

Long-term management of canine disseminated granulomatous meningoencephalitis with imatinib mesylate: a case report

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Abstract: A seven-year-old Toy Poodle was presented for progressive ataxia and seizure episodes. Magnetic resonance imaging revealed inflammatory lesions in the cerebrum and brainstem. Management with imatinib mesylate, prednisolone and hydroxyurea were initiated and resulted in complete resolution of the clinical signs. In regular magnetic resonance imaging scans, the overall appearance of the lesions deteriorated but improved again after an increase in the imatinib mesylate dose. The patient had not shown any neurological signs until death and survived for 1052 days after initial presentation. On histopathological examination, the patient was diagnosed with disseminated granulomatous meningoencephalitis involving the cerebrum and brainstem. Immunohistochemical staining was performed on the five types of tyrosine kinase (PDGFR- α , PDGFR- β , VEGFR-2, c-Kit and c-Abl proteins), which constitute therapeutic targets for conventional multitargeted tyrosine kinase inhibitors. The immunohistochemical analysis revealed that all these tyrosine kinases were expressed in the brain samples. The present report describes the first case of the use of imatinib mesylate therapy for granulomatous meningoencephalitis in the dog. Therapy with imatinib mesylate plus glucocorticoids appears promising as a new therapeutic intervention in meningoencephalitis of unknown aetiology.

Keywords: dog; immunohistochemical staining; magnetic resonance imaging; unknown aetiology; tyrosine kinase; tyrosine kinase inhibitor

Granulomatous meningoencephalitis (GME) is a common non-infectious inflammatory disease of the central nervous system (CNS) in dogs and is thought to be an autoimmune disorder (Fisher 2002; Matsuki et al. 2004; Granger et al. 2010). GME belongs to a category of diseases termed meningoencephalitis of unknown aetiology (MUE) and is characterised by focal or disseminated granulomatous lesions with perivascular mononuclear cuffing within the CNS.

Systemic medical therapy with a combination of a glucocorticoid and an immunosuppressant is the current mainstay of treatment despite the poorly understood pathogenic mechanisms. Although the efficacy of these therapies varies depending on the patient and the clinicopathological subtype, the overall reported median survival time for disseminated GME in dogs ranges from weeks to months (Munana and Luttgen 1998; O'Neill et al. 2005).

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Tyrosine kinases (TKs) are enzymes that phosphorylate other proteins on tyrosine residues and are key players in normal cell signal transduction, acting to tightly regulate cell growth and differentiation. It is well recognised that specific TKs are abnormally activated in malignant neoplasms and inflammatory processes (Mirshafiey et al. 2014; Bonkobara 2015). The efficacy of tyrosine kinase inhibitors (TKIs) in a variety of cancers in both human and veterinary medicine has also been well demonstrated. Recently, inhibition of specific TKs has shown clinical efficacy for the treatment of autoimmune diseases in numerous animal models and human studies (Akashi et al. 2011; Azizi and Mirshafiey 2013).

Up to now, no studies have investigated the role of TKs in dogs with immune-mediated diseases and there was no clinical trial to assess the efficacy of TKIs in the canine immune-mediated CNS inflammatory response. We here report the first case of the use of imatinib mesylate therapy for disseminated GME in a dog based on its medical record including clinical status, magnetic resonance imaging (MRI), histopathological and immunohistochemical findings.

Case description

A seven-year-old, intact female Toy Poodle weighing 3.2 kg was presented with a two-month history of ataxia in the hind limbs and an acute history of cluster tonic-seizure episodes. The owner had noticed a progressive ataxic gait over the preceding two months with the dog occasionally showing tetraparesis. The dog had been vaccinated, and there was no history of trauma or exposure to toxin.

