Case report

Effective treatment of anlotinib in giant delayed pulmonary metastasis of osteosarcoma: a case report and literature review

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Abstract: Tumor relapse and pulmonary metastasis, especially unresectable lesions, are the major cause of poor prognosis of patients with osteosarcoma. Anlotinib, a novel small-molecule tyrosine kinase inhibitor (TKI), has been proved to have desirable anti-tumor effects via blocking VEGFR2 and PDGFRβ phosphorylation in several tumors, including non-small cell lung cancer and soft tissue sarcoma. In this study, we presented a case of giant delayed pulmonary metastasis of osteosarcoma which was effectively treated by anlotinib. CT scan of this patient showed a giant neoplasm with the size of 1,366 cm^3 in the left lung, clinically diagnosed as pulmonary metastasis of osteosarcoma. Due to refusing to chemotherapy and not eligible for surgery of the giant neoplasm, anlotinib was recommended. As a result, the tumor volume decreased more than 82% during 24-week anlotinib administration, from 1,366 to 247 cm^3. Unfortunately, disease progression was observed at 27-week. Although argon-helium cryoablation (AHC) was performed followed by apatinib administration, the patient was dead in 16 weeks after disease progression. The progression-free survival (PFS) and overall survival since anlotinib administration of this patient was 27 weeks and 43 weeks, respectively. The toxicity included hypertension, fatigue and hand-foot skin syndrome in grade 1–2, which were controllable and well tolerated. Meanwhile, immunohistochemical staining showed that the expression of VEGFR2 and PDGFRβ was decreased significantly and the whole exon sequencing revealed that c-MYC was duplicated, which was potentially associated with anlotinib resistance. Anlotinib had promising anti-tumor efficiency in the treatment of delayed pulmonary metastatic osteosarcoma. However, the potential mechanism of anlotinib resistance and the subsequent therapy after resistance were still challengeable and needed further investigation.

Keywords: Osteosarcoma; delayed lung metastasis; anlotinib; tyrosine kinase inhibitor (TKI); tumor volume

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Introduction

Osteosarcoma is the most common primary bone sarcoma in adolescent and young adult which usually diagnosed with a peak incidence in the second decade of life. The major cause of death of these patients was lung metastasis which occurred in approximately 18% of patients at the time of initial diagnosis and about 30% of patients during treatment period. Patients who underwent standard multimodal therapeutic strategy, including pre- and postoperative

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chemotherapy combined with surgery, achieved a 5-year event-free survival (EFS) of 60–70% (1). However, the conventional chemotherapy regimens are not satisfactory for the treatment of metastatic osteosarcoma, with 5-year EFS of 28–33% (2). Even varieties of regimens including first- and second line chemotherapy had been considered, the dismal disease control rate (DCR) was was mostly under 50% with severe toxicity (3-6). Additionally, limited by the controversial results in osteosarcoma, the macrophage activator mifamurtide was not approved in metastatic patients (7). Therefore, how to effectively treat pulmonary metastasis of osteosarcoma remains a great challenge.

Angiogenesis plays a crucial role in tumor progression and other pathological process such as inflammatory. During the oncogenesis, tumor cells need blood supply via angiogenesis to supply nutrients and oxygen to form a tumor lesion, while release several angiogenic factors including VEGF, PDGF and chemokines (8). Therefore, reported by varieties of clinical trials, the anti-angiogenesis tyrosine kinase inhibitors (TKIs) had shown better antitumor efficiency in advanced primary bone and soft tissue sarcoma compared with conventional agents in the last two decades. Grignani *et al.* performed a phase II cohort trial with advanced osteosarcoma, and demonstrated that sorafenib or sorafenib plus everolimus had promising activity as a second-line treatment with the mean progression-free survival (PFS) of 4 months, even though it didn't achieve the prespecified target of 6-month PFS of 50% (45%, 95% CI, 28–61%) (9,10). What's more, in a double-blind, placebo-controlled phase 2 study of regorafenib in metastatic osteosarcoma, 8-weeks PFS rate in control group was 65% (17/26) compared with 0% (0/12) in placebo group (11).

