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1 **Canine intracranial glial tumors treated with radiotherapy: is there an inferior outcome in tumors**
2 **contacting the subventricular zone?**

3

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15

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23 Abstract

24 Post-treatment outcome in canine glial tumors is described with a broad range of survival times between
25 2-28 months. After surgery or radiation therapy, the tumors may progress locally or spread within the
26 central nervous system. It is unknown if tumor- or patient-specific factors influence prognosis. In humans,
27 glioblastoma involving the subventricular zone have been found to recur distantly, with shortened time to
28 progression and overall survival.

29 We included 32 dogs irradiated for a presumptive primary glial brain tumor in this retrospective cohort
30 study. Tumors were grouped relative to subventricular zone contact and overt ventricular invasion
31 assessing pre-treatment magnetic resonance images.

32 Median time to progression (TTP) for all cases was 534 days (95%CI, 310-758), with a significantly
33 shorter TTP in dogs with lesions at the subventricular zone (median TTP, 260 vs. 687 days; $P = 0.049$).

34 Tumors at the subventricular zone progressed more often ($P = 0.001$), and more likely as CNS-metastasis
35 (52.9% vs. 13.3%, $P = 0.028$).

36 Median overall survival (OS) was 489 days (95%CI, 147-831) and median tumor-specific survival 609
37 days (95%CI, 382-835). Involvement of the subventricular zone was significantly associated with a
38 shorter tumor-specific survival (median, 306 vs. 719 days; $P = 0.044$).

39 Glial tumors contacting the subventricular zone in dogs have a shorter tumor-specific survival and a
40 higher rate of progression and CNS-metastasis. Despite of local tumor control, metastasis must be
41 considered and should prompt further treatment approaches.

42

43 **Key words:** brain tumor, dog, drop metastasis, glioma, neuroepithelial tumors, radiation therapy

44 Introduction

45 Treatments for intracranial tumors in dogs have received a constant trickle of attention in the last
46 decades, without massive improvement in outcome.¹⁻³ For glial tumors **treated with radiation therapy**,
47 outcome is described with a rather broad range of median overall survival times between 8-23 months.⁴⁻⁶
48 **Median survival times after surgery range from 2-6 months^{7,8}, with more promising outcomes of 7-28**
49 **months⁹ when experimental unspecified therapy (chemo-, immuno-, gene-therapy) was added.** While
50 radiation therapy and surgery remain the main pillars of brain tumor treatment in dogs, the inherent
51 difficulties remain as well: Tumors are often not amenable to appropriately wide surgical excision² and
52 the needed "extent" of treatment with radiation therapy is unknown. If the extent of treatment, the so-
53 called treatment margins are too small, tumors will recur or continue their growth at this site after both
54 types of therapy. In contrary to surgery, wide margins to include zones of invasion are easily attained in
55 radiation treatment planning. But if the margins are too large, the delivered radiation dose could also lead
56 to debilitating toxicities.

57 Furthermore, it is unknown if tumor- or patient-specific factors consistently influence prognosis.
58 Non only are re-treatment biopsies rarely obtained, but also their interpretation can be difficult, and
59 prognostic information is not well established.^{10,11} In treated cases, standardized follow-up is often
60 lacking, with times and pattern of tumor progression or relapse remaining unknown in these dogs.

61 One single study mentioned site of progression in irradiated glial tumors: of the 11 dogs that
62 eventually succumbed to tumor-related death, 7/11 (64%) had local recurrence or progression and in 4/11
63 (36%) CNS-metastasis was suspected based on diagnostic imaging.⁵ In humans, the anatomic relationship
64 of glial tumors to certain brain regions predicts the pattern of progression and outcome. Lim et al., (2007)
65 determined such a relation of glioblastoma multiforme to the subventricular zone, an area under the
66 ependyma of the lateral ventricles (along the lateral aspects) (Figure 1) that contains the largest
67 population of neural stem cells. Their classification system describes the contact of the contrast-enhancing
68 lesion with this zone and in part predicts tumor recurrence pattern.¹² This system has since sparked the
69 interest of radiation oncologists and has even raised the question of including this subventricular zone into

70 the radiation target, such as the clinical target volume (e.g. the margin around the gross disease). This
71 matter, however, is still under investigation.^{13,14} While it is well known that ventricular contact is a
72 common finding in canine gliomas^{15,16} and this stem-cell rich subventricular zone is comparable in dogs¹⁷,
73 a relationship between tumor location at any ventricle, or specifically at the subventricular zone and
74 prognosis or outcome has not been investigated.

75 Classifying human glioma patients along this standardized spatial classification system revealed
76 differences in outcome for tumors **contacting** the subventricular zone.¹² Hence, we were intrigued to have
77 a second look at our dog glioma data in retrospect. We wanted to investigate whether a simple, imaging-
78 based, non-invasive spatial anatomic feature could anticipate a similar pattern of failure (e.g., type of
79 progression or relapse) or even be linked to an outcome variable such as overall survival in dogs
80 undergoing radiation therapy.

81 We hypothesized that a clinically detectable difference of outcome for time to progression (TTP), overall
82 survival (OS) and tumor-specific survival or both would occur between the presumed glial tumors
83 contacting the subventricular zone compared to the non-contacting tumors.

