# Immunohistochemical study of solitary fibrous tumor in the ear related to pazopanib use

**Short running title:** Immunohistochemistry of SFT in the ear

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## Abstract

### Objective

Solitary fibrous tumors (SFTs) account for 0.4 % of brain tumors originating from the dura of the brain. These tumors are more invasive than meningiomas and may metastasize

systemically. The objective was to examine the expression rate of immune-related antibodies during the course of treatment by immunostaining the tumor tissue during the course using pazopanib for SFT of the ear canal.

## Methods

A 73-year-old woman was admitted to our department with right-sided hearing loss and a mass in the ear canal. Pathological examination revealed that Grade III anaplastic SFT. Immunostaining the ear canal SFT three times–before, after using pazopanib, and after using nivolumab– we examined the expression status of p53, Ki67, PD-1, PD-L1, PD-L2, VEGF, CD4, CD8, Her2, and EGFR in tumors. The expression status of VFGF targeted by pazopanib and the expression status of other immune-related antibodies were compared over time.

## Results

Ki-67, PD-1, VEGF, CD8, and EGFR were all positive, but p53 was always negative. PD-L2 was positive before treatment with pazopanib, but became negative thereafter. PD-L1 became negative after treatment with pazopanib, but became positive again after administration of nivolumab. CD4 was positive until after pazopanib administration, but then became negative. Her2 was positive only after administration of pazopanib.

## Conclusion

Expression of VFGF targeted by pazopanib was positive during the course of treatment.

The VEGF antibody was positive throughout the course, suggesting that the prognosis could have been further prolonged if pazopanib could be administered without side effects.

**Keywords:** solitary fibrous tumor, pazopanib, vascular endothelial growth factor, Immunohistochemistry

## Key Clinical Message

Solitary fibrous tumor generated in the ear canal constantly expressed VEGF by immunohistochemical staining of tumor tissue during the treatment process of chemotherapy. It may support treatment with Pazopanib, an antitumor drug that targets VEGF.

**Level of evidence:** Level 4

## Introduction

Solitary fibrous tumor (SFT) was first reported in 1930 by Klemperer and Rabin as a soft tissue neoplasm.[1] The tumor, previously known as hemangiopericytoma (HPC), was unified into SFT by the World Health Organization (WHO) 2016 classification, because these

tumors have the same unique NAB2-STAT6 fusion.[2] The new WHO classification classified this tumor into the following three grades: Grade I (highly collagenous, relatively low-cellularity spindle cell lesion previously classified as SFT), Grade II (more cellular, less collagenous tumor previously diagnosed as HPC in the central nervous system), and Grade III (what was termed “anaplastic HPC” in the past; diagnosed on the basis of > 5 mitoses/10 HPF).[2] SFT originates from the mesenchymal tissue of the pleura, but can involve soft tissues anywhere in the body.[3] Although the major features of Grade I SFTs are low recurrence rate and systemic metastasis, Grade III SFTs have been reported to be prone to metastasis, and the treatment involves total resection.[4] There is no standard treatment for patients with advanced unresectable tumors; however, anthracycline- and ifosfamide-based chemotherapies are widely used. pazopanib was identified through the chemical screening of compounds to inhibit vascular endothelial growth factor (VEGF) 2, a key mediator of tumor angiogenesis.[5] pazopanib is also effective against advanced soft tissue sarcoma.[6] In addition, there are cases in which pazopanib has been effective for metastatic SFT.[7] Here, we describe a patient with metastatic Grade III SFT treated with pazopanib after surgery.

## Materials and Methods

### Case

A 73-year-old woman presented to our otolaryngology department complaining of a recent hearing loss in the right ear caused by a smooth surfaced painless mass located in the external auditory canal. A gamma knife radiosurgery (2-division irradiation, 19Gy) was performed 17 years before her first visit to our department for the treatment of right cranial fossa meningioma. Fourteen years later, magnetic resonance imaging (MRI) revealed recurrence. Gamma knife radiosurgery (14 Gy) was performed two more times, and the patient was followed up. She was referred to our department because of right-sided hearing loss and a mass filling the right external auditory meatus (Fig 1-1, Fig 1-2). The symptoms had commenced approximately 2–3 months prior the visit, including tinnitus and a slow-growing mass at the middle ear to the auditory canal level. MRI showed a low signal on T1-weighted image (T1WI) of 28×10 mm and a high signal on T2-weighted image (T2WI) in the right external auditory meatus, and a tumor with non-uniform contrast effect was observed (Fig 2-1, Fig 2-2). Computed tomography showed bone destruction of the foramen ovale, spinous foramen, and carotid canal (Fig 2-3). An ear canal biopsy was performed, and the diagnosis was likely malignant. The patient was admitted to our department for surgery and a definitive diagnosis. Resection of the right external ear canal tumor was performed. Intraoperative findings showed an increase in the tumor size compared to the initial diagnosis, and it was difficult to completely excise the tumor