Anlotinib (CTTQ Pharma, Lianyungang, China), a novel oral multi-targeted TKI of VEGFR1-3, PDGFRα/β, FGFR1-4, c-Kit, RET, c-FMS, which will block the downstream signal pathway and suppress the tumor proliferation, angiogenesis, migration and metastasis (12). In addition to be approved by China Food and Drug Administration for the third-line treatment of non-small cell lung cancer (13), this agent acquired the indication for advanced soft tissue sarcoma with the median PFS and overall survival of 5.6 and 12 months, respectively (14). In osteosarcoma, a preclinical trial using in vitro and vivo models performed by Wang *et al.* indicated that anlotinib can suppress tumor growth, increase chemosensitivity and inhibit migration and invasion (15). In this study, we presented a case of giant delayed lung metastasis of osteosarcoma treated by anlotinib with effictive response.

We present the following article in accordance with the CARE reporting checklist (available at http://dx.doi.org/10.21037/apm-20-1790).

Case presentation

Male, 18 years old, admitted on April 5, 2012, complaining of local distending pain without apparent trigger of right knee, rubefaction, warm, swelling, or edema. Plain radiographs showed an exogenous neoplasm of right distal femur with irregular bone destruction and periosteal reaction. Magnetic resonance imaging (MRI) revealed a mass with the size of 4×3.5×1.5 cm^3 which was hypointense on T1-weighted images and exhibited a heterogeneous high-intensity signal on fat-suppressed T2-weighted images, suggesting malignant tumor (*Figure 1A*). Computed tomography (CT) of the chest showed no pulmonary lesions. By histopathological examination, the patient was diagnosed as common osteosarcoma.

Neoadjuvant chemotherapy (NAC) was performed each three weeks following the strategy of AP regimen (doxorubicin, 60 mg/m^2, d1–d3; cisplatin, 100 mg/m^2, d1), alternating with IFO (10 g/m^2, d1–d5), and high-dose methotrexate (HD-MTX, 10 g/m^2, d1, rescued with calcium folinate) (the so-called MAPI regimen). According to the response evaluation with MRI, we concluded the disease was progressed by reason of 25% increasing of diameter after NAC (*Figure 1B*). Therefore, NAC was terminated and the patient received complete tumor resection (R0), and the pathological examnation revealed that the tumor necrosis rate was 60–70% (*Figure 1C*). Postoperative chemotherapy was performed for 9 cycles (AP alternating with IFO) after operation.

Ten months after initial surgery, imaging examination revealed a intramuscular mass with the size of 1.5×2 cm^2 beneath the surgical incision (*Figure 1D*). The mass was resected completely and the postoperative pathological diagnosis was osteosarcoma. Unfortunately, the patient had a second local recurrence two months later and received R0 resection. Whereafter, he was recruited in a randomized clinical trial to receive docetaxel (1 g/m^2, d1,8) combined with lobaplatin (75 mg/m^2, d1,8) for 5 cycles.

After a symptom-free interval of 5 years, the patient re-hospitalized due to increasing cough and dyspnea for two months. A chest CT revealed a giant neoplasm with the size of 13.2×10.7 cm^2 in the pulmo dexter crossing the upper and lower lobe with a small pleural effusion. At this time,