84

85 **Materials and Methods**

86 *Patient selection*

87 For this retrospective cohort study, we reviewed medical records of patients with an imaging diagnosis
88 suspicious of a primary glial brain tumor, treated with radiation therapy at XXX between 2015 and 2020.

89 For study inclusion all patients needed to have an MRI performed either at our or at the individual
90 referring institution. As a minimum dataset **of MRI-images**, post-contrast T1-weighted images (T1W),
91 T2-weighted images (T2W) and T2-weighted fluid attenuated inversion recovery images (T2-FLAIR) in
92 at least one plane each were required. Additional sequences such as diffusion-weighted imaging (DWI) or
93 sequences sensitive for hemorrhage such as susceptibility-weighted imaging (SWI) or T2*-weighted
94 images were desired, but not required for inclusion.¹⁸ As a minimum standard in this retrospective study,
95 however, the imaging study must have been interpreted by a board-certified veterinary radiologist, who

96 assigned at least the superclass “glial”¹⁹ to the suspected tumor diagnosis. Primary tumors of glial origins
97 are usually suspected in a contrast-enhancing and non-contrast-enhancing single intra-axial lesion with
98 mass effect. In cases of hemorrhagic and/or cystic lesions, an additional solitary part is usually present.
99 Usually there are no further signs of inflammation in MRI and CSF.¹⁵

100 This diagnosis was then used for treatment decision, as common practice in our Animal Hospital. Further,
101 the patient must have been followed-up for a minimum of three months after imaging, preferably until
102 death of any cause.

103

104 *Tumor topography and grouping*

105 Anatomical location relative to the subventricular zone as well as size (gross tumor volume, GTV) of
106 each tumor and contrast enhancement were recorded. **The dog's subventricular zone lines the ventricular**
107 **wall of the lateral ventricles and is laterally limited by the caudate nucleus, medially by the**
108 **interventricular septum and dorsally by the corpus callosum (Figure 1).**¹⁷ Dogs were grouped to have their
109 lesion either contacting the subventricular zone (GroupSVZ+) interpreted as tumor possibly infiltrating
110 the subventricular zone¹⁷) or not (GroupSVZ-). **As an additional factor, presence or absence of overt**
111 **ventricular invasion on the MRI images was reported.** All MR images were performed blinded by the
112 authors, and cases of disagreement were discussed among the authors until consensus was reached. All
113 images were reviewed on Horos Version 3.3.6.

114

115 *Treatment*

116 Dogs were treated based on owner's choice with either 20x2.5 Gy, 10x4 Gy or a 10x4 Gy protocol with a
117 15% boost to the GTV. The former two protocols had been found to yield non-different outcomes, even if
118 the lower biologically effective dose (BED) in the 10x4 Gy-fraction protocol implies a lower efficacy and
119 tumor control probability.^{6,20} The third protocol is currently offered to owners with the intent of
120 increasing the total dose, thereby improving outcome (ongoing project).

121 Target volumes and organs at risk (OAR), **such as whole brain**, were contoured in a facility internal
122 standardized manner as previously published, **allowing for volumetric measurements**.²⁰ In brief, the gross
123 tumor volume (GTV) was delineated using co-registered contrast-enhanced CT images or CT and MRI
124 images, in tumors with no contrast uptake T2 sequences were used for delineation of GTV. Clinical target
125 volume (CTV), accounting for subclinical microscopic disease extension of 4-8 mm was defined. The
126 CTV-margin was then extended three-dimensionally by 2 mm to define the planning target volume
127 (PTV), accounting for setup uncertainties in daily image-guided photon treatment. OAR were segmented
128 as described previously by our research team.^{xx} The recommendations for specifying dose and volumes
129 were adhered to as in the ICRU reports 50 and 62 for 3DCRT and ICRU report 83 for IMRT plans.²¹⁻²⁴ All
130 dogs were treated with a Varian Clinac iX 6MV linear accelerator (Varian Medical Systems, Palo Alto,
131 USA) with a four degree-of-freedom couch, a 120 leaf multi-leaf-collimator and the treatment planning
132 system ECLIPSE (version 10.0.28 or 15.1.25, Varian Oncology Systems, Palo Alto, USA) with AAA-
133 algorithm. Treatment was delivered with photons as 3-dimensional conformal radiation therapy (3DCRT)
134 or intensity-modulated radiation therapy (IMRT).
135 Daily positioning verification with kilovolt (kV) orthogonal radiographs and occasional kV-cone-beam
136 CTs were performed with the on-board imaging system (Varian On-Board Imager, Varian, Palo Alto,
137 USA) and matched by an experienced radiation therapist. Quality assurance of on-board imager and linear
138 accelerator was performed as required by institutional and federal guidelines.

139 Treatment was delivered with definitive-intent, on a daily Monday to Friday schedule, over 2 to 4
140 weeks, depending on the protocol.

141 Additional medical treatment before and after radiotherapy was not standardized but adapted to the
142 individual needs of the dogs. Medication was usually started at the day of diagnosis or beginning of
143 neurological signs and adapted according to the improvement of clinical signs and consisted mostly of
144 antiepileptic drugs and corticosteroids.

145

146 *Follow-up*

147 Results of follow-up examinations were retrieved from the medical files. At our institution we
148 recommend clinical re-checks 3 weeks and 3 months after the end of radiation therapy, and at three-
149 monthly intervals thereafter. In cases where clinical examination is not feasible, we usually contact the
150 owners by phone. Further, we recommend follow-up MRI 6 and 12 months after radiation therapy for
151 patients in clinically stable or improved condition and at any time in the case of neurologic deterioration.

