Appendix S1

Materials and methods

Statistical analysis. Statistical analyses were conducted using EZR software version 1.64 (Saitama Medical Center, Jichi Medical University) (29). The continuous variables exhibited non-normal distribution, warranting non-parametric tests, including the Mann-Whitney U and Kruskal-Wallis tests. The Holm method was used to adjust for multiple comparisons. The Fisher's exact test was used for dichotomous variables. $P<0.05$ was considered to indicate a statistically significant difference.

Contrast-enhanced computed tomography (CT) protocol. CT scans were performed using a SOMATOM Drive (Siemens K.K.; Siemens AG) or Aquilion ONE (Canon Medical Systems Corporation). The scan parameters were as follows: Voltage, 80-120 kVp; tube current, auto-milliampere; rotation time, 0.5 sec; slice thickness, 1 mm; and reconstruction interval, 1 mm. Iodinated contrast agents (iohexol or iopamidol; 300 mg iodine/ml; 600 mg iodine/kg) were intravenously administered for 70 sec for contrast enhancement. Image acquisition occurred 90 sec after the start of the injection for portal venous phase imaging.

Contrast-enhanced magnetic resonance imaging (MRI) protocol. Head MRI scans were obtained using a 1.5 or 3 T unit (Optima MR360, Signa HDx or Discovery MR750w; GE Healthcare). Post-contrast 3D T1-weighted images (repetition time, 500-502 msec; echo time, 9.38-11.14 msec; field of view, 256 mm; section thickness, 1 mm; and matrix, 256x256) were acquired 1-5 min after injection of a gadolinium-based contrast agent (0.1 ml/kg of gadobutrol; Gadovist, 5.0-7.5 ml).

Assessment of pazopanib treatment efficacy. All measurable lesions via CT and MRI were radiologically evaluated by two board-certified specialists, one with 21 and 8 years of experience in neurosurgery and radiology, respectively, and the other with 12 years of experience in radiology. The tumor longitudinal diameters, margin densities (CT attenuation values, Hounsfield units) and metastatic lymph node short diameters were measured using SYNAPSE version 5.5 (FUJIFILM Corporation) according to the Response Evaluation Criteria In Solid Tumors (version 1.1) (25) and Choi criteria (16) to determine the best overall response. Furthermore, to comprehensively evaluate the effects of pazopanib on multiple lesions in the 3 cases, the density and size of both target and non-target lesions were assessed. Even the smallest lesions were included as non-target lesions if they could be magnified and distinguished from other lesions on SYNAPSE and had a measurable density and size. The measurable lung lesions were identified using CT in a mediastinal setting. Purely bone lytic lesions were not included based on RECIST 1.1 criteria. In Case 3, bone lytic lesions were also excluded due to denosumab use; however, protruding tumors were measured as soft tissue lesions. Consequently, 20 intracranial and 30 extracranial lesions were eligible for assessment. These lesions were assessed using CT and MRI. In Case 3, only extracranial lesions were evaluated because of the lack of primary intracranial tumor recurrence after the initial resection. Tumor density and size changes were calculated using the following equation: Change ratio (%)=[(post-treatment change value)-(pre-pazopanib initiation value)]/pre-pazopanib initiation value x100.

Pathological diagnosis and protocols. The pathological diagnosis was performed by local or commissioned pathologists at each facility. Immunohistochemistry involved paraffin-embedded tissue samples with a thickness of 2 μm, no blocking reagent and no serum. The primary antibodies included STAT6 (Abcam; cat. no. ab32520; 1:100) and CD34 (Agilent Technologies, Inc.; cat. no. IR632; pre-diluted), which were incubated at 36˚C for 32 min. The secondary antibody was a horseradish peroxidase-labeled Multimer Secondary Antibody from the ultraView DAB Universal Kit (Roche Diagnostics; cat. no. 760-500), which was incubated at 36˚C for 8 min. A BX53 microscope (Olympus Corporation) was used for visualization, including a BenchMark ULTRA (Roche Diagnostics), in which the process from deparaffinization to staining was automated.

