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Endostar as a Perioperative Regimen with Chemotherapy in Osteosarcoma: A Review

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Abstract: This study was conducted to comprehensively review the efficacy and safety of endostar combined chemotherapy versus chemotherapy alone, for treating osteosarcoma patients. Osteosarcomas, which makes up less than 1% of all cancers in adult and about 13% of sarcomas, can rise from anywhere in the body. The current treatment for osteosarcoma comprises of surgery, radiotherapy and chemotherapy. As for resectable localized osteosarcoma, the standard treatment is surgical resection with adequate margins preserved. Despite of surgery, about half of the patients will suffer from recurrence and metastasis of osteosarcoma, thus requiring systemic chemotherapy as palliative treatment. Apart from Ewing's Sarcoma, osteosarcoma and rhabdomyosarcoma, most sarcomas are not sensitive enough to systemic chemotherapy resulting in cancer progression through blood circulation. Hence, the potential use of anti-angiogenic agent endostar in the treatment of sarcoma is being highly studied in clinical trials. Target therapy drug endostar was found to be both safe and effective when combined with chemotherapy as a perioperative regimen for resect-able advanced osteosarcoma and can benefit patients who cannot tolerate aggressive therapy signifying better tolerability of chemotherapeutic drugs when administered with endostar while also significantly improving the distant metastasis free survival and progression free survival in osteosarcoma patients. Confirmation of these results in larger double blinded controlled randomized trials is necessary before definite conclusions about this therapy can be drawn.

Keywords: Endostar, sarcoma, chemotherapy, Rh-endostatin, therapy.

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Introduction

Osteosarcoma, a cancerous mesenchymal tumour of the bone, makes up about 35% of primary bone cancers which are extremely rare neoplasms and make up approximately 13% of all sarcomas. Other common subtypes of primary bone tumors aside osteosarcoma include chondrosarcoma (30%), Ewing sarcoma (16%), malignant fibrous histiocytoma of bone (<5%), fibrosarcoma of bone (<2%) and giant cell tumor of bone [25, 29]. Osteosarcoma remains the most common bone sarcoma in children and adolescents. Recurrence occurs in about 30% of localized osteosarcoma patients and 80% of patients with metastatic sarcoma, are cancers that arise from the mesenchymal layer. Osteosarcoma makes up less than 3% of paediatric cancers, and less than 2% of adolescent and young cancer patients [1]. More than 100 different subtypes of sarcoma based on their histology, clinical presentation, age of incidence, aggressiveness, the way they metastasize, genetic changes, and their response to certain treatment have been identified in adults and paediatrics [2]. Sarcoma can arise from anywhere in the body, but most commonly happens in the extremities. Approximately 20% of patients with extremity sarcoma will have isolated pulmonary metastatic disease during the course of their disease [3]. Almost half of bone sarcomas and about a fifth of soft tissue cancers are diagnosed in people below thirty five years of age [4]. The 5-year relative survival rate was found to be approximately 65 percent for all sarcomas and Sun Min Lim et al reported no significant difference in overall Survival and Event Free Survival observed between adults and children [5, 51]. Only 16% of patients that undergo surgical resection alone survive for long period, indicating the existence of micrometastasis in a large majority of newly diagnosed patients [30, 31]. Prognosis and cure rate depend on the type of tumour, location, size and other factors [32]. In general the prognosis of recurrent osteosarcoma is poor, with long-term survival of less than 20%. The 2 year DFS rate for osteosarcoma patients with 3 or more metastatic lesions was 28% [31]. Up to date no effective Chemotherapy treatment is available for chondrosarcoma and for most subtypes of chordoma [32, 33]. Early preclinical studies in osteosarcoma have confirmed that the combination of Endostar with chemotherapy can significantly inhibit sarcoma progression and lung metastases [34-36].