152

153 *Statistical analysis*

154 Descriptive statistics were used in the analysis of dogs and tumor characteristics. When appropriate, data
155 were tested for normality by use of the Shapiro-Wilk normality test. Values were expressed as mean \pm SD
156 in case of normal distribution, or as median with a range in case of non-normal distribution. Follow-up
157 time was defined as the time from the first radiation treatment until death, loss to follow-up or time of
158 data analysis. The time to progression (TTP) was defined as the interval between start of RT and
159 discovery of new or progressive neurological signs or evidence of disease progression based on MRI.²⁵
160 Dogs dying without **clinical or image-based** evidence of disease progression were censored for TTP
161 analysis. The overall survival (OS) was defined as the interval between first RT until death of any cause;
162 for the disease-specific survival dogs that died of other causes were censored at the time of death. Dogs
163 still alive at the time of data evaluation or lost to follow-up were censored. Time to progression, overall
164 and disease-specific survival were coded and analyzed with Kaplan-Meier product-limit estimator,
165 accompanied by the log-rank or Breslow-Gehan-Wilcoxon tests. Survival estimates and median survival
166 time are reported with the corresponding 95% confidence intervals (95%CI). Cox's regression analysis
167 was used to determine whether co-variates showed an influence on TTP, OS and disease-specific survival.
168 The following variables were investigated for prognostic significance: sex, age (median used as cut-off),
169 weight (median used as cut-off), head conformance (brachycephalic or dolichocephalic), RT protocol
170 (10x4 Gy vs. 10x4Gy with 15% boost and 20x2.5 Gy), **contact to SVZ, ventricular invasion**, GTV
171 (median used as cut-off), brain volume (median used as cut-off) and GTV/brain volume ratio (median
172 used as cut-off).

173 The relationship between SVZ contact and **metastatic rate and death prevalence** was investigated by
174 means of Mann-Whitey *U* test or Chi-square test.
175 Data were analyzed with SPSS (IBM® SPSS® Statistics, Version 26, IBM Corp., Armonk, New York).
176 Results of statistical analyses with p-value <0.05 were considered statistically significant.

177

178 **Results**

179 *Patient and tumor characteristics*

180 Thirty-two dogs were included into this retrospective analysis and are presented in Table 1. All but one
181 patient had pre-contrast, all patients had post-contrast T1W images in at least one plane (29/32 had the
182 post-contrast T1W images in more than one plane). All patients had T2W images in at least two planes
183 (14/32 transversal + sagittal, 18/32 in all three planes) and T2W-FLAIR images in at least one plane
184 (29/32 in transversal or dorsal plane, 3 in different combinations of two orthogonal planes). DWI- and
185 SWI- or T2*-sequences were available in 15/32 patients. The majority of tumors (30/32) were localized in
186 the forebrain, with one each in brainstem and cerebellum. The majority of the tumors (25/32, 78.1%) were
187 in contact to the ventricular system, with 17/32 (53.1%) at the subventricular zone. **Eight tumors had**
188 **ventricular contact, but not in the area of the SVZ. In 6/32 (18.8%) ventricular invasion was suspected**
189 **based on the MRI images.** Overall, 22/32 (68.8%) of dogs had contrast-enhancing lesions, 15 (46.9%)
190 being in contact with the SVZ. In 10/32 (31.3%) dogs no contrast was detected within the lesion and 2
191 lesions (6.3%) were in contact with the SVZ.

192 Median tumor size GTV was 4.0 cm³ (range, 0.2-16.1 cm³). Tumors in GroupSVZ+ were significantly
193 larger (median, 6.1 cm³, range, 0.5-16.1) than tumors in GroupSVZ- (median, 3.0 cm³; range, 0.2-9.2
194 cm³; *P* = 0.008). Median brain volume was 90.4 cm³ (range, 46.9-120.9 cm³) corresponding to a median
195 GTV/BV-ratio of 5.4% (range, 0.2-14.1%). A significant difference between the two groups was noted in
196 GTV/BV ratio (6.6% vs. 3.4%; *P* = 0.006), but not in brain volume (Table 1).

197

198 *Treatment*

199 All dogs were irradiated with curative intent. Seventeen (53.1%) were treated with 10x4 Gy (8
200 GroupSVZ+, 9 GroupSVZ-), 8 with 10x4Gy and a 15% simultaneously integrated boost to the GTV 10x4
201 Gy (3 GroupSVZ+, 5 GroupSVZ-) and 7 were irradiated with 20x2.5 Gy (6 GroupSVZ+, 1 GroupSVZ-).
202 Applying an EQD2 calculation with an alpha/beta value of 10 for protocol comparison, the 10x4 Gy
203 protocol equals 46.7 Gy_{EQD2} (biologically effective dose, BED 56.0 Gy₁₀), the 10x4Gy with 15% boost
204 dose protocol equals 53.6 Gy_{EQD2} (biologically effective dose, BED 64.3 Gy₁₀) and the 20x2.5 Gy
205 protocol equals 52.8 Gy_{EQD2} (biologically effective dose, BED 62.5 Gy₁₀), rendering the two latter the
206 more effective protocols from a calculation point of view. The 17 tumors contacting the SVZ were
207 equally distributed between the BED levels, with 8/17 tumors in the lower BED group (10x4Gy)
208 contacting the SVZ, and 9/17 tumors in the higher BED group (10x4Gy +15% boost and the 20x2.5Gy)
209 contacting the SVZ.