because it continued up to the middle fossa. On pathological examination of the tissue excised by surgery, STAT6, CD99, bcl-2, and vimentin were positive, and H/E staining showed nine cell divisions in 10 fields and more than five cells. Thus, it was diagnosed as SFT Grade III (Fig 3). After the tumor was confirmed, a postoperative cyber knife treatment (40 Gy + 40 Gy) was performed. One year after surgery, bilateral lung metastases were observed; therefore, right lung partial resection and left lower lobectomy were performed at our department of respiratory surgery. The pathology reports confirmed the diagnosis of metastatic SFTs.

One month later, MRI showed an increase in the right ear canal tumor (Fig 4-1), and chemotherapy with cetuximab plus cisplatin (CDDP)/5-Fluorouracil (5-FU) was initiated. MRI showed tumor growth after 10 courses of cetuximab; therefore, we switched to pazopanib treatment (Fig 4-2). Pazopanib was started at a standard dose of 800 mg/day. Three weeks after starting the treatment, thrombocytopenia was observed, and oral administration was discontinued. Shortly thereafter, side effects such as hypertension and liver dysfunction were observed. After receiving targeted therapy, the patient resumed oral therapy of 400 mg/day 2 months after her symptoms subsided, but thrombocytopenia recurred, and the dose was reduced to 200 mg/day. Although there were no side effects for 4 months, malaise was observed after resuming the treatment, and administration was

deferred every other week. An MRI evaluation 3 months after the start of pazopanib showed that some portions of the tumor had shrunk (Fig 5-1, Fig 5-2). While the irregular mass in the external auditory meatus had decreased in size, the lesion extending from the left jugular vein to the sinusoidal sinus had increased, and the tumor extending from the right mid-cranial fossa to the foramen ovale to the outside of the skull had also increased. There were some side effects, and we were forced to reduce the dose of pazopanib. An MRI 6 months after the start showed further tumor growth (Fig 5-3). The dosage was changed every other day and oral administration was continued until 7 months later. Due to side effects and tumor growth, oral administration of pazopanib was discontinued. The patient was then treated with nivolumab for four courses; however, this treatment was discontinued because MRI showed further tumor growth 2 months later (Fig 6-1). The tumor increased further in size and the tumor bleeding and fatigue worsened (Fig 6-2). She died a month later.

### Immunohistochemical study

For immunostaining, the tissue samples were dehydrated through a graded series of ethanol, cleared in xylene, and embedded in paraffin. Serial paraffin sections (6-μm-thick) of tumor samples were prepared. After deparaffinization, the specimens were treated with

3% $H_2O_2$ for 20 min at room temperature (approximately 20 °C). Sections were treated with 5% normal goat serum in PBS for 30 min and then incubated with a rabbit anti-human antibody, diluted 1:1000 in 1% BSA–PBS, for 12 h at room temperature. After the sections were rinsed with PBS, they were incubated with ABC reagent (Vector Laboratories, Burlingame, CA, USA) for 1 h and developed in 0.05% 3,3-diaminobenzidine with 0.01% $H_2O_2$ substrate medium in 0.1 M phosphate buffer for 8 min. Regarding the expression of immunostaining, positive and negative judgments were made according to the following criteria:

### Immunostaining diagnostic criteria

Epidermal growth factor receptor (EGFR): It is positive when staining is observed on the cell membrane of tumor cells (based on the EGFR staining determination method in colorectal cancer tissue)[8]

Her2: Conformance to ASCO/CAP HER-2 guidelines for breast cancer and a positivity rate of 3+ or more [9]

p53: Positivity rates of 1% or more based on Zhang H's report on rectal cancer[10]