The rarity and the diversity of histologies render the diagnosis and treatment of sarcoma difficult. Sarcomas patients generally need multimodal therapy, consisting of aggressive chemotherapy, high-dose radiation and surgery wherever possible. Yet, metastatic sarcoma patients still endure a dismal prognosis, with low 5-year overall survival

(OS) rates not exceeding 30%. Since Rutkowski and colleagues showed that cytokine VEGF (vascular endothelial growth factor) level is high in soft tissue sarcoma, the potential use of anti-angiogenic agents in the treatment of sarcoma is being highly studied in clinical trials [6,7]. One of these anti-angiogenic agents is Endostar. Endostar though primarily inhibits angiogenesis, also inhibits lymphangiogenesis and lymph node metastasis [8, 9]. Previous studies also showed that Endostar was effective when used in combination with cytotoxic chemotherapy drug where Endostar exhibited synergistic effects [10].

This first review on Endostar on osteosarcoma patients comprehensively assesses from clinical trials the efficacy and the toxicity of anti-angiogenic agent Endostar in combination with chemotherapy for the treatment of osteosarcoma. A deeper analysis on the chemotherapy regimen combined and the adverse events will provide essential guides for clinicians to avoid certain cytotoxic drug in case the patients cannot tolerate these specific adverse events such as myelosuppression.

1. Target therapy drug Endostar

Rh-endostatin (code number YH-16, commercial name Endostar), commercialized by Simcere-Medgenn Bioengineering Co. Ltd located in China, is a different and more stable recombinant human endostatin (Rh-endostatin) compared to the original endostatin studied by O'Reilly[8,11]. Endostar (YH-16) gained approval from the China's State Food and Drug Administration (SFDA) for treating NSCLC in 2005 [12, 13]. Ever since, Endostar, the N-terminal modified recombinant human endostatin, is regarded as one of the most effective angiogenesis inhibitors. Angiogenesis consists of a dynamic balance between proangiogenic factors such as vascular endothelial growth factor (VEGF) and anti-angiogenic factors such as thrombospondin-1 [14, 15]. Endostar can suppress tumors vascular endothelial cell proliferation, inhibiting angiogenesis and by depriving the tumor of vascular supply inhibit tumor growth [16]. As shown in many preclinical studies, the combination of anti-angiogenic therapy in combination with chemotherapy may be a good strategy for sarcoma.

A comprehensive electronic search on databases such as pubmed, google scholar, Wanfangdata, CNKI and Embase using the following keywords "Sarcoma", "osteosarcoma, "bone tumour" both in English and Chinese were effected before January 2016 by at least two researchers before analysing and extracting the necessary data. Only clinical trials were included, and only of them is a randomized controlled trials.

2. Pre-clinical studies of Endostar against osteosarcoma

2.1 Cellular model research

The study of endostar combined with Adriamycin on osteosarcoma OS-732 cells demonstrated inhibiting effect against tumour cells. Other experiments found that Endostar inhibited the proliferation of rhabdomyosarcoma cells and plays a key role in suppressing the expression and production of VEGF in rhabdomyosarcoma PLA-802 cells.. Another study by Liming Zhang, Rh-endostatin had anti-cancer effect on MG-63 osteosarcoma cells both independently and in combination with cisplatin and therefore further research on these promising studies can be vital to prolong lifespan of cancer patients [17-19].

2.2 Animal model research

One study by Xian-liao Zhang and Xin Shi et al demonstrated that Endostar had anti-tumour effect on sarcoma xenograft decreasing cancer angiogenesis, infiltration and metastasis in the process. Additional study demonstrated synergistic effect of Rh-endostatin with either Adriamycin or vincristine in the inhibition of tumor growth by approximately 64.8% on osteosarcoma mouse model. Wang TB et al also showed significant ($P < 0.0001$) tumor regression in Endostar treated group with reduced level of MVD and bcl-2-positive rate in the intervention group concluding that Endostar can supress angiogenesis, favour cell apoptosis, and supress the proliferation of sarcoma xenograft. Mingwei Yang's study showed that endostar combined with radiotherapy group decreased the MVD (microvessel density) and angiogenesis [20-24].