On conducting physical and neurological examinations, moderate head tilt to the left and hind limb ataxia were identified. The other responses, including postural reactions, cranial nerve reflexes and spinal reflexes, were within normal limits. The result of complete blood count, serum chemistry profiling and radiography were not remarkable. On the basis of clinical signs and a neurological examination, an intracranial CNS lesion was highly suspected.

To identify the intracranial lesion, we performed a brain MRI scan using 0.4-T scanner (Aperto; Hitachi Medical Corporation, Tokyo, Japan), and cerebrospinal fluid (CSF) analysis (obtained from the atlanto-occipital cistern tap using a 22-gauge

needle). T1-weighted (T1W) images, T2-weighted (T2W) images, fluid-attenuated inversion recovery (FLAIR) images and contrast-enhanced T1-weighted (CET1W) images were obtained on the MRI scan. The CET1W images were obtained after the intravenous injection of Omniscan (Gadolinium EDTA; GE-Healthcare, Little Chalfont, United Kingdom) in a dosage of 0.20 mmol/kg body weight. On MRI images (Figure 1), it was possible to identify a focal, ill-defined lesion within the right thalamus region. This lesion appeared hyperintense on T2W and FLAIR images and iso- to hypointense on T1W images. There was moderate non-uniform enhancement on CET1W images. On slightly right parasagittal T2W and T1W images, a broad, ill-defined brainstem lesion that extended from the pons to the medulla oblongata and longitudinal cervical syringomyelia which were suspected to be secondary to the existing lesions were identified. Examination of the CSF revealed an increased nucleated cell count of 18 cells/ μ l (reference range, < 5 cells/ μ l) and a protein concentration of 30 mg/dl (reference range, < 25 mg/dl). Cytological examination of CSF revealed a mononuclear cell pleocytosis. Based on the result of the MRI scan and CSF analysis, we tentatively diagnosed the patient with MUE.

However, we could not absolutely rule out the possibility of a brain tumour at that time. We thus decided to prescribe a TKI, which can be effective against both brain tumours and immune-mediated CNS inflammation based on previous research (Azizi and Mirshafiey 2013; Jung et al. 2014). Treatment with imatinib mesylate (Glivec[®], Novartis Pharm., Stein, Switzerland; 10 mg/kg per day orally) and prednisolone (Prednisolone, Korea Pharm., Seoul, Republic of Korea; 1 mg/kg twice a day orally) was initiated. Moreover, we added hydroxyurea (Hydroxyurea, Korea United pharm., Seoul, Republic of Korea; 50 mg/kg every other day orally), which is known to show synergism with TKIs in specific brain tumours (Reardon et al. 2012). The neurological signs including seizure, head tilt and ataxia rapidly improved. One month after the initiation of treatment, the neurological abnormalities had disappeared completely, and a second MRI scan was made to assess the patient's response to therapy. On this MRI scan (Figure 2B), the lesions involving the right thalamus and brainstem were markedly improved. In addition, the pre-existing syringomyelia in the cervical spinal cord