Ki-67 (MIB-1): Positivity rates (Ki-67 labeling index) of 20% or more per 500 tumor cells[11]

Programmed cell death 1 (PD-1): Positivity rates of >8% based on Konishi et al.'s report for lung cancer[12]

Programmed cell death-ligand 1 (PD-L1): Positivity rates of 5% or more according to the PD-L1 staining judgment manual of Dako[13]

Programmed cell death-ligand 2 (PD-L2): Based on the report by Yearly et al., divided into five stages: 0=negative, 5=very high, and positivity rates of 3 or more[14]

CD4, CD8: Positive if there are 100 T cells expressing CD4 per 1000 cells[15]

VEGF: Nayak S, et al. reported that positive if 25% or more of VEGF is expressed in tumor cells[16]

## Results

Immunohistological analysis

P53 (tumor suppressor gene) was negative at all time points. Ki67 was positive, but the expression was slightly weaker after treatment with pazopanib. All samples were positive for PD-1, a type of immune checkpoint receptor on the surface of cytotoxic T cells. PD-L1 and PD-L2 became negative after pazopanib treatment. VEGF, that is affected by pazopanib, was positive in all tissues. CD4 and CD8 were positive in many tissues due to the activation of immune cells against cancer. Many samples were negative for Her2, and a positive finding was seen only after using pazopanib. EGFR was found to be positive, as in many cases of head and neck cancer. Cetuximab was used because the tumor was EGFR positive, but the tumor growth was stimulated by the treatment and the expression

increased (Fig 7-1, Fig 7-2).

## Discussion

The WHO states that the histological appearance and clinical behavior of SFTs and HPCs are similar, and this is a widely shared view.[17] Currently, the word HPC was integrated into the disease concept of SFT. Histological diagnosis, especially by immunostaining, is important for the definite diagnosis of SFTs. On histopathological examination SFT shows irregularly arranged oval to spindle-shaped cells , and several slit-shaped vascular cavities called “staghorn” are recognized.[18] The fact that 90–95% of SFTs are CD34 positive on immunohistochemical analysis is particularly useful for differential diagnosis.[19] In addition, bcl-2 and MIC-2 (CD99) are also frequently positive, and have the molecular genetically specific fusion gene NAB2-STAT6, and STAT6 strongly expressed in the nucleus.[20, 21] In this case, as STAT6, CD34, bcl-2, CD99, and STAT-6 were positive, SFT was diagnosed. Moreover, as a criterion for malignancy, four or more mitotic divisions in 10 visual fields should be observed under a microscope.[22] In this case, 9 or more mitotic divisions were observed in 10 visual fields; therefore, a diagnosis of Grade III SFT was made.

Pazopanib is a multi-kinase inhibitor that inhibits VEGFR, platelet-derived growth factor receptor, and hepatocyte factor receptor (c-kit). VEGF is found in many types of malignant

soft tissue tumors, and pazopanib acts on VEGFR to suppress the survival and growth of cancer cells.[23] In Japan, it was approved in September 2012 for the treatment of malignant soft tissue tumors. In a phase 3 trial (PALETTE) in patients with advanced malignant soft tissue tumors who had progressed on standard therapy, pazopanib extended the progression-free survival by about 3 months (4.6 months vs. 1.5 months) compared to placebo.[24] Serious side effects include liver dysfunction, high blood pressure, bleeding, and thrombocytopenia.[25] Targeted therapy such as drug withdrawal, dose reduction, administration of antihypertensive agents, and hemostatic treatment are performed. In the present case, thrombocytopenia became the biggest complication, and general malaise became stronger thereafter. Therefore, the dosage was reduced and treatment continued. Pazopanib administration lasted for 7 months; however, if the adverse reactions were controllable, the prognosis could have been extended.

