;5:1-30. doi:10.1111/j.1476-
836 5829.2011.00283.x.
- 837 54. Vail DM, Michels GM, Khanna C, Selting K a, London C a. Response evaluation
838 criteria for peripheral nodal lymphoma in dogs (v1.0)--a Veterinary Cooperative
839 Oncology Group (VCOG) consensus document. *Vet Comp Oncol.* 2010;8(1):28-37.
840 doi:10.1111/j.1476-5829.2009.00200.x.
- 841 55. Kaiser CI, Fidel JL, Roos M, Kaser-Hotz B. Reevaluation of the University of
842 Wisconsin 2-year protocol for treating canine lymphosarcoma. *J Am Anim Hosp*

- 843 *Assoc.* 2007;43(2):85-92. doi:10.5326/0430085.
- 844 56. Simon D, Moreno SN, Hirschberger J, et al. Efficacy of a continuous, multiagent
845 chemotherapeutic protocol versus a short-term single-agent protocol in dogs with
846 lymphoma. *J Am Vet Med Assoc.* 2008;232(6):879-885. doi:10.2460/javma.232.6.879.
- 847 57. Boyce KL, Kitchell BE. Treatment of canine lymphoma with COPLA/LVP. *J Am*
848 *Anim Hosp Assoc.* 2000;36(5):395-403. doi:10.5326/15473317-36-5-395.
- 849 58. Elliott JW, Cripps P, Marrington AM, Grant IA, Blackwood L. Epirubicin as part of a
850 multi-agent chemotherapy protocol for canine lymphoma. *Vet Comp Oncol.*
851 2013;11(3):185-198. doi:10.1111/j.1476-5829.2011.00311.x.
- 852 59. Aresu L, Martini V, Rossi F, et al. Canine indolent and aggressive lymphoma: Clinical
853 spectrum with histologic correlation. *Vet Comp Oncol.* 2015;13(4):348-362.
854 doi:10.1111/vco.12048.
- 855 60. Moore AS, Cotter SM, Rand WM, et al. Evaluation of a discontinuous treatment
856 protocol (VELCAP-S) for canine lymphoma. *J Vet Intern Med.* 2001;15(4):348-354.
- 857 61. Marconato L, Stefanello D, Valenti P, et al. Predictors of long-term survival in dogs
858 With High-Grade Multicentric Lymphoma. *JAVMA.* 2011;238(4):480-485.
- 859 62. Abbo AH, Lucroy MD. Assessment of anemia as an independent predictor of response
860 to chemotherapy and survival in dogs with lymphoma: 96 cases (1993-2006). *J Am Vet*
861 *Med Assoc.* 2007;231(12):1836-1842. doi:10.2460/javma.231.12.1836.
- 862 63. Lucroy M, Christopher M, Kraegel S. Anaemia associated with canine lymphoma.
863 *Comp Haematol, Int.* 1998;8:1-6.
- 864 64. Gentilini F, Calzolari C, Turba M, Al. E. Prognostic value of serum vascular
865 endothelial growth factor (VEGF) and plasma activity of matrix metalloproteinase
866 (MMP) 2 and 9 in lympho- ma-affected dogs. *Leuk Res.* 2005;29:1263-1269.
- 867 65. Wrobel T, Poreba M, Mazur G, Al. E. Angiogenic and coagulation-fibrinolysis factors
868 in non Hodgkin's lymphoma. *Neoplasia.* 2006;53:253-258.
- 869 66. Wang SL, Lee JJ, Liao a. T. Chemotherapy-induced neutropenia is associated with
870 prolonged remission duration and survival time in canine lymphoma. *Vet J.*
871 2015;205(1):69-73. doi:10.1016/j.tvjl.2015.04.032.
- 872 67. Vaughan A, Johnson JL, Williams LE. Impact of chemotherapeutic dose intensity and
873 hematologic toxicity on first remission duration in dogs with lymphoma treated with a
874 chemoradiotherapy protocol. *J Vet Intern Med.* 2007;21(6):1332-1339.
875 doi:10.1892/06-197.1.