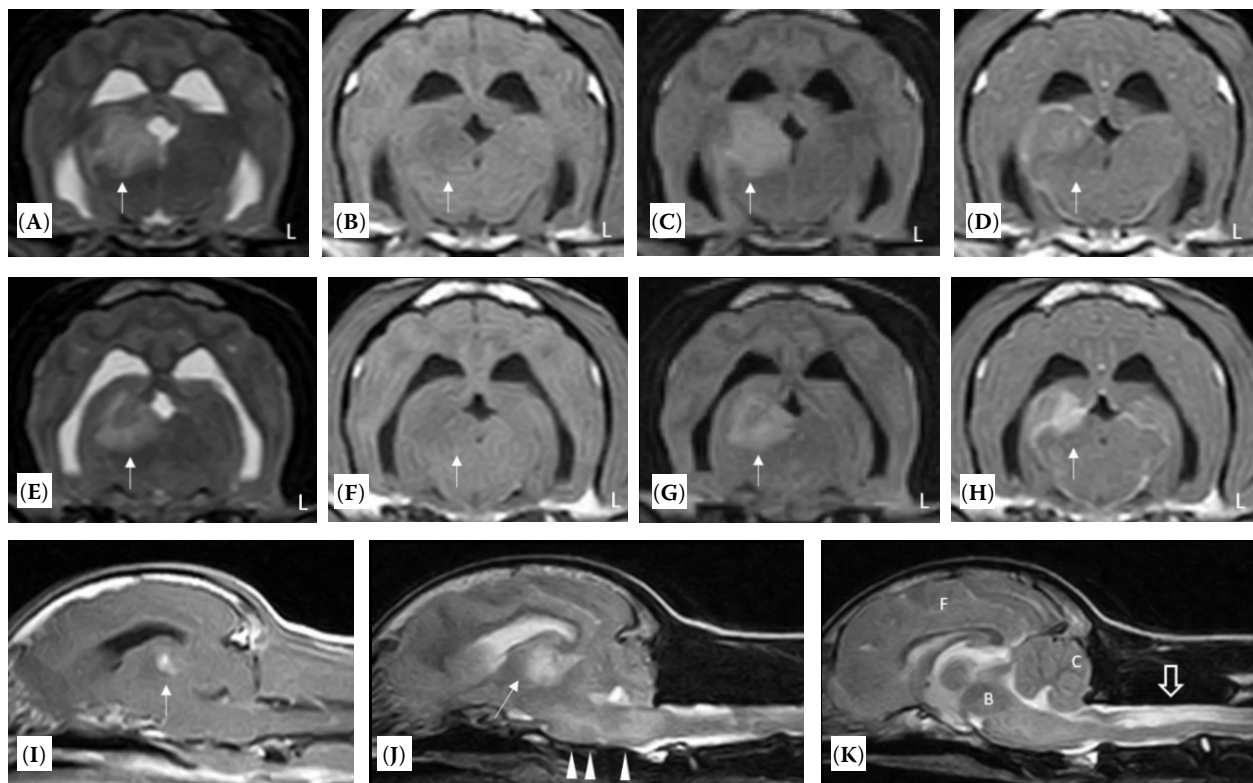


Figure 1. Transverse (A–H), slightly right parasagittal (I and J) and midsagittal (K) images on magnetic resonance imaging scan at initial examination. (A, E, J and K) T2-weighted images, (B and F) T1-weighted image, (C and G) fluid-attenuated inversion recovery image, (D, H and I) contrast-enhanced T1-weighted image. (A–C, E–G and J) A focal, ill-defined T2-, FLAIR hyperintense and T1- iso- to hypointense lesion (arrows) was evident in the right thalamus. (D, H and I) Heterogeneous non-uniform enhancement was identified in the same region. (J) A broad, ill-defined lesion (arrow heads) stretching from the pons to the medulla oblongata with an increased signal intensity was observed. (K) Longitudinal syringomyelia (empty arrow) in the cervical spinal cord segment was identified

B = brainstem; C = cerebellum; F = forebrain; L = left

segment was no longer detected. The same therapy with imatinib mesylate plus hydroxyurea was maintained, and the prednisolone dosage was tapered slowly to 0.15 mg/kg once daily without any relapse of clinical signs. A third MRI scan was performed 21 months after initial presentation. This MRI scan (Figure 2C) identified further ill-defined hyperintense lesions on both temporal lobes.

Approximately 23 months after initial presentation, the patient was presented with an acute history of vomiting. On complete blood count, leukopenia ($3.8 \times 10^9/l$; reference range: 6 to $17 \times 10^9/l$) was identified. We stopped prescribing hydroxyurea and decreased imatinib mesylate dosage to 8 mg/kg per once daily. The vomiting stopped and the leukopenia was gradually alleviated (after two weeks, $8.8 \times 10^9/l$; reference range: 6– $17 \times 10^9/l$).