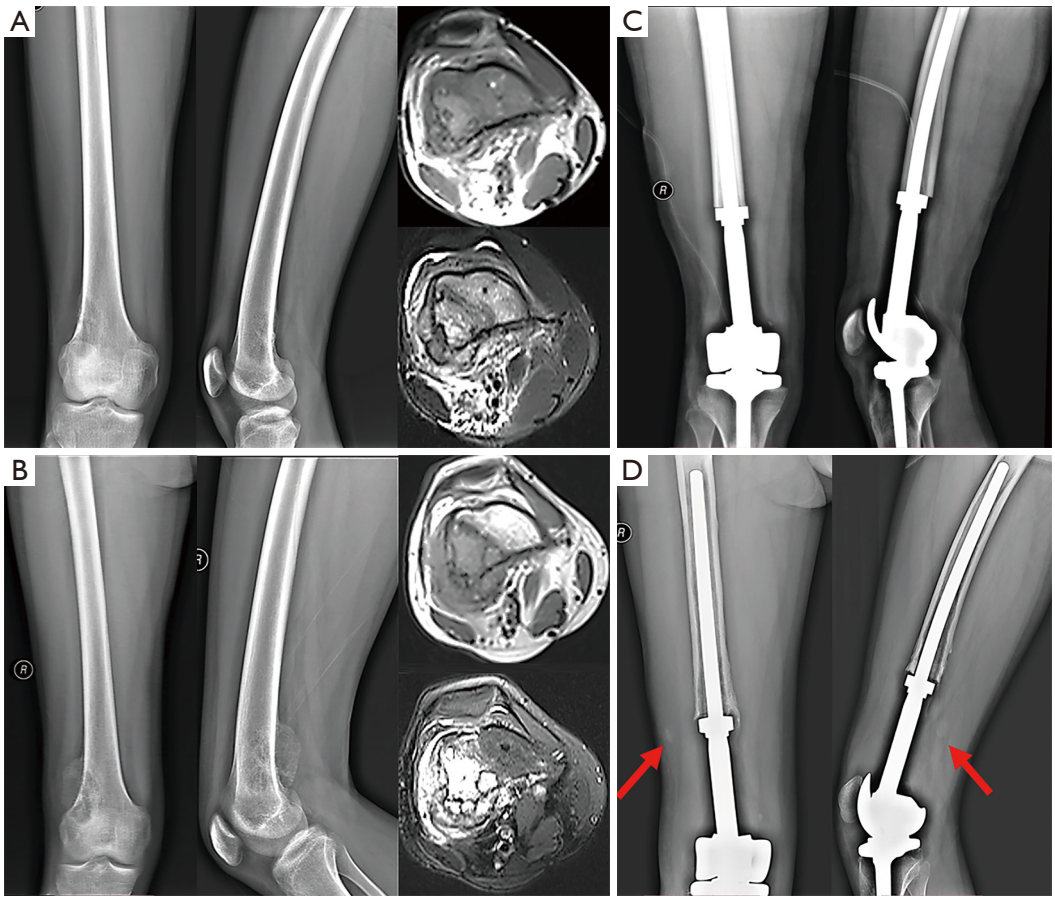

Figure 1 Images of the patient. (A) X-rays and MRI before NAC. (B) X-rays and MRI after 3 times chemotherapy. (C) Postoperative X-rays. (D) X-rays of the first local recurrence at 10 months after surgery. Red arrows indicated the lesion of relapse.

the Eastern Cooperative Oncology Group Performance Status (ECOG PS) of this patient was 3. Based on his medical history and CT imaging features, the patient was highly considered as delayed pulmonary metastasis of osteosarcoma. Considering patient's poor physical condition which was intolerable for invasive surgery and his refusal to receive chemotherapy, multidisciplinary consultation recommended anlotinib with the schedule of 12 mg once daily on a 2-week on and 1-week off. Treatment response was evaluated by the unidimensional criteria extrapolated to volumes using Mimics Medical 20.0 software to reconstruct the 3D model of pulmonary neoplasm. Complete response (CR) indicated tumor disappearance, partial response (PR) indicated greater than 65% reduction in tumor volume, progressive disease (PD) indicated greater than 73% increase in tumor volume, and stable disease (SD) represented less than 65% reduction to less than 73% increase in tumor volume (16). Six weeks after anlotinib administration, SD with 30% shrinkage of volume was observed in this patient. CT images showed punctate calcifications, liquefactive necrosis and heterogeneous density. The lesion shrank persistently and more than 82% reduction considered as PR after 24 weeks treatment compared to the initial volume before anlotinib treatment, from 1,366 to 247 cm^3 (*Figure 2A*). Meanwhile, ECOG PS was recovered from 3 to 1.

Twenty-seven weeks after anlotinib administration, the tumor volume unfortunately increased 80% compared to that at 24-weeks, indicating the response result was PD. Reexamination of enhanced CT showed that the solid part of tumor was surrounded by liquid. Consequently, we concluded that the patient had developed drug resistance to anlotinib, and then the administration of anlotinib was suspended.