210 Thirteen of 32 dogs (40.6%) were treated with a conformal photon plan (3DCRT), and 19/32 (59.4%)
211 with an intensity-modulated radiation therapy plan (IMRT). **At the start of radiation therapy, all but one**
212 **patients received corticosteroids at a median dosage of 0.73 mg/kg sid (range 0-2.0 mg/kg) and all but**
213 **two patients were treated at a median dosage of 0.49 mg/kg sid (range 0-1.4mg/kg) at the end of radiation**
214 **therapy.** All 26 dogs with reported seizures received antiepileptic medical treatment. At the end of
215 radiation therapy, a total of 21 dogs received phenobarbital at a median dosage of 2.6mg/kg bid (range
216 1.1-3.8 mg/kg) and a total of 14 dogs received levetiracetam at a median dosage of 21.0 mg/kg tid (range
217 9.1-35.6 mg/kg). One dog received potassium bromide (8.9 mg/kg bid). As a monotherapy, 12 dogs
218 received phenobarbital at a median dosage of 2.8 mg/kg bid (range 1.6-3.8 mg/kg) and 5 dogs received
219 levetiracetam at a median dosage of 23.8 mg/kg tid (range 19.2-35.6 mg/kg), respectively. More than one
220 antiepileptic drug (combinations of phenobarbital, levetiracetam and potassium bromide) was prescribed
221 to 9 patients. In general, phenobarbital dose was titrated based on blood-levels to the upper recommended
222 range (25-30 mg/L)²⁶ and levetiracetam was added if seizure control was insufficient, or if dogs did not
223 tolerate phenobarbital.

224

225 *Follow-up*

226 Eighteen patients (56.3%) had at least one follow-up MRI after radiation therapy. With a total number of
227 30 MRIs performed, 9 were performed in patients that showed neurologic deterioration and 21 in dogs
228 presented for a regular re-check without any suspicion of neurologic deterioration. Six patients had the
229 MRI three months after radiation therapy (4/6 as routine re-check, 2/6 with neurologic deterioration), 13
230 at six months (11/13 as a routine re-check, 2/13 with neurologic deterioration) 4 at 12 months (all as a
231 routine re-check), two at 18 months (one as a routine re-check, one with neurologic worsening) and one
232 24 months (routine re-check) after radiation therapy. In four patients an MRI was repeated 1-3 months
233 after a routine MRI re-check without any signs of tumor progression or metastases, because of sudden
234 onset of signs of neurologic worsening/suspected tumor progression.

235

236 *Outcome*

237 The median follow-up time was 341 days (range, 102-861 days). For a subset of patients (10/32),
238 outcome was already published in an earlier study.^{xx} At the time of writing, 8/32 dogs (25%) were still
239 alive.

240 Because of deterioration of neurological signs or evidence of disease progression based on MRI
241 in 22/32 (68.8%) dogs during the follow-up period, tumor progression was suspected (8/32) or confirmed
242 (14/32) (Table 1). Hence, in 8 dogs (25.0%) tumor progression was clinically suspected but not imaging-
243 confirmed, but not imaging-confirmed. In 3 dogs (9.4%), only local progression was found. In 11 dogs
244 (34.4%) CNS-metastasis was part of the progressive pattern, with 8 patients (25%) exhibiting CNS-
245 metastasis despite of local tumor control. In 4/11 (36%) of dogs with CNS-metastasis, lesions were
246 limited to the spinal cord, and in 6/11 (54%), lesions were found to affect both, spinal cord and brain. In
247 6/11 (54%) cases, metastases were found within the ventricular system and 2 of these 6 were in contact
248 with the subventricular zone (in only two of these 6 patients, the intraventricular lesion was a single
249 lesion, in the other 4 dogs multifocal subarachnoid or diffuse meningeal lesions were present. In one of
250 these cases, an additional intra-axial lesion was present). Diffuse meningeal infiltration at the level of the

251 brain stem and cranial spinal cord was found in 1/11 (9%) of dogs with metastasis. Overall, 9 dogs
252 developed neurological signs again consistent with the clinical signs or neuroanatomical localization at
253 initial presentation. Clinical signs included behavioral changes and pacing (2), uncontrolled seizures (5)
254 or status epilepticus (2) (after prior medical seizure control). In the 9 dogs with neurological deterioration
255 consistent with the initial neuroanatomical localization, CNS-metastasis were confirmed by MRI in 3
256 cases (one with additional histopathology) and progression of the initial tumor by MRI or postmortem in
257 2 cases. Other 9 dogs were presented with additional neurological deficits consistent with an additional
258 neuroanatomical localization. Of these 9 dogs 4 were presented with spinal cord localization (C1-C5 (2),
259 Th3-L3 (2)), 3 with multifocal neurological signs, 1 with forebrain signs and 1 with central vestibular
260 signs. In 3 dogs, progression was reported by the owner or referring vets, but detailed neurological
261 examinations were not available. In all but 1 dog with additional neurological deficits MRI confirmed
262 CNS-metastasis.