A fourth MRI scan was performed 28 months after initial presentation. The patient maintained good

clinical status without any neurological signs. At this time, however, the lesions had generally progressed to a more diffuse stage than at the third MRI check (Figure 2D). Further, a focal lesion within the left thalamus, which was not identified previously, was detected. We again increased the imatinib mesylate dosage to 10 mg/kg once daily. A fifth MRI scan was performed 35 months after initial presentation for follow-up check. At that time the patient had not shown any clinical signs. Compared with the preceding MRI (Figure 2E), the lesions involving the cerebrum improved again but the lesions within the two temporo-parietal lobes remained identical.

Unfortunately, the patient did not recover from the anaesthesia and the owner requested euthanasia. We surmised that multifocal lesions of the cerebrum and brainstem may have been associated with the failure to recover from anaesthesia. The patient was donated by the owner, and we per-

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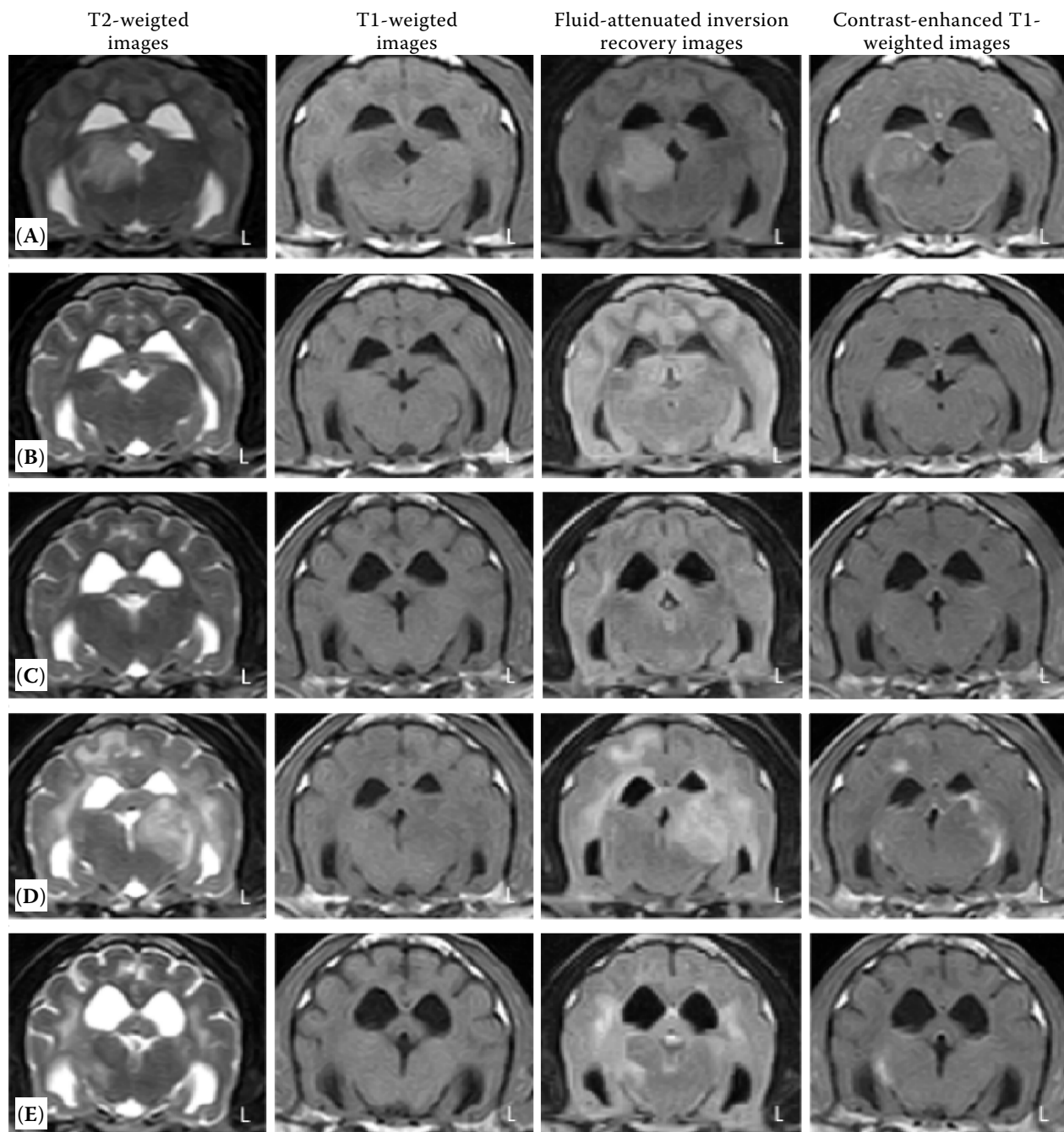