3. Clinical research of Endostar on osteosarcoma

A first non-randomized phase I clinical trial on 17 osteosarcoma patients by Zhou Xing and al comparing the clinical benefit response, ECOG quality of life scores and adverse reactions showed that Endostar exhibited anti-tumor activity with high clinical benefit response in osteosarcoma patients without overt mestastasis. The experimental group was treated with a 6-weeks cycle of perioperative regimen with one preoperative cycle and 3 cycles of postoperative therapy consisting of 15mg $2.4x10^5$ units Endostar, methotrexate, cisplatin, doxorubicin/pirarubicin, and cyclophosphamide. The Clinical benefit rate (CBR) of the experimental group was 100% while the control group could only reached 71.4%. Both the results of the CBR and the Quality of life scores from the experimental group was not statistically

significant which could be because of the few cases investigated. The main grade 3 and grade 4 symptoms reported were leukopenia, neutropenia, hemoglobinemia, thrombocytopenia and rise in liver enzymes AST and ALT. The comparison of adverse events between the two groups was not statistically different.[37] A following Phase II randomized clinical trial by Zhang Yan et al on 76 osteosarcoma patients demonstrated a clinical effective rate of 71% based on WHO evaluation criteria in the experimental group compared to 51.3% in the control group (P =0.049). Seventy five percent of the patients who in the observation group were treated with 2 cycles of neoadjuvant 15 mg Endostar c.i.v d1-14 combined with DIA regimen, had Enneking stage II osteosarcoma and 29% of them achieved complete response after resection and chemotherapy. The 5 year overall survival of about 55% was significantly higher in the test group (P>0.048) though more patient cases in the test group were lost and remained unaccounted during the follow up. In comparing the limb function based on the Musculoskeletal Tumour Society 93 evaluation criteria, the Endostar treated group had a superior post chemo limb function that was statistically significant (x2 = 2.92, P = 0.04). The main adverse events observed in the experiment group were leukopenia, nausea and vomiting, alopecia as well as liver and renal damage and comparison between the two groups was not statistically significant (P>0.05) [38].

In a larger phase II trial conducted by Meng Xu and colleagues on the effects of endostar combined chemotherapy in the treatment of 116 high-grade stage II osteosarcoma patients showed an increase in event free survival in the endostar treatment group. Perioperative regimen included 2 cycles of preoperative therapy and 2 cycles of postoperative therapy comprising of doxorubicin 60 mg/m2 per day IV bolus ×2 days; cisplatin 120 mg/m2 per day × 2 days; methotrexate 12 g/m2 per day and Endostar 15 mg per day × 5 days in one cycle. The 5 year overall survival rate in the endostar treatment group was 76% (p=0.338) while the 5 year event free survival rate 5-yr EFS, at 70% in the test group compared to 56% in the control group, was significantly increased by the Endostar treatment (P = 0.043). Additionally, the study showed that the metastasis rate in the Endostar treated group was 24%, which was significantly lower than the 34% in the control group [39]. The individual's age, tumor location, and histologic response were link more strongly to Metastasis Free Survival (MFS) in osteosarcoma model than gender, size of neoplasm, or pathologic fracture in a study by Koichi Ogura et al but no significant difference was found in respect to most of these characteristics between the two groups in this clinical trial. No data on adverse events was available [52]. Immunohistochemistry of the pre-chemotherapy biopsy and post-chemotherapy tumor resection specimens by Meng Xu et al showed that Endostar administration significantly inhibits the expression of VEGF and the increased microvessel density leading to a lower metastasis rate in Endostar treated patients [39, 53]. An earlier preclinical trial did demonstrate that the inhibition of VEGF signaling can drastically decreased pulmonary metastases in an osteosarcoma model [46]. Kaya et al. who reported significantly higher serum level of VEGF in patients with pulmonary metastases compared to those who did not develop metastases also showed similar increase of VEGF expression in metastatic sarcoma compared to non-metastatic sarcoma [44, 45]. Studies have demonstrated that hypoxia due to tumor necrosis by chemotherapy can promote the up-regulation of VEGF by viable tumor cells [47]. The combinations of Endostar with chemotherapy can downregulate the VEGF level induced by the viable hypoxic tumor cells after necrosis-induced hypoxia due to traditional chemotherapy drugs. Chemotherapy was shown to increase angiogenesis while Endostar treatment can significantly inhibit angiogenesis induced by chemotherapy in osteosarcoma patients [39].