263 Of the 22 dogs (68.8%) with progressive disease, 16 dogs were in the GroupSVZ+ (94.1%, thereof) and 6
264 dogs in the GroupSVZ- (37.5%) ($P = 0.001$). Also, dogs with the GroupSVZ+ were more likely to
265 succumb to CNS-metastasis (52.9% vs 13.3%, $P = 0.028$).

266 The median TTP for all cases was 534 days (95%CI, 310-758). TTP was significantly shorter in
267 dogs with lesions at the SVZ compared with GroupSVZ- (median TTP, 260 vs. 687 days; $P = 0.049$)
268 (Figure 2). Also was median TTP significantly shorter for the dogs with clear ventricular invasion
269 (median TTP, 203 vs. 609 days; $P = 0.013$)

270 At the time of analysis 24/32 (75.0%) dogs were dead. Of these, 21/24 (87.5%) died of tumor-specific
271 causes, including 15 in GroupSVZ+ (88.2%) and 6 in GroupSVZ- (40%) ($P = 0.008$). Of the 8 patients
272 still alive, 7 (87.5%) had tumors not in contact to the SVZ ($P = 0.013$). Median OS was 489 days (95%CI,
273 147-831). Median tumor-specific survival was 609 days (95%CI, 382-835). Involvement of the
274 subventricular zone was significantly associated with a shorter tumor-specific survival (median, 306 vs.
275 719 days; $P = 0.044$) (Figure 3) and influenced OS (median, 226 vs. 609 days; $P = 0.066$). OS was

276 significantly shorter in dogs with clear ventricular invasion (171 vs. 536 days; $P = 0.010$) and so was
277 tumor-specific survival (224 vs. 609 days; $P = 0.037$).

278 The variables significantly associated with an increased risk of tumor progression included a
279 larger GTV (HR = 2.8; 95% CI = 1.1-7.3; $P = 0.037$) and ventricular invasion (HR = 3.6; 95% CI = 1.2-
280 10.5; $P = 0.019$), whereas a tendency was observed for SVZ contact (HR = 2.5; 95% CI = 0.9-6.6; $P =$
281 0.057). The only variable significantly associated with an increased risk of disease-specific death was
282 ventricular invasion (HR = 3.1; 95% CI = 1.1-8.8; $P = 0.038$) and a tendency was observed for SVZ
283 contact (HR = 2.3; 95% CI = 0.9-6.1; $P = 0.081$).

284 Ventricular invasion was the only variable significantly associated with an increased risk of death for any
285 cause (HR = 2.3; 95% CI = 0.9-6.1; $P = 0.081$).

286

287 Discussion

288 In humans, glial tumors involving the subventricular zone have been found to recur at locations
289 distant from the initial lesion, with shortened time to progression and overall survival. Hence, it was
290 suggested that tumors involving this zone are more invasive and migratory than tumors originating from
291 other regions.^{12,14} Here, we investigated the outcomes of a series of 32 dogs based on this described
292 spatial classification system. In 78.1%, the majority of our convenience sample, the tumors were in
293 contact with the ventricular system, with 53.1% at the subventricular zone. Ventricular contact of canine
294 glioma is common, described in up to 90% of dogs¹⁶ and tumors frequently involve the subventricular
295 zone. Disruption of the ependyma of the wall of the lateral ventricles has sporadically been associated
296 with cerebrospinal fluid (CSF) drop metastasis²⁷, but systematic investigations of progression are lacking.
297 In our case sample, dogs with tumors located at the subventricular zone had a tendency for earlier
298 progression and a significantly shorter tumor-specific survival. Comparable to 36% in the prior small
299 sample⁵, 11/32 (34.4%) of all tumors progressed distantly as CNS-metastases. Furthermore, the 6 dogs
300 with obvious ventricular invasion, had a shorter TTP and OS. Five of these 6 tumors progressed into
301 CNS-metastasis. In many of the cases with known site of progression, however, radiation therapy

302 provided a long-term local control with primary tumors in remission or stable after radiation therapy and
303 only in three patients simultaneous local *and* distant progression was found.

304 In humans, one of the main reasons for glial cell tumors to recur is the high migratory capacity of
305 a small subpopulation of tumor cells, the so-called brain tumor stem cells.^{28,29} These brain tumor stem
306 cells are more resistant to treatment and share common features with neuronal stem cells, indicating
307 potential transformation of neurogenic stem cells into brain tumor stem cells: both possess self-renewal
308 and multipotential capabilities and are highly migratory. Gliomas can be induced from subventricular
309 zone cells in animal models, supporting this theory.²⁹ In the adult brain, neuronal stem cells reside within
310 specialized neurogenic niches. The largest of these niches in mammals is the subventricular zone. This
311 subventricular zone is therefore not only a source of the neuronal stem cells, but potentially also of brain
312 tumor stem cells and its microenvironment supports tumorigenesis.³⁰ Neoplastic glial tumors, however,
313 can not only arise from stem cell regions, but also be initiated by neoplastic transformation of non-
314 subventricular progenitor cells or mature glial cells that have undergone dedifferentiation.²⁹ And while
315 many malignant brain tumors arise distant to the subventricular zone, findings support the more invasive
316 nature of tumors arising from this zone, with such tumors occurring multifocally at initial diagnosis and
317 recurring distantly from the initial tumor.¹² A second pattern of recurrence beside the parenchymal
318 infiltration described above, is dissemination via CSF. This type of recurrence has not been investigated
319 as deeply as the parenchymal migration. Localization near the CSF pathways seems to be more important
320 than histological grade in CSF spread of human gliomas.^{31,32}