Subsequently, the argon-helium cryoablation (AHC), American Superconductor argon-helium surgical system

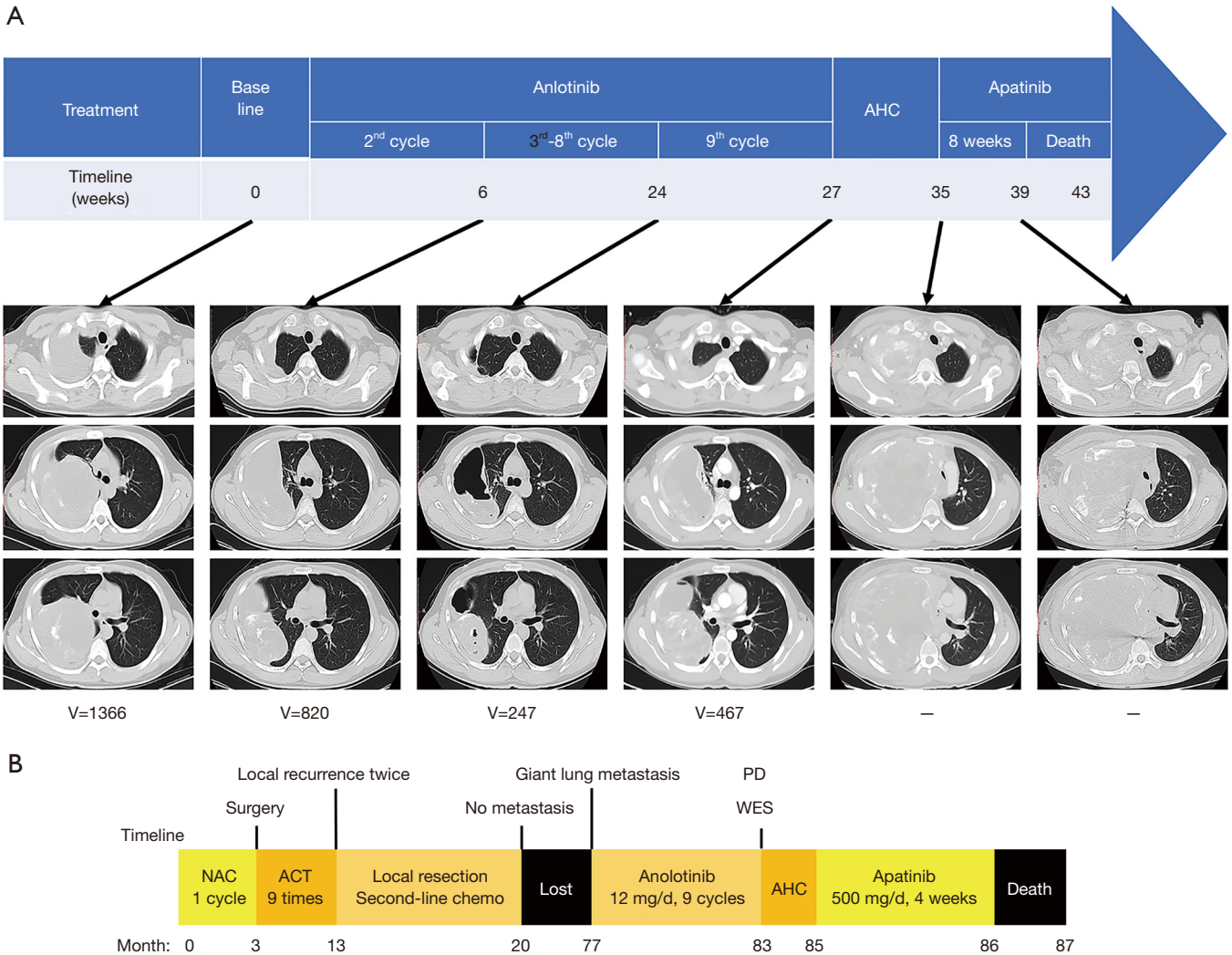

Figure 2 Therapeutic process of the patient. (A) CT imaging showed a shrinkage of tumor volume compared with the baseline at 2 and 6 months after anlotinib administration; PD at 7 and 9 months; SD at 10 months. (B) The various treatments the patients received as well as the duration of each treatment. NAC, neoadjuvant chemotherapy. ACT, adjuvant chemotherapy. PD, progression disease. WES, whole exome sequencing; AHC, argon-helium cryoablation.

equipped with 5 mm freezing probes, was recommended to reduce the tumor burden. It was frustrated that no symptom improvement was observed after AHC. In the meantime, ECOG PS deteriorated from 2 to 4, and the patient couldn't walk or work due to progressive chest pain and panting. Based on the results of an open label phase II clinical trial that showed apatinib, another TKI agent was sensitive to advanced osteosarcoma with a high response rate after failure of chemotherapy (17), the patient received apatinib, 500 mg/d. Although the CT scan revealed SD for 4 weeks, ECOG PS wasn't improved. Increasing dyspnea cause the death of the patient after 8 weeks of apatinib administration (*Figure 2B*).