Immunohistochemical studies were performed three times during the course of the illness to examine the reactivity of the tumor to chemotherapy. Tissues were collected during surgery before using pazopanib, after using pazopanib, and after using nivolumab. Expression of p53, a typical tumor suppressor gene,[26] was suppressed in all three samples. Ki67, a cell cycle-related nuclear protein, is a marker for cell proliferation and cell cycle, and for detecting proliferating cells in tumor tissue.[27] At every time point, tissues were positive

for PD-1, a type of immune checkpoint receptor on the surface of cytotoxic T cells.[28] PD-L1 and PD-L2, expressed on the surface of cancer cells by the ligand,[28] became negative after treatment with pazopanib. VEGF, which is also involved in the process of malignant transformation such as tumor angiogenesis and metastasis,[29] was detected in all tissues. CD4 and CD8 were positive in several tissues due to the activation of immune cells against cancer.[30,31] With regard to CD4 after nivolumab treatment, it is presumed that immunocompetence was weakened as the tumor continued to increase in size. Her2 is a non-ligand binding member of the EGFR family and exerts its activity through heterodimerization with other family members. Functional activation of Her2 promotes carcinogenesis, leading to the investigation of Her2-directed drugs in Her2-altered cancers.[32] The first two samples were negative for Her2, but the third sample, after using pazopanib was positive. EGFR is also found in many cases of head and neck cancer,[33] and all the samples were positive for this biomarker. We used Cetuximab because the tumor was EGFR positive; however, the actual effect was poor, and the results were stimulated by the treatment and the expression increased. SFT presents the possibility of angiogenesis inhibitor treatment, due to the activation of T cells such as CD4 and CD8.[34] In addition, it has been reported that three patients who received pazopanib for metastatic HPC had tumor shrinkage >30%, survival >5 months, and stable disease >8 months of

treatment.[35] In our case, we used pazopanib for HPC and confirmed stable disease for at least 4 months. However, we continued to reduce the dose due to the appearance of side effects, which may have led to an increase in the tumor size.

## Conclusion

In conclusion, we report a case of SFT in the ear canal after treatment of a meningioma in the middle fossa. This is the first report to examine the induction of immune cells in SFTs during the course of pazopanib treatment. Although pazopanib was used for SFT and prognosis was prolonged for approximately 4 months, tumor growth was observed because of dose reduction due to side effects. The immunostaining of SFT revealed that the expression rate of immune-related antibodies changed over time, and we considered that pazopanib was effective; however VEGF was always expressed. Since it is said that VEGF expression is basically high in the head and neck, pazopanib, which is a VEGF antibody, may be effective regardless of the type of cancer. [36] There are also reports on the use of pazopanib as the first-line treatment for SFTs.[37] In the future, pazopanib may be used as the first-line treatment for SFTs, and the indication may be expanded to the head and neck malignancy in addition to soft tissue tumors.

References:

1. Klemperer P, Coleman BR. Primary neoplasms of the pleura. Primary neoplasms of the pleura. A report of five cases. Am J Ind Med. 1992 ; 22:1-31.

2 Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella–Branger D, Cavenee WK, Ohgaki H, Wiestler OD, Kleihues P, Ellison DW. The 2016 World Health Organization Classification of Tumors of the Central Nervous System : a summary. Acta Neuropathol. 2016;131:803–820.

3 England DM, Hochholzer L, McCarthy MJ. Localized benign and malignant fibrous tumors of the pleura. A clinicopathologic review of 223 cases. Am J Surg Pathol. 1989;13:640-58.

4 Kim BS, Kim Y, Kong DS, Nam DH, Lee JI, Suh YL, Seol HJ. Clinical outcomes of intracranial solitary fibrous tumor and hemangiopericytoma: analysis according to the 2016 WHO classification of central nervous system tumors. J Neurosurg. 2018;129(6):1384-96.

5 Harris PA, Boloor A, Cheung M, Kumar R, Crosby RM, Davis-Ward RG, Epperly AH, Hinkle KW, Hunter RN 3rd, Johnson JH, Knick VB, Laudeman CP, Luttrell DK, Mook RA, Nolte RT, Rudolph SK, Szewczyk JR, Truesdale AT, Veal JM, Wang L, Stafford JA. Discovery of 5-[[4-[(2,3-Dimethyl-2*H*-indazol-6-yl)methylamino]-2-pyrimidinyl]amino]-2-methyl-benzenesulfonamide (Pazopanib), a Novel and Potent Vascular Endothelial Growth Factor Receptor Inhibitor. J Med Chem. 2008;51(15):4632-40

6 Lee ATJ, Jones RL, Huang PH. Pazopanib in advanced soft tissue sarcomas. Signal Transduct Target Ther 2019;4:16. doi: 10.1038/s41392-019-0049-6. eCollection

7 Lee SJ, Kim ST, Park SH, Choi YL, Park JB, Kim SJ, Lee J. Successful use of pazopanib for treatment of refractory metastatic hemangiopericytoma. Clin Sarcoma Res 2014;4:13 doi:10.1186/2045-3329-4-13.eCollection

8 Cunningham D, Humblet Y, Siena S, et al.: Cetuximab monotherapy and cetuximab plus in irrinotecan-refractory metastatic colorectal cancer. N Engl J Med. 2004;351:337-345.