Figure 2. Serial transverse magnetic resonance images at the level of the thalamus. Initial presentation (A), one month (B), 21 months (C), 28 months (D) and 35 months (E) after initial presentation. After reduction of imatinib mesylate dosage and discontinuation of the hydroxyurea, the lesions became markedly more diffuse (D). After the dosage of imatinib mesylate was once again increased, the lesions improved again (E)

formed necropsy and histopathological examination of the brain tissue.

At necropsy, gross findings from the brain showed generalised cerebrovascular congestion. On multiple sections of the brain, multifocal discolourations of the white matter in the cerebrum and brainstem were observed.

On histopathological examination (Figure 3A), multifocal perivascular cuffing lesions were distributed widely throughout the white matter of the cerebrum and brainstem. These inflammatory lesions mainly consisted of macrophages and lymphocytes. Based on these findings, the patient was definitively diagnosed with disseminated GME.

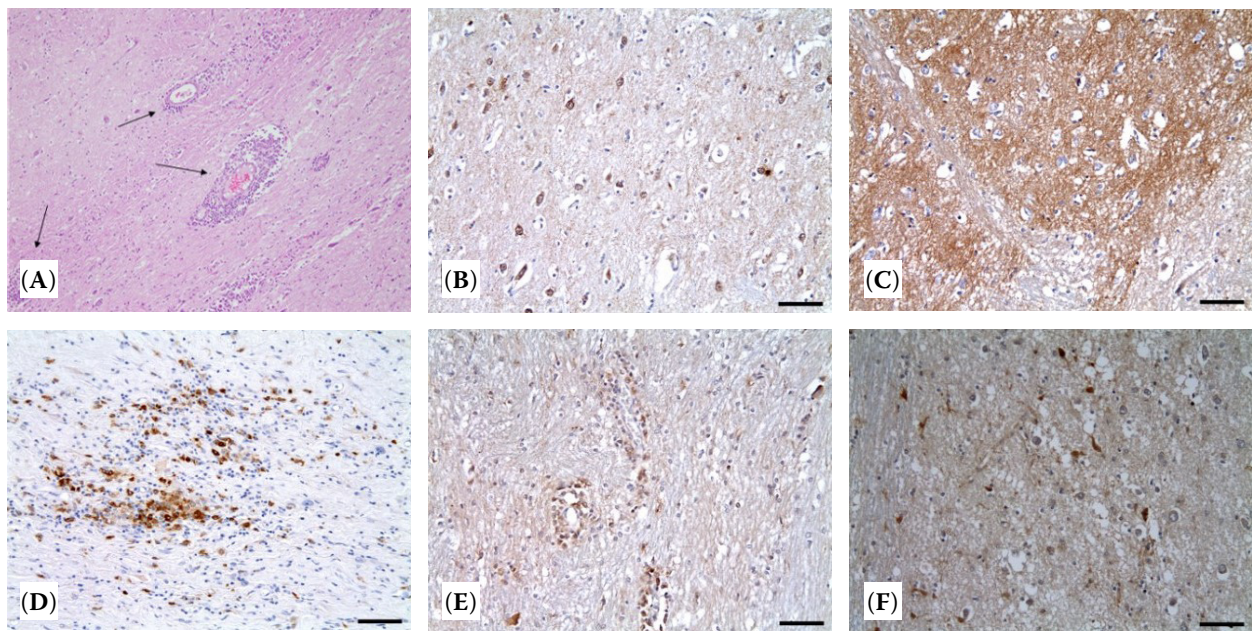


Figure 3. Histopathological and immunohistochemical findings. (A) Haematoxylin and eosin staining, $\times 100$. Histopathologically, multifocal perivascular cuffing lesions (arrows) were distributed widely throughout the white matter of the cerebrum and brainstem. (B–F) $\times 200$, scale bar size; 70 μm . Immunohistochemical analysis showing PDGFR- α (B), PDGFR- β (C), VEGFR-2 (D), c-Kit (E) and c-Abl (F) expression

Immunohistochemical staining was performed for five different TKs using antibodies specific for the PDGFR- α , PDGFR- β , VEGFR-2, c-Abl and c-Kit proteins: a polyclonal rabbit PDGFR- α (Lifespan BioSciences, Seattle, USA), a polyclonal rabbit PDGFR- β (Abcam, Tokyo, Japan), a polyclonal rabbit VEGFR-2 (Abcam, Tokyo, Japan), a polyclonal rabbit c-Abl (Santa Cruz Biotechnology, Santa Cruz, USA) and a polyclonal affinity isolated rabbit c-Kit (DAKO, Glostrup, Denmark). These proteins are typical TKs that are inhibited by conventional multi-targeted TKIs (e.g., imatinib, toceranib and masitinib). The intensity and distribution of TK expression in each lesion were analysed by means of a semi-quantitative scale. The intensity of TK expression was graded as follows: 0, negative; 1+, mild; 2+, moderate and 3+, strong, and the distribution of positively stained cells was graded as follows: 0 (0%, negative), 1+ (< 10%, scant), 2+ (< 50%,