Hairong Xu et al conducted a non-randomized control trial on 388 stage II osteosarcoma patients showing increased 1-year and 3-years metastasis-free survival rates of 93% and 77% in Endostar combined chemotherapy group compared to 79% and 65% respectively in the control group (P =0.045). The difference in 3-years overall survival rate was not statistically significant (P = 0.220) which could be because of cytotoxic drug resistance among other reasons but the 3-years progression free survival rate was statistically significant at 74% versus 60% (P = 0.025). The 1-, 2-, 3-year distant metastasis free survival rates (DMFS) was statistically significant at 90%, 86% and 77% respectively (P = 0.045) with more than 85% of cases being lung metastasis. Both arms were given two to four cycles of neoadjuvant therapy and 12-18 cycles of postoperative adjuvant therapy consisting of $10g/m^2$ methotrexate, 3g/m2 ifosfamide, 120mg/m2 cisplatin and 30 mg/m2 Adriamycin while 4 cycles of 15 mg Endostar was added to the test group postoperatively. Amputed patients who made up about 16% of the test group did not receive neoadjuvant treatment. Endostar was well tolerated as the difference in side effects between the two arms was not statistically significant. Adverse events reported were predominantly mild symptoms and grade 3-4

symptoms were mainly leukopenia, anemia, liver and renal damage, nausea and vomiting [40].

The quality of life of cancer patients and toxicity induced by chemotherapy are as important as efficacy, the consideration of tolerability and therapy discontinuation events are key in this setting. Xu Hairong et al and Zhang Yan et al reported Grade I and Grade II toxicities as being predominant in their studies. The reported symptoms measured in the different trials that involved different chemotherapy regimens were leukopenia, nausea and vomiting, liver and renal dysfunction, alopecia and mucosal lesion. Leukopenia was the most common adverse event among all the trials. No drug related death or discontinuation due to adverse events occurred among the different trials. Since no statistical significance in toxicity was observed between the test and control groups of the different clinical trials, the symptoms observed may not be related to Endostar. Actually the Endostar treated groups had a smaller percentage of patients reporting severe adverse events, possibly indicating better tolerance of chemotherapy when administered together with Endostar [37, 38, 40]. A similar observation where Endostar treated patients suffered less adverse events compared to control group was made by Rong Baoxue et al in a systematic review of Endostar combined chemotherapy in treating non-small cell lung cancer [50]. Endostar demonstrated cytotoxicity as a single agent, but was also found to be effective when used in combination with cytotoxic chemotherapy drug. Anti-angiogenic agents seem to lower the hydrostatic pressure of the tumour tissue, allowing the chemotherapy drugs to further drain into the tumour tissue. Another proposed mechanism is that suppression of angiogenesis in solid tumours develops a hypoxic microenvironment that increases the tumour cells' sensitivity to chemotherapy treatment [46]. This may have led to a smaller percentage of patients reported to suffer from mild to severe adverse events in the Endostar combined chemotherapy treated groups [38,40]. Hence Endostar combined with chemotherapy have shown synergistic effects that inhibit the growth of tumors by decreasing the formation of micro vessels and enabling greater delivery of chemotherapy drug into the tumor tissue. This can lead to a better local control of the malignant tumors.

Discussion

Progress in the field has historically been hampered by the relative rarity of osteosarcoma, and the significant heterogeneity that exists in histology [39]. Doxorubucin as single agent or in combination regimens remains the treatment of choice for advanced non-resectable soft tissue sarcoma. In a study regrouping three randomized clinical trials Ian Judson and al discouraged the use of combination therapy of doxorubicin and ifosfamide as palliative therapy for advanced sarcoma due to increased toxicity despite demonstrating an overall survival of 14.3 months which was not significantly superior to the overall survival of 12.4 months from doxorubicin alone in the EORTC trial. Similarly several other doxorubicin based chemotherapy regimens proved ineffective to significantly increase median overall survival due to increased adverse events and poor quality of life [51,52]. In the Hellenic Cooperative Oncology Group combined therapy trial of PL-Doxorubicin and paclitaxel in advanced STS achieved a median time to progression of 5.7 months and a median overall survival of 13.2 months [53,54]. The Aldoxorubicin trial did achieved a median Overall survival of 14.7 months for the Doxorubicin arm [55]. Seungcheol Kang and colleagues reported a 2-year survival of 47.1 ± 4.8% for advanced soft tissue sarcoma though 33% of the patients underwent metastectomy and multimodality treatment was available to all patients in the study while the patients that participated in the Endostar treated trial by Lu Ping Zhang and al were all unresectable advanced STS patients and yet achieved a two year survival rate of 30.2% [27, 56, 57].