9 Tchrakian N, Flanagan L, Harford J, Gannon JM, Quinn CM. New ASCO/CAP guideline

recommendations for HER2 testing increase the proportion of reflex in situ hybridization tests and of HER2 positive breast cancers. Virchows Arch. 2016;468(2):207-11.

10 Zhang G, Xu Q, Wang Z, Sun L, Lv Z, Liu J, Xing C, Yuan Y. p53 protein expression affected by TP53 polymorphism is associated with the biological behavior and prognosis of low rectal cancer. Oncol Lett. 2019;18(6):6807-21.

11 Dowsett M, Nielsen TO, A'Hern R, Bartlett J, Coombes RC, Cuzick J, Ellis M, Henry NL, Hugh JC, Lively T, McShane L, Paik S, Penault-Llorca F, Prudkin L, Regan M, Salter J, Sotiriou C, Smith IE, Viale G, Zujewski JA, Hayes DF. Assessment of Ki67 in Breast Cancer: Recommendations from the International Ki67 in Breast Cancer Working Group. J Natl Cancer Inst. 2011;103(22):1656-64.

12 Konishi J, Yamazaki K, Azuma M, Kinoshita I, Dosaka-Akita H, Nishimura M. B7-H1 expression on non-small cell lung cancer cells and its relationship with tumor-infiltrating lymphocytes and their PD-1 expression. Clin Cancer Res. 2004;10(15):5094-100.

13 Ilie M, Khambata-Ford S, Copie-Bergman C, Huang L, Juco J, Hofman V, Hofman P. Use of the 22C3 anti-PD-L1 antibody to determine PD-L1 expression in multiple automated

immunohistochemistry platforms. PLoS One. 2017; 10;12(8):e0183023.

doi: 10.1371/journal.pone.0183023. eCollection.

14 Yearley JH, Gibson C, Yu N, Moon C, Murphy E, Juco J, Lunceford J, Cheng J, Chow LQM, Seiwert TY, Handa M, Tomassini JE, McClanahan T. PD-L2 Expression in Human Tumors: Relevance to Anti-PD-1 Therapy in Cancer. Clin Cancer Res. 2017;23(12):3158-67.

15 Banat GA, Tretyn A, Pullamsetti SS, Wilhelm J, Weigert A, Olesch C, Ebel K, Stiewe T, Grimminger F, Seeger W, Fink L, Savai R. Immune and Inflammatory Cell Composition of Human Lung Cancer Stroma. PLoS One. 2015;10(9):e0139073. doi: 10.1371/journal.pone.0139073. eCollection.

16 Nayak S, Goel MM, Bhatia V, Chandra S, Makker A, Kumar S, Agrawal SP, Mehrotra D, Rath SK. Molecular and phenotypic expression of decorin as modulator of angiogenesis in human potentially malignant oral lesions and oral squamous cell carcinomas. Indian J Pathol Microbiol. 2013;56(3):204-10.

17 Carneiro SS, Scheithauer BW, Nascimento AG, Hirose T, Davis DH. Solitary fibrous

tumor of the meninges: a lesion distinct from fibrous meningioma. A clinicopathologic and immunohistochemical study. Am J Clin Pathol. 1996;106:217-24.

18 Gold JS, Antonescu CR, Hajdu C, Ferrone CR, Hussain M, Lewis JJ, Brennan MF, Coit DG. Clinicopathologic correlates of solitary fibrous tumor. Cancer. 2002;94:1057-68.

19 Thompson LD, Miettinen M, Wenig BM. Sinonasal-type hemangiopericytoma: a clinicopathologic and immunophenotypic analysis of 104 cases showing perivascular myoid differentiation. Am J Surg Pathol. 2003;27:737-49.