During the treatment of AHC, biopsy specimen was obtained for the whole exome sequencing (WES) in order to detect potential sensitive drugs for this patient. The results of WES showed that varieties of genes were duplicated such as *ZFHX4*, *HNF4G*, *PKIA* which were associated with ovarian serous cystadenocarcinoma, pancreatic or cervical cancer (18-20), while the mutations of *VEGFR2*, *PDGFRβ*, *FGFR1* and *c-Kit* were negative in whether single nucleotide polymorphism (SNP) or copy number variation

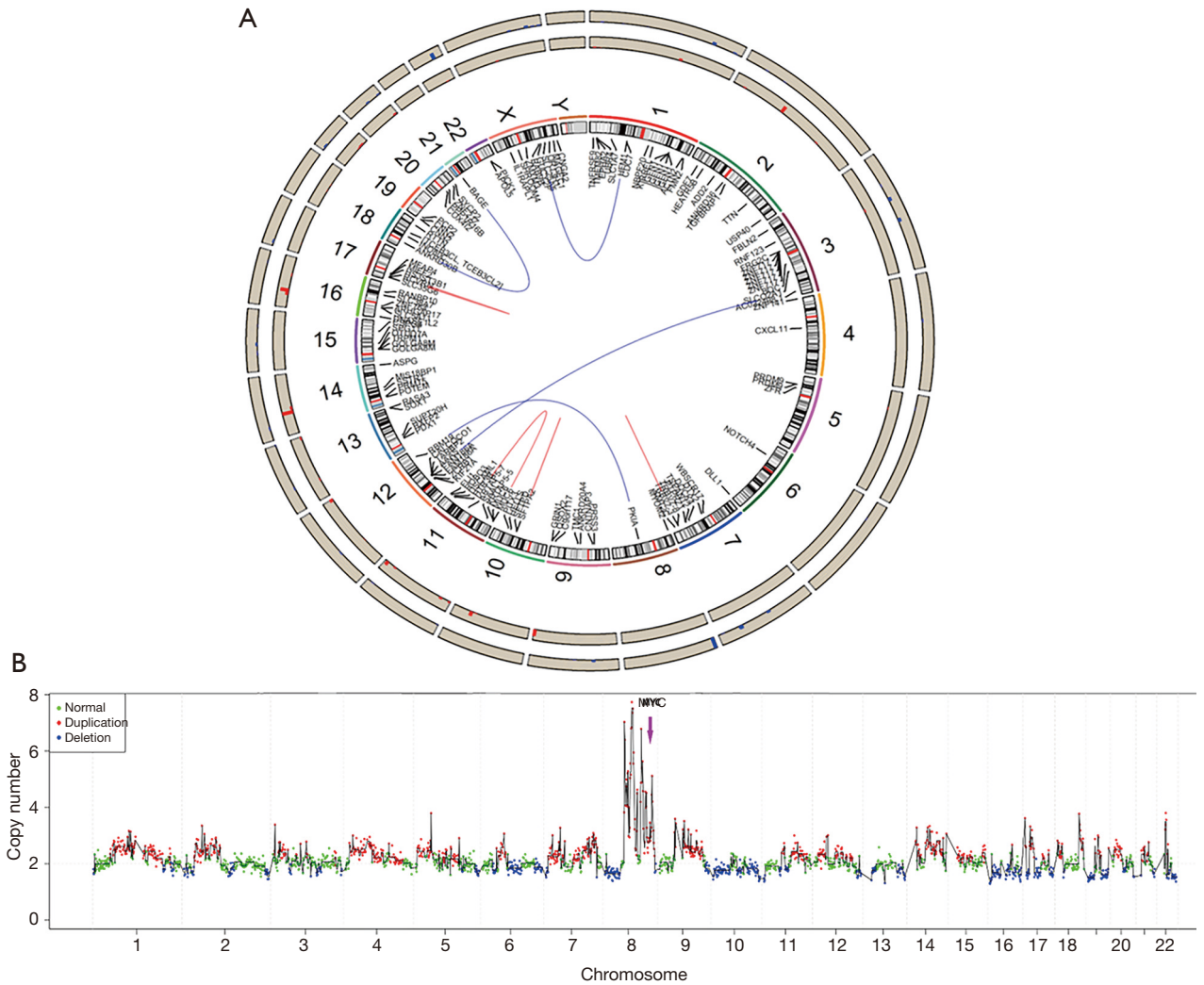

Figure 3 Circos and CNV plots of the patient sequenced by WES. The gene of c-MYC was duplicated with CNV >2. CNV, copy number variation; WES, whole exome sequencing.