Bacci and al who reported a 5 year overall survival of 66.6% across all types of primary bone tumors demonstrated that the 5 year event-free survival DFS was significantly associated with the histological reponse to chemotherapy[32]. For example, the EICESS Study Group reports a 5 year relapse-free survival RFS of 22% for Ewing's Sarcoma Family of Tumor (ESFT) patients [58,59]. The PALETTE trial has formally demonstrated the benefit of treating sarcoma patients with anti-angiogenic agent over placebo in terms of PFS in a Phase III setting [60]. With current treatment, localized osteosarcoma patients can hope for a 5-year survival rate in the range of 60% to 80%. In this review, one trial on 116 high-grade stage Ⅱ osteosarcoma patients reported a 5-year overall survival rate of 76%. Most importantly, a statistically significant 5-year Event Free survival of 70% and a significantly better limb function based on the Musculoskeletal Tumor Society functional score MSTS 93 evaluation criteria was achieved which can relate to a better quality of life in Endostar treated localized osteosarcoma patients. If the osteosarcoma has already metastasize upon diagnosis, the 5-year survival rate is about 15% to 30% but to date no such clinical trial on Endostar in treating advanced osteosarcoma are yet published.

Based on this review of trials conducted on localized osteosarcoma, Endostar combined with chemotherapy significantly increases the event-free survival rate as a perioperative regimen and in addition to enhancing the limb function also prevented metastases in resected osteosarcoma patients.

Conclusion

Target therapy drug Endostar is safe and effective when combined with chemotherapy for the treatment of late stage soft tissue sarcoma including patients who cannot tolerate aggressive therapies. The combination of chemotherapy and Endostar as a perioperative regimen can significantly improve the distant metastasis free survival and progression free survival in osteosarcoma patients. Larger double blinded randomized controlled trials need to be carried out for these conclusions to be confirmed.

Conflict of interest

The authors declare that they have no competing interests.

References

[1] Amankwah E, Conley P, Reed D. Epidemiology and therapies for metastatic sarcoma. Clin Epidemiol 2013; 5: 14.

[2] Roberts S, Chou A, Kong NV Cheung. Immunotherapy of Childhood Sarcomas. Front Oncol 2015; 5: 181.

[3] Liebl L, Elson F, Quaas A, Gawad K and Izbicki JR. Value of repeat resection for survival in pulmonary metastases from soft tissue sarcoma. Anticancer Res 2007; 27:2897-2902.

[4] Bethesda M, Ries L, Melbert D, Krapcho M, Mariotto A, Miller B et al. SEER Cancer Statistics Rev 1975-2004, National Cancer Institute website.

[5] Lim S, Yoo C, Han J, Cho YJ, Kim S, Ahn J et al. Incidence and Survival of Pediatric Soft Tissue Sarcomas: Comparison between Adults and Children. Cancer Res Treat 2015; 47(1):9-17.

[6] Rutkowski P, Kaminska J, Kowalska M, Ruka W, Steffen J. Cytokine serum levels in soft tissue sarcoma patients: correlations with clinico-pathological features and prognosis. Int J Cancer 2002; 100(4):463-71.

[7] Ling Y, Yang Y, Lu N, You Q, Wang S, Gao Y et al. Endostar, a novel recombinant human endostatin, exerts antiangiogenic effect via blocking VEGF-induced tyrosine phosphorylation of KDR/Flk-1 of endothelial cells. Biochem Biophys Res Commun 2007; 361(1):79-84.

[8] Jia Y, Liu M, Huang W, Wang Z, He Y, Wu J et al. Recombinant Human Endostatin Endostar Inhibits Tumor Growth and Metastasis in a Mouse Xenograft Model of Colon Cancer. Pathol Oncol Res 2012; 18:315–323.