For histology and staining, fixation was performed with 10% neutral-buffered formalin for Cases 1 and 2 and 10% non-buffered formalin for Case 3, fixed at room temperature for 24-48 h. The section thickness was 2 μm, and hematoxylin and eosin (HE) staining was performed at room temperature, with 5 min for hematoxylin and 5 min for eosin staining. An Olympus BX53 microscope was used for visualization.

Results

Whole-body imaging. Baseline whole-body contrast-enhanced CT images for the 3 cases, collected before pazopanib initiation, are shown in Fig. S1. The lesion characteristics and imaging periods correspond to those listed in Tables I and SI, respectively. Contrast-enhanced MRI findings were used as a reference to identify intracranial lesions via SYNAPSE and correlate them with the CT findings. It was aimed to detect whole-body lesions and distinguish them from other lesions as precisely as possible, even if they were small and classified as non-target lesions. As a result, 50 lesions were identified and analyzed.

Pathological findings. Representative pathological images are shown in Fig. S2. Each of the 3 cases was evaluated for hypercellularity, necrosis and mitosis using HE staining. All 3 cases exhibited hypercellularity (Fig. S2A, B, D, E, G and H), with necrosis observed in Cases 1 and 2, while Case 3 showed no necrosis (Fig. S2A, D, G and H). Representative mitoses are shown in Fig. S2B, E, G and H. The mitotic counts were 9 mitoses/10 high-power fields (HPFs) in Case 1, 19 mitoses/10 HPF in Case 2, and 4 mitoses/10 HPF in Case 3. Immunohistochemistry for STAT6 was performed in Cases 1 and 2, which showed nuclear positivity (Fig. S2C and F). Therefore, based on the 2021 World Health Organization (WHO) classification of central nervous system (CNS) tumors (1), Cases 1 and 2 were diagnosed as solitary fibrous tumor (SFT), WHO grade 3. Herein, the specimens for Cases 1 and 2 were obtained from a pelvic cavity tumor during surgery in March 2023 and from a left supratentorial tumor during surgery in February 2023, respectively; therefore, both specimens were relatively recent and suitable for investigation according to the 2021 WHO classification. However, the specimen for Case 3 was an older sacrum biopsy sample, diagnosed in October 2018, for which STAT6 immunostaining was not performed due to unavailability of the anti-STAT6 antibody at the facility at that time. Furthermore, the specimen was donated to another facility for further investigation, preventing any additional analyses on this sample. Case 3 underwent a liver biopsy at that facility for comprehensive gene panel

testing in August 2021, which revealed non-significant gene alterations. During this procedure, STAT6 immunostaining confirmed nuclear positivity, reaffirming the diagnosis of SFT. The pathology report, which includes the nuclear-positive STAT6 immunostaining image, is not available for publication due to the proprietary rights of the aforementioned facility. According to the 2016 (4th edition) (30) and 2021 (5th edition) WHO classifications of CNS tumors, CD34 staining is generally diffusely positive in grade 1 SFT, while little (focal) or no expression is associated with higher grades. The presence of hypercellularity also contributes to higher grades according to the 2016 WHO classification. Therefore, the CD34 immunostaining findings for Case 3 have been included Fig. S2I. Since Case 3 exhibited focal CD34 positivity, the facility's pathologist diagnosed it in October 2018 as metastatic SFT/hemangiopericytoma, WHO grade 2, along with the presence of hypercellularity, absence of necrosis (Fig. S2G and H), and 4 mitoses/10 HPF.

Figure S1. Baseline contrast-enhanced whole-body CT images for the three cases prior to pazopanib initiation. (A) Case 1, extracranial lesions: right cervical lymph node, liver, right sacrum and pelvic cavity. Reactive hyperplasia was considered as a differential diagnosis concerning the right cervical lymph node lesion (gray arrow). (B) Case 2, extracranial lesions: left skull base, C5, left scapula, T1, T5, left lung and L1. The other left scapula lesion (arrowhead), diagnosed as a purely bone lytic lesion, was not included for pazopanib treatment evaluation. (C) Case 3, extracranial lesions: right second rib, left fourth rib, left seventh rib, liver, bilateral kidneys and ilium. Arrows: tumors. Scale bar, 1 cm per division. CT, computed tomography; C, cervical vertebra; T, thoracic vertebra; L, lumbar vertebra.