321 Glial tumors in dogs commonly recur or progress after surgical or radiation treatment.^{2,5,6,9} When
322 considering the published literature, the site of progression was only mentioned for a few dogs and was
323 either local, distant but within the CNS (intraventricular or spinal spreading, "drop metastasis"), or
324 both.^{5,6,27} In our cohort, CNS-metastasis based on MRI findings were diagnosed in 10 cases. Of these 10
325 cases, only 3 dogs experienced additional local progression. MR features are commonly the only access to
326 information on canine intracranial tumors treated with radiation therapy, as pretreatment histopathologic
327 evaluation is uncommon.^{4-6,33-35} MRI is the preferred modality for the evaluation of intracranial disease

328 with a 70% accuracy of predicting the type of primary brain tumors.³⁶⁻³⁸ While MR features provide a
329 multitude of valuable information, no features allow to reliably differentiate tumors on a cellular level,
330 such as astrocytoma from oligodendroglioma or provide a tumor grade.^{15,16} No prognostic factors on MRI
331 have been consistently identified in canine glioma patients.^{5,6} One group, however, recently described
332 cystic tumors of presumed glial origin to have shorter OS.⁴ We found an increased risk for tumor
333 progression in larger tumors. This was in line with findings in dogs with glioma treated with radiation
334 therapy, where the risk of death was increasing with relatively larger tumors.⁴

335 The correlation described in Lim et al. that tumor location relative to the SVZ predicts the pattern
336 of progression and outcome focused on glioblastoma multiforme, while in our study histopathology is
337 lacking and only imaging was available to characterize the brain tumors. The tumors described herein
338 most likely represent a heterogenous glioma population. Possibly, tumors contacting the subventricular
339 zone could represent less migratory tumor types in dogs, and thus glial tumors of lower aggressive
340 histotypes. Three quarters (76-80%) of glial tumor in dogs with survival data, however, were described of
341 grade III or IV (high grade).^{5,9} Median survival time after surgery is not well described and found to be
342 low with 2-6 months^{7,8}, but one group found surgery and additional unspecified adjuvant immunotherapy
343 to result in 6.7 months for grade IV and 10 months for grade III tumors. The most common cause of death
344 was tumor recurrence (50/86, 58.1%), without specification of the site.⁹ This survival time is comparable
345 to the 7.5 months we described after radiation therapy earlier, where in 5/12 (41.6%) glial tumors local
346 recurrence or progression was observed, whereof 4/12 (33.3%) had suspected CNS-metastasis
347 (unpublished result).⁶

348 With 26-40% of glial tumors recurring or progressing after therapy^{5,6,9}, and – at least after
349 radiation therapy – one third with CNS-metastasis^{5,6}, it would be clinically relevant to better identify this
350 subpopulation of patients in the future. Canine glioma is often marketed as a model for the human disease
351 counterpart.^{9,11,39,40} With this in mind, backward extrapolations from human glioblastoma therapy to dogs
352 could be made as well: glioblastoma treatment usually includes maximal safe resection, followed by
353 radiation therapy and temozolomide chemotherapy (reviewed in Nam and de Groot, 2017).⁴¹ Several

354 radiation oncologists have now even started exploring the subventricular zone as a potential target for
355 therapeutic intervention with radiotherapy in humans. While some found improved outcome when
356 including the ipsilateral subventricular zone into the high-dose area, others have not seen any associations
357 and currently findings remain inconclusive.¹⁴

358 Apart from the lack of consensus on how to operate or irradiate dogs with glioma, adjuvant
359 chemotherapy has only been very sporadically used. No survival advantage could be found with
360 lomustine at maximally tolerated doses of 90 mg/m² every four weeks⁴² or with subtherapeutic doses of
361 either lomustine (mean 60.6 mg/m², every 3 to 6 weeks) or temozolomide (65 mg/m² daily in a five-day
362 cycle).^{5,43} The maximally tolerated dose for temozolomide for dogs is now established as 150 mg/m² daily
363 in a five-day cycle and should serve as a future reference dose.⁴⁴ Whether the addition of temozolomide is
364 useful for dogs with glioma remains to be explored in parallel with tumor-specific markers from biopsies.
365 For example, the methylation status of the repair protein O⁶-alkylguanine DNA alkyl-transferase, encoded
366 by the O-6-methylguanine-DNA methyltransferase (MGMT) gene is of highest relevance, when treating
367 with temozolomide.⁴⁵⁻⁴⁷ In glioblastoma patients with methylated (epigenetically silenced) MGMT
368 promoter and therefore incomplete DNA-repair capacity, chemoradiation treatment is more damaging and
369 leads to a survival advantage.⁴⁸⁻⁵⁰ In dogs, the possible importance of the MGMT methylation has neither
370 been investigated on an *in vitro* nor *in vivo* level up to date, presenting a future outlook for research.