[9] Fan J, Du J, Wu J, Fu S, Hu D, Wan Q. Antitumor effects of different administration sequences of cisplatin and Endostar on Lewis lung carcinoma. Oncol Lett 2015; 9(2): 822–828.

[10] O'Reilly M, Boehm T, Shing Y, Fukai N, Vasios G, Lane W et al. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. Cell 1997; 88: 277–85.

[11] O'Reilly M, Holmgren L, Shing Y, Chen C, Rosenthal R, Moses M et al. Angiostatin:a novel angiogenesis inhibitor that mediates the suppression of metastasis by a Lewis lung carcinoma. Cell 1994; 79: 315–28.

[12] Xu X, Mao W, Chen Q, Zhuang Q, Wang L, Dai J et al. Endostar, a novel recombinant humanendostatin, exerts antiangiogenic effect via blocking VEGF-induced tyrosine phosphorylation of KDR/Flk-1 of endothelial cells. Biochem Biophys Res Commun 2007; 361:79-84.

[13] Song H, Liu X, Zhang H, Zhu B, Yuan S, Liu S, Tang Z. Pharmacokinetics of His-tag recombinant human endostatin in Rhesus monkeys. Acta Pharmacol Sin 2005; 26:124-128

[14] Castro Junior G, Puglisi F, Azambuja E, El Saghir N, Awada A. Angiogenesis and cancer: a cross-talk between basic science and clinical trials (the "do ut des" paradigm). Critical Reviews in Oncology/Hematology 2006; 59(1):140–50.

[15] Folkman JN. Tumor angiogenesis: therapeutic implications. Engl J Med 1971; 285:1182-1186.

[16] Sun Y, Wang J, Liu Y, Song X, Zhang Y, Li K et al. Results of phase III trial of rh-endostatin (YH-16) in advanced non-small cell lung cancer (NSCLC) patient Journal of Clinical Oncology. 2005 ASCO Annual Meeting Proceedings 2005; 23(16S):7138.

[17] Xu H, Tian W, Niu X. In vitro study of recombinant human endostatin in combination with adriamycin on human osteosarcoma cell OS-732. Chin J Bone Tumor Bone Dis 2008; 7(3):51-59.

[18] Yang QP, Zhou Z, Yin Q, Luo X, Li S, Xiang M. To investigate the inhibitory effect of Endostar, an inhibitor of human vascular endothelium, on the growth of rhadomyosarcoma. Chin J Biologicals 2010; 11:1230-35.

[19] Zhang L. Effects of recombinant human vascular endostatin combined with cisplatin on the expression of VEGF and the proliferation and invasion ability of M G-63 osteosarcoma cells in vitro. Hebei Med J 2015; 17:850-854.

[20] Shi X, Zhang X. The effect of recombinant human endostatin combined with cisplatin on C57BL/6 mouse on sarcoma. Chinese Clin Oncol 2010; 33:127.

[21] Xu H, Niu X. Synergistic antitumor efficacy by combining adriamycin with recombinant human endostatin in an osteosarcoma model. Oncol Lett 2011; 2(5):773–778.

[22] Yang Q , Zhou Z , Lu B. Combined application of small dosages of vincristine and endostar inhibits transplanted rhabdomyosarcoma in nude mice. Chin J Biologicals 2012; 9:1156-1160.

[23] Wang T, Wei X, Lin W, Shi H, Dong W. The inhibition of Endostar on the angiogenesis and growth of gastrointestinal stromal tumor xenograft. Clin Exp Med 2012; 12(2):89-95.

[24] Yang M, Yang L. Enhancement of endostar on the radiosensitivity of human rhabdomyosarcoma xenograft. Chinese Clin Oncol 2013; 12:1066-05.

[25] Burningham Z, Hashibe M, Spector L Schiffman J. The Epidemiology of Sarcoma. Clin Sarcoma Res 2012; 2:14-23.

[26] Judson I, Peake D, and Soddon B. Guidelines for the Management of Soft Tissue Sarcomas Robert Grimer. Sarcoma Vol 2010; 50:6182.