(CNV). Particularly, *MYC* (8q24.21), a protein oncogene that can promote tumorigenesis in a wide range of tissues, was duplicated with CNV >2 (*Figure 3*). Retrospectively, we collected the tissues of this patient from the first surgery, first recurrence, last recurrence and lung metastasis after anlotinib administration to examine the expression of VEGFR2 and PDGFRβ via immunohistochemical (IHC) stain.

The anlotinib-related toxicities that patient had experienced include hypertension (grade 2, G2), fatigue (G2), hand-foot skin syndrome (G1), diarrhea (G1), serum apoliporotein A (G1) and thyroid stimulating hormone (G1) increase, and thyroxine reduction (G1). All adverse events were in grade 1–2 and tolerable. During apatinib administration, the patient suffered from the complications of fatigue and abdominal pain in grade 1.

All interventions performed in studies involving human participants were approved by the Ethics Review Board of Xijing Hospital (YS20181014-C-1) and in accordance with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient.

Discussion

With the development of multidisciplinary treatment, the standardized strategy of primary osteosarcoma is

based on the complete excision and sufficient pre- and postoperative multidrug chemotherapy, including HD-MTX, doxorubicin, cisplatin and IFO. Unfortunately, about 30–40% of patients with primary osteosarcoma will develop a local recurrence or/and pulmonary metastasis, which was usually occurred in the first 2–3 years after biopsy (median 1.6 years, range, 0.1–14.3 years), and 5% and 0.7% of recurrences develop in 6–10 years and thereafter (21). Long-term survival of patients with metastatic osteosarcoma is generally dismal. Durnali *et al.* reported that the 5-year and 10-year post-metastasis overall survival was 17% and 15% on patients with lung metastatic osteosarcoma (22). As the optimal strategy, complete pulmonary metastasectomy could improve survival time significantly (Hazard radio: 0.26; 95% CI: 0.13–0.51; P<0.001) (23). However, for patients with unresectable lesions, the role of chemotherapy is still controversial and no controlled studies are available. Whether combination of IFO and etoposide or carboplatin and etoposide, a better post-relapse survival was not observed in spite of the improvement of 6-month post-relapse EFS (from 0% to 9%) (24). Therefore, chemotherapy and radiotherapy are considered as palliative treatment for patients with unresectable lesions.

Although long-term cure is presently unachievable in unresectable disease, it's worthwhile to explore innovative approaches and take efforts to extend the survival. Anlotinib, a novel oral multi-targeted TKI that is domestically made in China, can not only target against PDGFRβ, c-Kit, and FGFR1, but also block VEGFR2 phosphorylation with high selectivity (median inhibition concentration <1 nmol/L). Lin *et al.* have reported that anlotinib inhibited angiogenesis via blocking tyrosine kinase phosphorylation and effecting on the expression of VEGFR2, PDGFR-β and FGFR1 (25). Recently, a retrospective experience of two centers in China, including 32 osteosarcoma and 78 other sarcomas, suggested that anlotinib had limited effectiveness in osteosarcoma patients compared with apatinib (objective response rate 7.69% *vs.* 15.79%; DCR 23.08% *vs.* 63.16%; median PFS 2.67±1.60 *vs.* 4.67±3.01) (26). However, in 2020 ASCO, a phase II study of anlotinib revealed the PFS and DCR in treating 29 patients with relapsed or metastatic osteosarcoma was 4.83 months and 79.3%, respectively (27). Although didn't reach the median PFS comparable to apatinib in the most of studies (7.9 months), anlotinib still achieved the similar DCR and lower incidence of severe adverse events (82.0–86.8% DCR in apatinib) (28,29). In this case, the volume of lung neoplasm was decreased sequentially during anlotinib administration with gradually improved ECOG PS. Overall, the overall survival of the patient was 7 years after diagnosis, and at least 10 months from lung metastasis. The PFS of anlotinib was 6.75 months compared with less than 3.5 months of second-line chemotherapy (4). Accordingly, the PFS in this case was similar with clinical studies of anlotinib and other TKIs (PFS of anlotinib, sorafenib, regorafenib and apatinib was 4.83, 4, 4.1 and 7.9 months, respectively) which indicated that anlotinib was alternative for the treatment of lung metastatic osteosarcoma (9, 11,27-29).