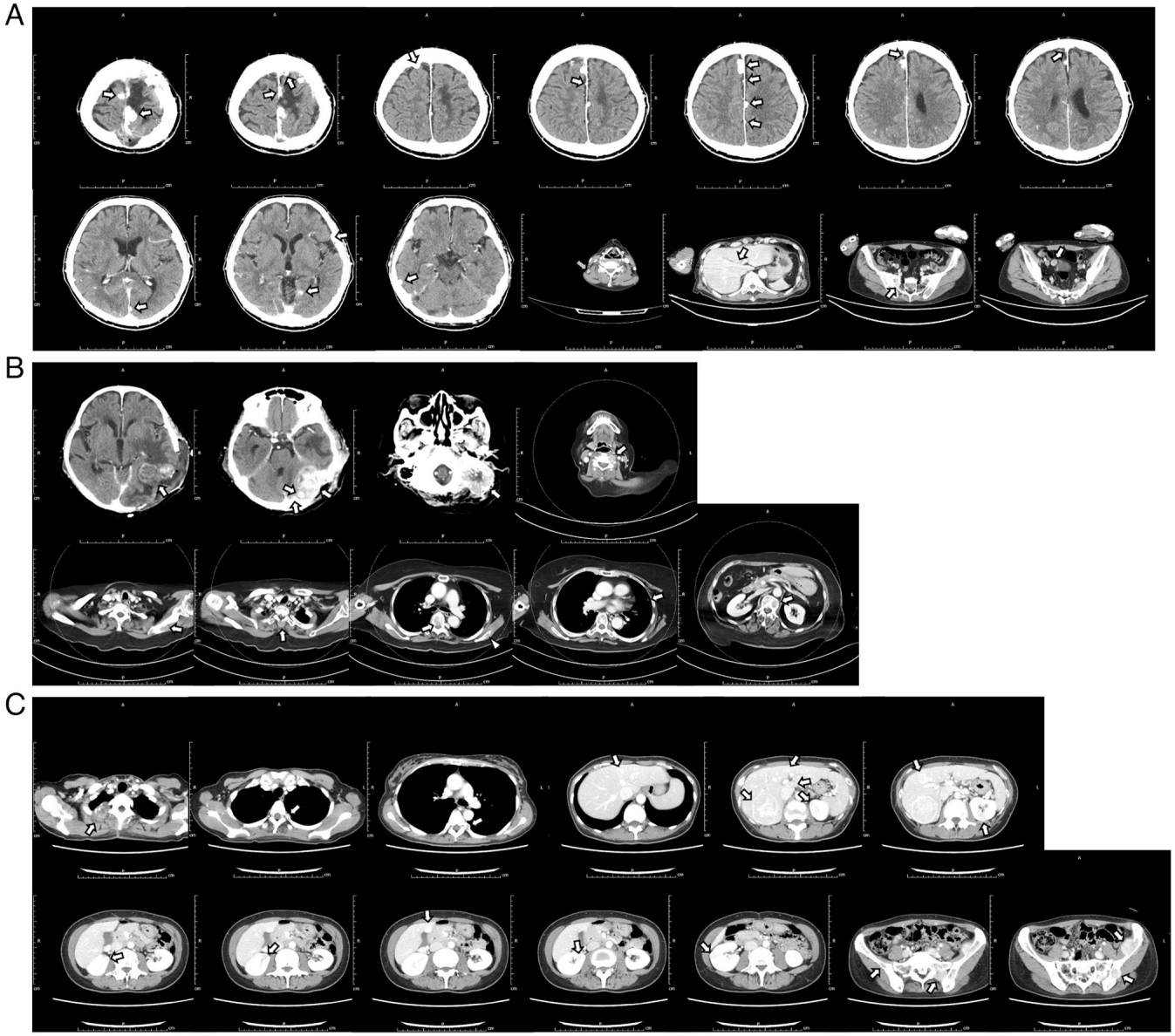

Figure S2. Representative pathological findings for the three cases. (A-C) Case 1; (D-F) Case 2; (G-I) Case 3. (A, B, D, E, G and H) hematoxylin and eosin staining. (C and F) STAT6 immunostaining. (I) CD34 immunostaining. Arrows: mitoses.