20 Chmielecki J, Crago AM, Rosenberg M, O'Connor R, Walker SR, Ambrogio L, Auclair D, McKenna A, Heinrich MC, Frank DA, Meyerson M. Whole-exome sequencing identifies a recurrent NAB2-STAT6 fusion in solitary fibrous tumors. Nat Genet. 2013;45(2):131–2.

21 Robinson DR, Wu YM, Kalyana-Sundaram S, Cao X, Lonigro RJ, Sung YS, Chen CL, Zhang L, Wang R, Su F, Iyer MK, Roychowdhury S, Siddiqui J, Pienta KJ, Kunju LP, Talpaz M, Mosquera JM, Singer S, Schuetze SM, Antonescu CR, Chinnaiyan AM.

Identification of recurrent NAB2- STAT6 gene fusions in solitary fibrous tumor by integrative sequencing. Nat Genet. 2013;45(2):180–5.

22 Demicco EG, Park MS, Araujo DM, Fox PS, Bassett RL, Pollock RE, Lazar AJ, Wang WL. Solitary fibrous tumor. A clinicopathological study of 110 cases and proposed risk assessment model. Mod Pathol. 2012;25(9):1298-306.

23 Kumar R, Knick VB, Rudolph SK, Johnson JH, Crosby RM, Crouthamel MC, Hopper TM, Miller CG, Harrington LE, Onori JA, Mullin RJ, Gilmer TM, Truesdale AT, Epperly AH, Boloor A, Stafford JA, Luttrell DK, Cheung M. Pharmacokinetic-pharmacodynamic correlation from mouse to human with pazopanib, a multikinase angiogenesis inhibitor with potent antitumor and antiangiogenic activity. Mol Cancer Ther. 2007;6(7):2012-21.

24 van der Graaf WT, Blay JY, Chawla SP, Kim DW, Bui-Nguyen B, Casali PG, Schöffski P, Aglietta M, Staddon AP, Beppu Y, Le Cesne A, Gelderblom H, Judson IR, Araki N, Ouali M, Marreaud S, Hodge R, Dewji MR, Coens C, Demetri GD, Fletcher CD, Dei Tos AP, Hohenberger P; EORTC Soft Tissue and Bone Sarcoma Group; PALETTE study group. Pazopanib for metastatic soft-tissue sarcoma (PALETTE): a randomised, double-blind,

placebo-controlled phase 3 trial. Lancet. 2012;379(9829):1879-86.

25 Miyamoto S, Kakutani S, Sato Y, Hanashi A, Kinoshita Y, Ishikawa A. Drug review: Pazopanib. Jpn J Clin Oncol. 2018;48(6):503-13.

26 Luo Q, Beaver JM, Liu Y, Zhang Z. Dynamics of p53: A Master Decider of Cell Fate. Genes. 2017;8:66. doi: 10.3390/genes8020066.

27 Rioux-Leclercq N, Turlin B, Bansard J, Patard J, Manunta A, Moulinoux JP, et al. Value of immunohistochemical Ki-67 and p53 determinations as predictive factors of outcome in renal cell carcinoma. Urology. 2000;55(4):501–5.

28 Okazaki T, Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. Int Immunol. 2007;19:813-24.

29 Yamazaki Y, Morita T. Molecular and functional diversity of vascular endothelial growth factors. Mol Divers. 2006;10(4):515-27.

30 He S, Fei M, Wu Y, Zheng D, Wan D, Wang L, Li D. Distribution and clinical significance

of Th17 cells in the tumor microenvironment and peripheral blood of pancreatic cancer patients. Int J Mol Sci. 2011;12:7424–37.

31 Hiraoka K, Miyamoto M, Cho Y, Suzuoki M, Oshikiri T, Nakakubo Y, Itoh T, Ohbuchi T, Kondo S, Katoh H. Concurrent infiltration by CD8+ T cells and CD4+ T cells is a favourable prognostic factor in non-small-cell lung carcinoma. Br J Cancer. 2006;94:275–80.

32 Connell CM, Doherty GJ. Activating HER2 mutations as emerging targets in multiple solid cancers ESMO Open. 2017;2(5):e000279. doi: 10.1136/esmoopen-2017-000279.