In spite of excellent efficiency, the limited response duration was urgently needed to be overcome which was challengeable and unavoidable in the application of anlotinib or other TKIs. However, there had no significant biomarker, as glutathione S-transferase P1 in mediating the chemosensitivity of osteosarcoma cells, which could promote the resistance of TKIs (30). In this patient, the response to apatinib was negligible after anlotinib resistance. In order to explore the mechanism of drug resistance, the priority was to characterize and validate the potential targets to predict therapeutic response. Chen *et al.* analyzed two gastric PDX models using apatinib and reveled that microvessel density (marked by CD31) was not only positively related to drug response, but also reduced after treatment. And afatinib inhibited tumor growth in the PDX models with EGFR amplification, EGFR overexpression or HER2 amplification (31). In osteosarcoma, afatinib could significiantly inhibit cell viabilitay and decrease phosphorylation of key components in the ErbB signaling pathway (32). Similar as previous research, we found that the positive rate of VEGFR2 and PDGFRβ decreased significantly after anlotinib administration (P<0.0001 and P<0.0001, respectively) which indicated that drug resistance might be caused by the reduction of these targets (*Figure 4*). Nevertheless, the variation of targets expression after anlotinib administration and its association with drug resistance needed further investigation.

MYC, a family of regulator genes and proto-oncogenes, is known to regulate biological functions such as cell cycle, apoptosis, and metabolism (33). *c-MYC* amplification occurres in more than 10% cases, which was more upregulated in metastatic samples and associated with poor prognosis, and played a role in osteosarcoma development and promotes cell invasion by activating MEK-ERK pathway (34,35). High expression of *MYC* promotes osteosarcoma cell proliferation, migration, clonogenicity and spheroid formation, which could be successfully inhibited by targeting *MYC*-driven super enhancers (CDK7