- 876 68. Flory AB, Rassnick KM, Erb HN, et al. Evaluation of factors associated with second
877 remission in dogs with lymphoma undergoing retreatment with a cyclophosphamide,
878 doxorubicin, vincristine, and prednisone chemotherapy protocol: 95 cases (2000-
879 2007). *J Am Vet Med Assoc.* 2011;238(4):501-506. doi:10.2460/javma.238.4.501.
- 880 69. Alvarez FJ, Kisseberth WC, Gallant SL, Couto CG. Dexamethasone, Melphalan,
881 Actinomycin D, Cytosine Arabinoside (DMAC) Protocol for Dogs with Relapsed
882 Lymphoma. *J Vet Intern Med.* 2006;20:1178-1183.
- 883 70. Lucroy MD, Phillips BS, Kraegel S a, Simonson ER, Madewell BR. Evaluation of
884 single-agent mitoxantrone as chemotherapy for relapsing canine lymphoma. *J Vet*
885 *Intern Med.* 1998;12:325-329.
- 886 71. Moore AS, London CA, Wood CA, et al. Lomustine (CCNU) for the treatment of
887 resistant lymphoma in dogs. *J Vet Intern Med.* 1999;13(5):395-398. doi:10.1892/0891-
888 6640(1999)013<0395:LFTTOR>2.3.CO;2.
- 889 72. Saba CF, Hafeman SD, Vail DM, Thamm DH. Combination Chemotherapy with
890 Continuous L-Asparaginase, Lomustine, and Prednisolone for Relapsed Canine
891 Lymphoma. *J Vet Intern Med.* 2009;23:1058-1063.
- 892 73. Grivennikov SI, Greten FR, Karin M. Immunity, Inflammation, and Cancer. *Cell.*
893 2010;140(6):883-899. doi:10.1016/j.cell.2010.01.025.Immunity.
- 894 74. Skor O, Fuchs-Baumgartinger A, Tichy A, Kleiter M, Schwendenwein I. Pretreatment
895 leukocyte ratios and concentrations as predictors of outcome in dogs with cutaneous
896 mast cell tumours. *Vet Comp Oncol.* 2016:1-13. doi:10.1111/vco.12274.
- 897 75. Li Y, Pan Y, Jiao Y, Al. E. Peripheral blood lymphocyte/monocyte ratio predicts
898 outcome for patients with diffuse large B cell lymphoma after standard first-line
899 regimens. *Ann Hematol.* 2014;93:617.
- 900 76. Marconato L, Martini V, Stefanello D, et al. Peripheral blood lymphocyte/monocyte
901 ratio as a useful prognostic factor in dogs with diffuse large B-cell lymphoma
902 receiving chemoimmunotherapy. *Vet J.* 2015;206(2):226-230.
903 doi:10.1016/j.tvjl.2015.07.009.
- 904 77. Wilkerson MJ, Dolce K, Koopman T, et al. Lineage differentiation of canine
905 lymphoma/leukemias and aberrant expression of CD molecules. *Vet Immunol*
906 *Immunopathol.* 2005;106(3-4):179-196. doi:10.1016/j.vetimm.2005.02.020.
- 907 78. Frantz AM, Sarver AL, Ito D, et al. Molecular Profiling Reveals Prognostically
908 Significant Subtypes of Canine Lymphoma. *Vet Pathol.* 2013;50(4):1-11.
909 doi:10.1177/0300985812465325.

910 **Legends to Tables & Figures**

911

912 **Table 1: Pre-treatment absolute leukocyte concentrations and ratios from the study**
913 **population (n=25).** *The two ratios which were found to have a significant effect on outcome
914 have been marked in bold; NLR was independently predictive for PFS1 in ($P=0.025$), and OS
915 ($P=0.009$). LMR was independently predictive of OS ($P=0.031$).

916

917 **Table 2: Signalment and cellular markers of dogs with aberrant immunophenotypes.** The
918 aberrant markers for each dog are placed in bold. “+” = present, “-“ = absent. FN = female
919 neutered, ME = male entire, MN= male neutered. Statistical analysis of individual
920 aberrations was not performed due to small sample sizes.

921

922 **Table 3: Rescue protocols used after COP-type and CHOP induction protocols (n=42).**

923 *Bleomycin / mitoxantrone.

924 #Masitinib.

925

926 **Table 4 : Distribution of the total number of dogs receiving different numbers of doxorubicin**
927 **doses and the placement of doxorubicin therapy through induction and rescue treatment.**

928

929 **Table 5 : Significant differences between the total number of doxorubicin doses received and**

930 **OS.** Median OS values are given for each dose. Significant p values are marked in bold.

931 Hazard Ratios (HR) are given for categories where significant differences in OS were found.

932 *Only one dog received 7 doses of doxorubicin hence the OS value is actual rather than
933 median.