371 We acknowledge limitations to this study: 1) All patients were treated based on an imaging
372 diagnosis only, hence there is a lack of histopathological information such as type or grade. Yet, we deem
373 our findings of relevance as many of our pet owners do not consider a biopsy due to associated risks
374 and/or costs. 2) **In two dogs of our sample, the tumors were not located in the forebrain, but in the**
375 **cerebellum and in the brainstem area, respectively. Tumor location in dog's glioma has not been described**
376 **to be of prognostic impact, and hence we decided to include these patients, even they were very unlikely**
377 **to invade the SVZ for anatomical reasons.** 3) The retrospective nature of the study precludes standardized
378 imaging protocols at initial diagnosis and standardized follow-up (imaging and postmortem examination).
379 Including advanced imaging in the initial work up (such as diffusion and perfusion sequences),

380 performing standardized imaging follow-up and availability of postmortem examinations could add
 381 essential information about the involvement of the subventricular zone in progression of canine glioma. 4)
 382 the three factors, "tumors located at the subventricular zone", "ventricular contact" and "ventricular
 383 invasion", partially overlap. Furthermore, based on the available imaging quality and to the general
 384 resolution of MR-images, ventricular invasion can't be excluded in many cases with ventricular contact.
 385 We believe it is important to further investigate possible prognostic factors, as a future approach to canine
 386 glioma treatment will include a shift in treatment or the addition of adjuvant therapies.

387

388 **Conclusion**

389 Treatment of glial tumors in dogs can be recommended irrespective of tumor location, with the caveat of
 390 a possible shorter time-to-progression, tumor-specific survival and a higher rate of CNS-metastasis in
 391 tumors contacting the subventricular zone and in tumors with overt ventricular invasion. After radiation
 392 therapy, the pattern of failure seems to frequently involve CNS-metastasis. CNS-metastasis despite local
 393 tumor control should be addressed in future treatment approaches.

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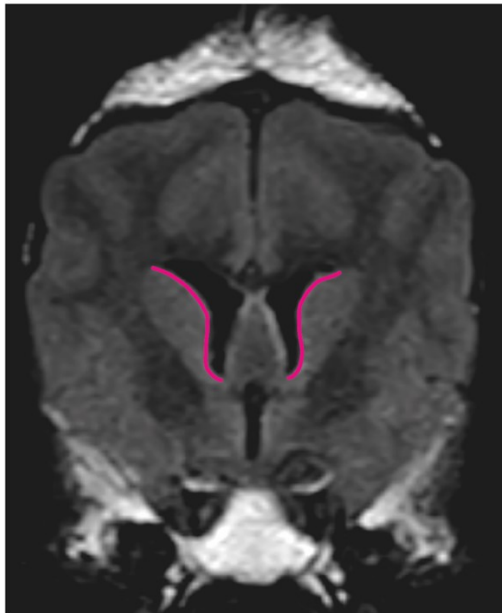
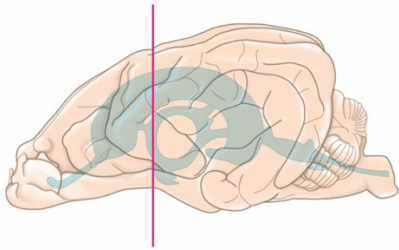
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522

523 **Figure Legends**

A

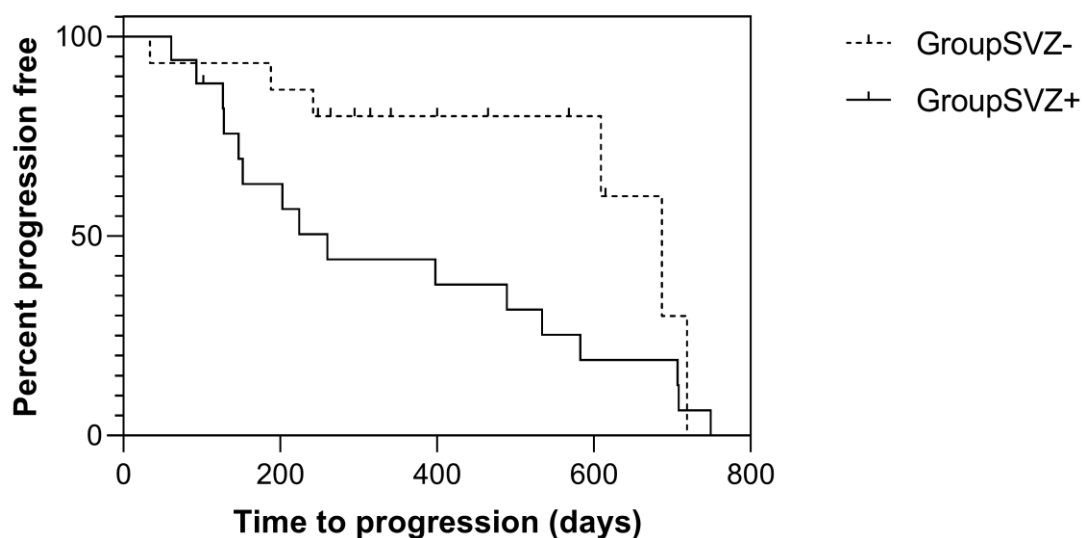


524

525 **Figure 1:** Transverse MR image of the brain. The transverse pink line indicates the rostrocaudal location.