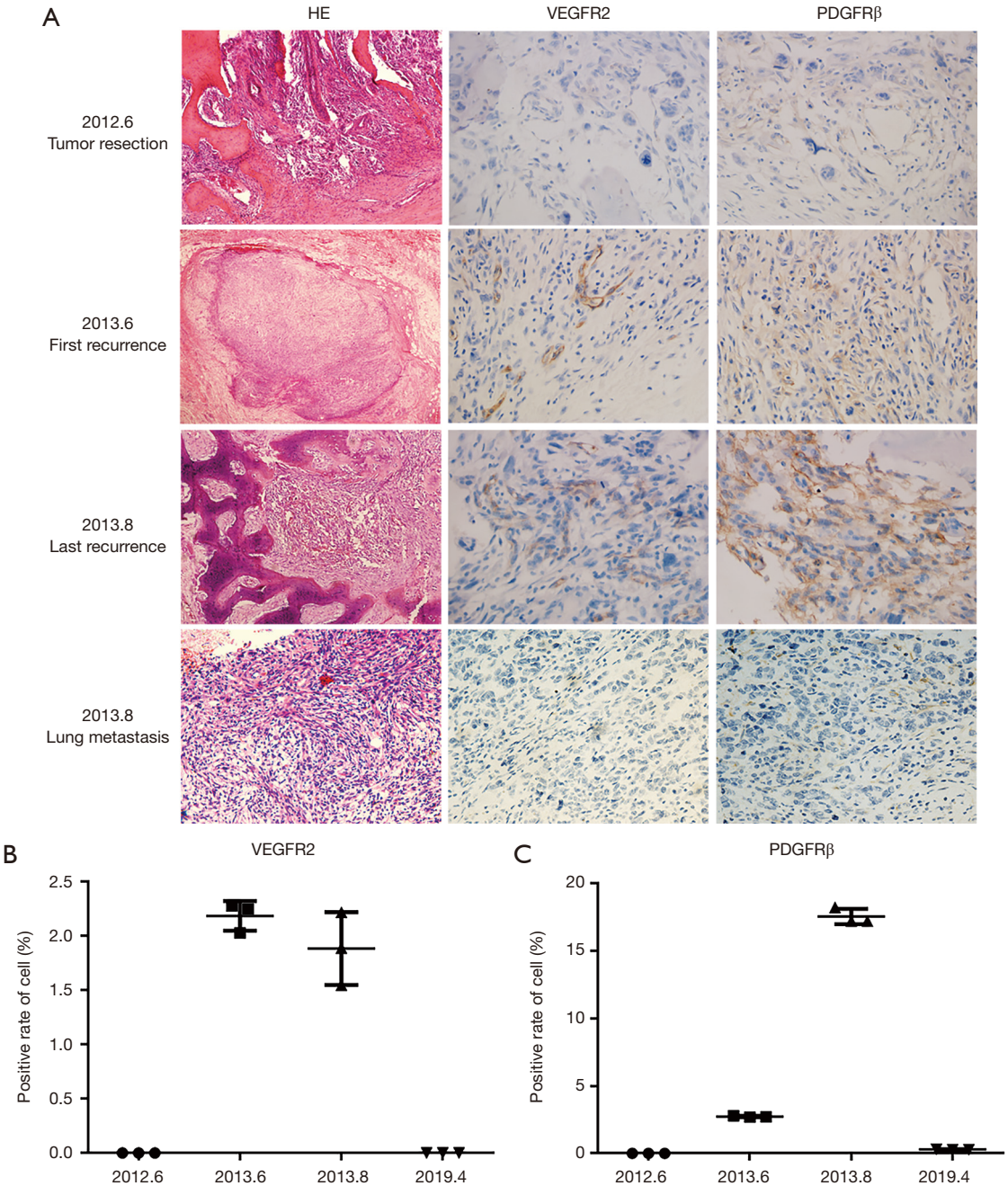

Figure 4 Histology and immunohistochemistry of tumor tissue from the first sugery, the first recurrence, the last recurrence and lung metastasis after anlotinib administration. (A) HE stain and immunohistochemistry of VEGFR2 and PDGFRβ, 200× magnification. The tumor cells are fiocally positive for VEGFR2 and PDGFRβ before anlotinib (×200, magnification), but the expression of these two targets were decreased after anlotinib. (B) Unpaired *t*-test analysis of VEGFR2 before and after anlotinib administration. (Average counting of positive cells: 0 *vs.* 37 *vs.* 70 *vs.* 0, P<0.0001). (C) Unpaired *t*-test analysis of PDGFRβ before and after anlotinib administration. (Average counting of positive cells: 0 *vs.* 315 *vs.* 190 *vs.* 67, P<0.0001).

and BET family) (36-38). Additionally, *MYC* amplification is potentially involved in mediating secondary resistance to anti-tumor therapies. Strippoli *et al.* evaluated the *c-MYC* expression in 121 metastatic colorectal cancer before treatment with anti-EGFR+Folfiri therapy and in 33 subsequent metastases collected during target therapy or in resistance phase. Patients with higher *c-MYC* expression, more frequent in the metastases after target therapy, showed a significant lower PFS and OS compared to low expression which indicated that *c-MYC* pathway regulation and the downstream *c-MYC* effector genes could provide a new possible target to overcome the anti-EGFR resistance (39). WES result of the patient in this case showed a large amount CNV in chromosome 8 but none of tumor-related gene except *MYC* (8q24.21, CNV >2) after anlotinib administration. However, a population-based analysis would be needed to explore the relevance of *c-MYC* and resistance of anlotinib or other TKIs in osteosarcoma.

The limitation of this study was the absence of pathological tissue prior to anlotinib administration. In addition, it was urgent to exploring the alternative therapies after drug resistance, and our attempts including AHC and other TKI showed no obvious improvement under this circumstance.

The results of this report suggested that lung metastasis of osteosarcoma would be benefited from anlotinib and the potential possibility of predicting response via targets expression levels. More efforts are needed to explore the correlation between targets expression and drug efficiency to identify the patients who will be benefited from anlotinib. Moreover, prolonging the duration of response and developing the treatment after drug resistance needs further investigation and c-MYC associated genes was a potential target to overcome TKI resistance in osteosarcoma.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/apm-20-1790). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All interventions performed in studies involving human participants were approved by the Ethics Review Board of Xijing Hospital (YS20181014-C-1) and in accordance with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient.

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