moderate), and 3+ (> 50%, widespread) for each core. The immunohistochemical analysis revealed that the brain samples were positive for the expression of all these TKs (Figures 3B–3F and Table 1). Also, immunohistochemical staining of PDGFR- β showed relatively strong intensity and widespread distribution.

DISCUSSION AND CONCLUSIONS

The role of TKIs in autoimmune disorders, especially rheumatoid arthritis and multiple sclerosis (MS), has been suggested, and their efficacy in numerous animal models and clinical studies in human medicine has already been demonstrated (Eklund and Joensuu 2003; Akashi et al. 2011; Coffey et al. 2012; Vermersch et al. 2012; Azizi et al. 2014). MS is a human inflammatory disease characterised by the T-cell mediated autoimmune response specific for myelin antigens of the CNS (Sospedra and Martin 2016). The T-cell mediated immune response also plays a key role in canine GME and necrotising encephalitis, and it is also likely that these disorders are characterised by autoimmunity against the CNS (Suzuki et al. 2003; Matsuki et al. 2004; Park et al. 2012). According to a previous immunologi-

Table 1. The intensity and distribution of the expression of five different tyrosine kinases in immunohistochemical staining

	PDGFR- α	PDGFR- β	VEGFR-2	c-Kit	c-Abl
Intensity	1+	3+	1+	1+	2+
Distribution	2+	3+	1+	2+	2+

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cal study, canine GME and necrotising encephalitis are considered similar to MS, which shares many of the clinical, neuropathological and immunological characteristics of these conditions (Braund 1985; Greer et al. 2010). Multiple signal transduction pathways involving TKs are implicated in the pathogenesis of MS. (Azizi et al. 2014; Mirshafiey et al. 2014). Uncontrolled signalling of these TKs results in aberrant inflammatory responses including a T-cell mediated immune response. Although pathogenetic studies regarding role of TKs in canine GME and necrotising encephalitis have not yet been reported, we could diagnose GME and identify expression of five types of TK in the present case. This result suggests that some proteins which act as ligand agonists for these TKs could be involved in key aspects of the pathogenesis of GME, and multi-targeted therapy for over-expressed TKs may have potential as an effective treatment modality for GME.