[27] Lu-PZ, Liao XY, Xu YM, Yan GF, Wang XX et al. 2013 Efficacy and Safety of Endostar® Combined with Chemotherapy in Patients with Advanced Soft Tissue Sarcomas. Asian Pacific J Cancer Prev 2013; 14(7):4255-4259.

[28] Tuma RS. Sometimes Size Doesn't Matter: Reevaluating RECIST and Tumor Response Rate Endpoints. J Natl Cancer 2006; 98(18):1272-1274.

[29] Biermann JS, Farooki A, Fournier M, Gralow J. Bone Cancer. J Natl Compr Canc Netw 2013; 11:688-723.

[30] Link MP, Goorin AM, Miser AW, Green A, Pratt C, Belasco J et al. The effect of adjuvant chemotherapy on relapse-free survival in patients with osteosarcoma of the extremity. N Engl J Med 1986; 314(25):1600–1606.

[31] Eilber F, Giuliano A, Eckardt J, Patterson K, Moseley S, Goodnight J. Adjuvant chemotherapy for osteosarcoma: a randomized prospective trial. J Clin Oncol 1987; 5(1):21–26.

[32] Bacci G, Mercuri M Mercuri M, Longhi A, Ferrari S, Bertoni F et al. Grade of chemotherapy-induced necrosis as a predictor of local and systemic control in 881 patients with non-metastatic osteosarcoma of the extremities treated with neoadjuvant chemotherapy in a single institution. Eur J Cancer 2005; 41(14):2079-85.

[33] Bacci G, Briccoli A, Ferrari S, Saeter G, Donati D, Longhi A et al. Neoadjuvant chemotherapy for osteosarcoma of the extremities with synchronous lung metastases: treatment with