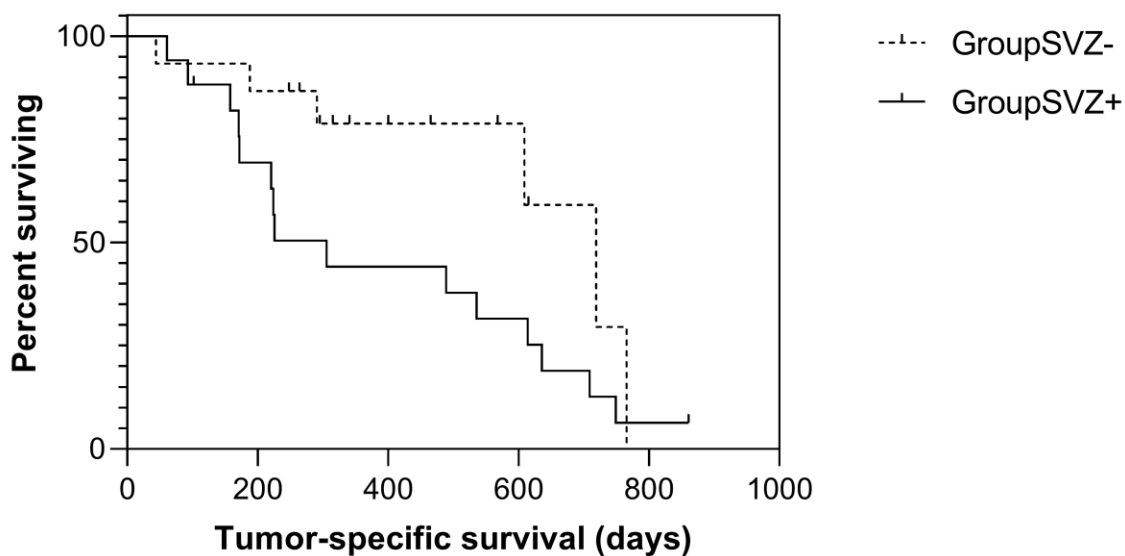
526 A, The pink curve between the nucleus caudatus and lateral aspect of the lateral ventricle on this FLAIR

527 image illustrates the subventricular zone.¹⁷



528

529 **Figure 2:** Kaplan-Meier plot showing time to progression for the 32 dogs with glial tumors after
 530 radiotherapy: Median TTP for all cases was 534 days (95%CI, 310-758). TTP was significantly shorter in
 531 dogs with lesions at the SVZ compared with GroupSVZ- (median TTP, 260 vs. 687 days; $P = 0.049$).



532

533 **Figure 3:** Kaplan-Meier plot showing tumor-specific survival for the 32 dogs with glial tumors after
 534 radiotherapy: Median tumor-specific survival was 609 days (95%CI, 382-835). Involvement of the

535 subventricular zone was significantly associated with a shorter tumor-specific survival (median, 306 vs.
536 719 days; $P = 0.044$).

537 **Table 1.** Distribution of patient and tumor characteristics in 32 dogs with intracranial glial tumors.

538 Tumors were either considered as a whole or stratified according to whether they contacted the

539 subventricular zone (SVZ) or not.

	Total (n=32)	GroupSVZ+ [lesion contacting subventricular zone] (n=17)	GroupSVZ- [lesion NOT contacting subventricular zone] (n=15)	P
Age (years)**	7.6 (1.5-13.5)	7 (1.5-10.8)	8.2 (5.8-13.5)	0.007
Weight (kg)**	15.6 (3.4-39.5)	26 (3.4-38)	14.2 (7.9-39.5)	0.417
Sex				0.526
female, intact	4 (12.5%)	2 (11.8%)	2 (13.3%)	
female, spayed	11 (34.4%)	7 (41.2%)	4 (26.7%)	
male, intact	10 (31.3%)	6 (35.2%)	4 (26.7%)	
male, castrated	7 (21.9%)	2 (11.8%)	5 (33.3%)	
Head conformance				0.678
brachycephalic	25 (78.1%)	14 (82.4%)	11 (73.3%)	
dolichocephalic	7 (21.9%)	3 (17.6%)	4 (26.7%)	
GTV (cm³)**	4 (0.2-16.1)	6.1 (0.5-16.1)	3 (0.2-9.2)	0.008*
Brain volume (cm³)**	90.4 (46.0-120.9)	95.9 (46.9-119.5)	86.6 (56.4-120.9)	0.115
GTV/brain volume (%)**	5.4 (0.2-14.1)	6.6 (0.8-14.1)	3.4 (0.2-10.2)	0.006*
Progression (any)				0.001*
yes	22 (68.8%)	16 (94.1%)	6 (40%)	
no	11 (34.4%)	1 (5.9%)	9 (60%)	
CNS metastasis				0.028*
yes	11 (34.4%)	9 (52.9%)	2 (13.3%)	
no	21 (65.6%)	8 (47.1%)	13 (86.7%)	
Dead, any cause				0.013*
yes	24 (75.0%)	16 (94.1%)	8 (53.3%)	
no	8 (25.0%)	1 (5.9%)	7 (46.7%)	
Dead, tumor-related				0.035*
yes	21 (65.6%)	15 (88.2%)	6 (40.0%)	
no	11 (34.3%)	2 (11.8%)	9 (60.0%)	
Median tumor progression (95% CI) (days)	534 (310-758)	260 (150-370)	687 (569-805)	0.049*
Median overall survival (95% CI) (days)	489 (147-831)	226 (112-340)	609 (274-944)	0.066
Median tumor-specific survival (95% CI) (days)	609 (382-835)	306 (148-464)	719 (552-886)	0.044*

540 * = significant; ** = values expressed as median and range; GTV = gross tumor volume;
541 CNS = central nervous system.