Imatinib mesylate is a selective multi-targeted TKI, which can inhibit BCR/Abl, PDGFR, c-KIT, c-fms, TCR/Abl, Lck, FLT-3 and MAPK activities in various cell types (Azizi and Mirshafiey 2013). The five types of TK which were expressed in this case are all included in the inhibition spectrum of imatinib mesylate. Among these, PDGFR- β , which plays an important role in MS pathogenesis and is effectively inhibited by the imatinib mesylate, was relatively intensely and broadly expressed in this case. Therefore, the clinically relevant effect of imatinib mesylate in the present case appears to be at least partly based on inhibition of PDGFR- β . Other TKs also appear to be highly associated with the disease process based on previous MS studies (Mirshafiey et al. 2014) and the expression patterns observed in this study. However, further studies will be required to fully understand the relationship between the expression of each TK and immune-mediated neuroinflammation in dogs. Furthermore, in our experience (unpublished data), although immunohistochemical staining for TKs was not investigated, some MUE patients were not responsive to multi-TK-targeted therapy using imatinib mesylate. We thus surmised that expression of specific TKs and expression patterns might influence the efficacy of imatinib mesylate in dogs with MUE as in tumours.

The major limitation of this report is that the therapy did not solely consist of imatinib mesylate. Hydroxyurea is a ribonucleotide reductase

inhibitor which is used as an antineoplastic drug. Synergism of hydroxyurea with some TKIs has been documented in certain neoplastic conditions via inhibition of the enzyme ribonucleotide reductase (Tipping et al. 2002; Shah et al. 2007; Reardon et al. 2012). Hydroxyurea also has immunomodulatory effects which include reduction of T-cell proliferation and direct reduction of levels of inflammatory cytokines (Lori 1999; Inayat et al. 2010). According to these pharmacological studies, we surmised that these properties of hydroxyurea might have accounted for the good clinical status and long-term survival time with other drugs in the present case. However, since hydroxyurea is not a commonly used drug in patients with immune-mediated inflammation, its efficacy has remained unclear, and a further well-designed placebo-controlled study will be needed.

The prognosis for GME is generally guarded to poor but highly depends on the modality of the disease and on the treatment protocol followed. According to previous studies, dogs with disseminated GME (weeks to months) had significantly shorter survival times than those with focal GME (weeks to years) (Munana and Luttgen 1998; O'Neill et al. 2005; Coates 2007). This patient's survival time (survival for 1052 days with treatment) was relatively long compared those in previously reported cases with disseminated GME, and improvement of clinical signs and maintenance were satisfactory. In other words, therapy using imatinib mesylate might represent a good therapeutic alternative to the other adjunctive immunosuppressive drugs due to its efficacy and lower adverse effects.

In conclusion, to the authors' knowledge, the present report describes the first case of the use of imatinib mesylate therapy for GME in the dog. The good response to the therapy and the results of the immunohistochemical staining suggest that these kinases may play a role in the pathogenesis and development of GME. Therapy with multi-targeted TKIs plus glucocorticoids appears promising as a new therapeutic intervention in MUE. Furthermore, since multi-targeted TKIs inhibit diverse signal transduction pathways underlying aberrant immune responses that cannot be controlled by conventional immunosuppression, it may be useful to combine these drugs with conventional therapy of MUE. However, further long-term prospective studies in a larger population of dogs with

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GME and necrotising encephalitis are required for estimation of the clinical efficacy and survival times with imatinib mesylate treatment.

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