33 Maiti GP, Mondal P, Mukherjee N, Ghosh A, Ghosh S, Dey S, et al. Overexpression of EGFR in head and neck squamous cell carcinoma is associated with inactivation of SH3GL2 and CDC25A genes. PloS one. 2013;8(5):e63440 10.1371/journal.pone.0063440.

34 Tazzari M, Negri T, Rini F, Vergani B, Huber V, Villa A, Dagrada P, Colombo C, Fiore M, Gronchi A, Stacchiotti S, Casali PG, Pilotti S, Rivoltini L, Castelli C. Adaptive immune contexture at the tumour site and downmodulation of circulating myeloid-derived suppressor cells in the response of solitary fibrous tumour patients to anti-angiogenic therapy. Br J Cancer. 2014;111(7):1350-62.

35 Lee SJ, Kim ST, Park SH, Choi YL, Park JB, Kim SJ, Lee J. Successful use of pazopanib for treatment of refractory metastatic hemangiopericytoma. Clin Sarcoma Res. 2014;4:13. doi: 10.1186/2045-3329-4-13. eCollection.

36 Denhart BC, Guidi AJ, Tognazzi K, Dvorak HF and Brown LF: Vascular permeability factor/vascular endothelial growth factor and its receptors in oral and laryngeal squamous cell carcinoma and dysplasia. Lab Invest. 1997;77:659–64.

37 Maruzzo M, Martin-Liberal J, Messiou C, Miah A, Thway K, Alvarado R, Judson I, Benson C. Pazopanib as first line treatment for solitary fibrous tumours: the Royal Marsden Hospital experience Clin Sarcoma Res. 2015;5:5. doi: 10.1186/s13569-015-0022-2. eCollection.

Figure 1-1: White, elastic, and soft mass occupying the right ear canal.

Figure 1-2: A pure tone hearing test shows a mild sensorineural threshold increase in the left ear and an advanced conductive deafness with an average threshold of 100 dB in the right ear.

Figure 2-1, 2-2: In the right external auditory meatus, T1WI low signal (Fig 2-1) and a T2WI high signal shows a TWI 28×10 mm tumor lesion with non-uniform contrast effect (Fig 2-2).

Figure 2-3: A lesion of 18×25 mm is observed from the base of the middle fossa to the external auditory meatus and the opening of foramen ovale. Note the destruction of bone around the foramen ovale, spinous foramen, and carotid canal.

Figure 3

CD34(-), STAT6(+), CD99(+), bcl-2(+), vimentin (+), and hematoxylin-eosin staining shows a large number of cell divisions (9 cell divisions/HPF). The diagnosis is Solitary fibrous tumor, Grade III.

Figure 4-1: One month after two cyber knife surgeries were performed, magnetic resonance imaging shows a tendency for tumor growth from the middle fossa to the

external auditory meatus.

Figure 4-2: Further tumor growth is observed on magnetic resonance imaging after 10 courses of Cetuximab.

Figure 5-1: Magnetic resonance imaging before using pazopanib.

Figure 5-2: A magnetic resonance image 3 months after the start of pazopanib shows an increase in the tumor mass from the right middle cranial fossa to the foramen ovale, but the irregular mass in the external auditory meatus has decreased.

Figure 5-3: A further tumor growth is observed on magnetic resonance imaging 6 months after starting the treatment, when the dose of pazopanib has been reduced due to side effects.

Figure 6-1: After four courses of nivolumab treatment, magnetic resonance imaging shows further tumor growth.

Figure 6-2: The tumor has significantly increased from the ear canal to the outside and

shows a tendency to bleed.

Figure 7-1: The upper row shows immunostaining findings of solitary fibrous tumor tissue before pazopanib treatment, the middle row after pazopanib treatment, and the lower row after nivolumab treatment.

Figure 7-2: The positive and negative immunostaining findings during the course are tabulated. Ki-67, programmed cell death-ligand 1 (PD-L1), vascular endothelial growth factor (VEGF), CD8, and epidermal growth factor receptor (EGFR) are all positive, but p53 is negative. PD-L2 is positive before treatment with pazopanib, but becomes negative thereafter. PD-L1 becomes negative once after treatment with pazopanib, but becomes positive after administration of nivolumab. CD4 is positive until after pazopanib administration, but then becomes negative. Her2 is positive only after administration of pazopanib.