cisplatin, adriamycin and high dose of methotrexate and ifosfamide. Oncol Reports 2000; 7(2):339-342.
[34] Fuchs B, Dickey ID, Yaszemski MJ, Inwards C, Sim FH. Operative management of sacral chordoma. J Bone Joint Surg Am 2005; 87:2211-2216.
[35] Fleming GF, Heimann PS, Stephens JK, Simon M, Ferguson M, Benjamin R et al. Dedifferentiated Chordoma. Response to aggressive chemotherapy in two cases. Cancer 1993;72:714-718
[36] Denny CT. Gene rearrangements in Ewing's sarcoma. Cancer Invest 1996; 14: 83-88.
[37] Wittig JC, Bickels J, Priebat D, Jelinek J, Kellar-Graney K, Shmookler B et al. Osteosarcoma: a multidisciplinary approach to diagnosis and treatment. Am Fam Physician 2002; 65:1123-1132.
[38] Daldrup-Link HE, Franzius C, Link T, Laukamp D, Sciuk J, Jurgens H et al. Whole body MR imaging for detection of bone metastases in children and young adults. Roentgenol 2001; 177:229-236.
[39] Zhou X , Shi X , Wu S. A clinical observation of rh-endostatin(endostar) combined with chemotherapy for osteosarcoma. Journal of Medical Postgraduates 2012; 1:39-44.
[40] Zhang Y, Ren L. Clinical Analysis of the Recombinant Human Endostatin Combined with DIA in the Treatment of Osteosarcoma. Anti-tumor Pharmacy 2013; 5-0368-74.
[41] Xu M, Xu CX, Bi WZ, Song ZG, Jia JP, Chai W et al. Effects of endostar combined multidrug chemotherapy in osteosarcoma. Bone 2013; 57(1):111-5.
[42] Ogura K, Fujiwara T, Yasunaga H, Matsui H, Jeon DG, Cho WH et al. Development and external validation of nomograms predicting distant metastases and overall survival after neoadjuvant chemotherapy and surgery for patients with nonmetastatic osteosarcoma: A multi-institutional study. Cancer 2015; 121(21):3844-52.
[43] Matthew LB, Jonathan C, Clark M, Damian EM. The Molecular Pathogenesis of Osteosarcoma: A Review. Sarcoma Vol 2011; 95;924-8.
[44] Kaya M, Wada T, Nagoya S, Kawaguchi S, Isu K, Yamashita T. Concomitant tumour resistance in patients with osteosarcoma A clue to a new therapeutic strategy. J Bone Joint Surg [Br] 2004; 86-B:143-7.
[45] Kaya M, Wada T, Nagoya S. The level of vascular endothelial growth factor as a predictor of a poor prognosis in osteosarcoma. J Bone Joint Surg [Br] 2009; 91-B:784-8.
[46] Xu H , Li B , Huang Z, Zhang Q, Niu XH. A controlled non--randomized clinical research on chemotherapy combined with rh·-endostatin for stage II B osteosarcoma. Chinese Clinical Oncology 2013;5:421-25.
[47] Rossi B, Schinzari G, Maccauro G, Scaramuzzo L, Signorelli D, Rosa MA. Neoadjuvant multidrug chemotherapy including High-Dose Methotrexate modifies VEGF expression in Osteosarcoma: an immunohistochemical analysis. BMC Musculoskelet Disord. 2010; 11: 34.
[48] Fan J, Du JR, Wu J, Fu S, Hu D, Wan Q. Antitumor effects of different administration sequences of cisplatin and Endostar on
Lewis lung carcinoma. Oncol Lett 2015; 9(2): 822–828.
[49] Rong B, Yang S, Li W, Zhang W, and Ming Z. Systematic review and meta-analysis of Endostar (rh-endostatin) combined with chemotherapy versus chemotherapy alone for treating advanced non-small cell lung cancer. World J Surg Oncol 2012; 10:170.
[50] Moureau-Zabotto L, Delannes M, Le Péchoux C, Sunyach MP, Kantor G, Sargos P et al. Management of soft tissues sarcoma of the limbs by external beam radiation therapy. Cancer Radiother 2016; 20(2):133-140.
[51] Randall L, Cable MG. The role of molecular testing in soft tissue sarcoma diagnosis. Lancet Oncol 2016; 204; (15):625-7.
[52] Vincenzi B, Frezza AM, Schiavon G, Santini D, Dileo P, Silletta M et al. Bone metastases in soft tissue sarcoma: a survey of natural history, prognostic value and treatment options. Clin Sarcoma Res 2013; 17;3(1):6.
[53] Judson I, Verweij J, G elderbrom H, Hartmann J, Schoffski P, Blay JY et al. Doxorubicin alone versus intensified doxorubicin plus ifosfamide for first-line treatment of advanced or metastatic soft-tissue sarcoma: a randomised controlled phase 3 trial. Lancet Oncol 2014;15(4):415-423.
[54] Chawla SP, Papai Z, Mukhametshina G, Sankhala K, Vasylyev L, Fedenko A et al. First-Line Aldoxorubicin vs Doxorubicin in Metastatic or Locally Advanced Unresectable Soft-Tissue Sarcoma: A Phase 2b Randomized Clinical Trial. JAMA Oncol 2015; 1(9):1272-80.
[55] García Franco CE, Torre W, Tamura A, Guillén-Grima F, San-Julian M, Martin-Algarra S et al. Long-term results after resection for bone sarcoma pulmonary metastases. Eur J Cardiothorac Surg 2010; 37(5):1205-8.
[56] Kang SC, Kim HS, Kim SJ. Post-metastasis survival in extremity soft tissue sarcoma: A recursive partitioning analysis of prognostic factors. Eur J Cancer 2014; 50(9):1649-56.
[57] Cotterill SJ, Ahrens S, Paulussen M, Jurgens HF, Voute PA, Gadner H et al. Prognostic factors in Ewing's tumor of bone: analysis of 975 patients from the European Intergroup Cooperative Ewing's Sarcoma Study Group. J Clin Oncol 2000; 18:3108-3114.
[58] Tsuchiya H. Basic science and clinical frontiers of sarcoma treatment. Int J Clin Oncol 2011; 16(2):82-83.
[59] Sharma S, Takyar S, Manson S, Powell S, Penel N. Efficacy and safety of pharmacological interventions in second- or later-line treatment of patients with advanced soft tissue sarcoma: a systematic review. BMC Cancer 2013;13:385.
[60] Janeway KA, Maki RG. New strategies in sarcoma therapy: linking biology and novel agents. Clin Cancer Res 2012; 18(21):5837-44.