934

935 **Figure 1: Kaplan Meier curves for the first progression free survival times (PFS1) of dogs**

936 **with mDLBCL-CB (n=28); presence of anaemia at diagnosis was independently predictive of**

937 **the length of first remission on cox hazard regression.** Dogs who were anaemic at diagnosis

938 (dotted line) had a median PFS1 of 147 days (95% CI: 22-272 days) and an increased hazard
939 of remission ending (hazard ratio 5.6) compared with those that were not anaemic on
940 presentation that had a median PFS of 254 days (95% CI: 189-319 days), $P=0.002$.

941

942 **Figure 2: Kaplan Meier curves for the first progression free survival times (PFS1) of dogs**
943 **with mDLBCL-CB (n=42); induction protocol was independently predictive of the length of**
944 **first remission on cox hazard regression.** Dogs who were treated with the CHOP protocol
945 (solid line) had a median PFS1 of 251 days (95% CI: 215-293 days) and decreased hazard of
946 remission ending (hazard ratio 0.22) compared with a median PFS1 of 147 days (95%CI: 73-
947 221 days) for those that were treated with the COP-type protocol ($P=0.000$).

948

949 **Figure 3: Kaplan Meier curves for the overall survival times (OS) of dogs with mDLBCL-**
950 **CB (n=28); pre-treatment lymphocyte:monocyte Ratio (LMR) was independently predictive**
951 **of OS length on cox hazard regression ($P=0.031$).** Dogs who had a LMR above a cut-off of
952 1.43 had a median OS of 353 days (95% CI: 208-498 days), while those who had an LMR
953 below 1.43 had a median OS of 174 days (94-254), $P=0.01$; hazard ratio 0.315.

954

955 **Figure 4: Kaplan Meier curves for the overall survival times (OS) of dogs with mDLBCL-**
956 **CB (n=28); pre-treatment Neutrophil : Lymphocyte Ratio (NLR) was independently**
957 **predictive of OS length on cox hazard regression ($P=0.009$).** Dogs who had a NLR above a
958 cut-off of 11.44 had a median OS of 128 days (0-325), while those who had an NLR below
959 11.44 had a median OS of 322 days (95% CI: 241-403 days); $P=0.000$, hazard ratio 7.7.

960

961 **Figure 5: Kaplan Meier curves for the overall survival times (OS) of dogs with mDLBCL-**
962 **CB (n=28); combination of total treatment was independently predictive of OS length on Cox**
963 **hazard regression ($P=0.030$).** In comparison with dogs that received CHOP induction
964 followed by no rescue therapy, dogs who received a COP-type protocol with no rescue had an
965 increased hazard ratio for death of 4.2 ($P=0.024$), whereas dogs who received CHOP

966 induction followed by doxorubicin-based rescue therapy had a reduced hazard ratio for death
967 of 0.330 ($P=0.04$). No significant differences were seen between dogs that received COP-
968 type induction followed by doxorubicin-based rescue, dogs that received CHOP induction
969 with no rescue protocol and dogs that received CHOP induction followed by a non-
970 doxorubicin-based rescue protocol. The two dogs that received COP-type rescue after COP-
971 type induction have been admitted from this graph for clarity.

972
973 **Figure 6: Kaplan Meier curves for the overall survival times (OS) of dogs with mDLBCL-**
974 **CB (n=37); the number of doses of doxorubicin received throughout induction and all rescue**
975 **therapy was independently predictive of OS on cox hazard regression ($P=0.002$).** Compared
976 with 1-3 doses of doxorubicin, dogs who received 5-8 doses had a decreased hazard ratio of
977 death of 0.399 ($P=0.049$). There was no statistically significant difference between dogs that
978 received 4 doses and dogs that received 1-3 doses.

979

980 **Supplementary Table 1: The CHOP protocol.** The Madison Wisconsin 19-week CHOP
981 protocol consisted of 4 repetitions of the above cycle of cytotoxic drugs; a tapering course of
982 prednisolone is given in the first cycle, with discontinuation of the drug at the start by the start
983 of the second cycle. The Madison Wisconsin 25-week CHOP protocol involved a two-week
984 break between cytotoxic drug treatments in the third and fourth cycles. No maintenance
985 chemotherapy was given with this protocol. *mg/kg dosing was used for doxorubicin below a
986 bodyweight of 10kg.

987

988 **Supplementary Table 2: The COP-type protocol.** Prednisolone was given throughout
989 induction and maintenance phases of the protocol. *A single dose of cytarabine was given
990 subcutaneously on the first day of the treatment in some dogs.

991

992 **Supplementary Table 3: The DMAC Protocol.** This protocol consists of ongoing 2-week
993 cycles as described in the table.

994 **Supplementary Table 4: The single-agent protocols.**

995