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Chapter

Treatment of Children with Osteosarcoma

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Abstract

Osteosarcoma accounts for 3% of all malignant tumors, 35–50% of all malignant bone tumors in pediatric patients. The chapter contains statistical data describing the incidence of the child population of osteosarcomas, classification of osteosarcomas, staging principles, a description of the main localizations, as well as a detailed description of the existing treatment protocols for children with osteosarcomas, including personalized therapy. The literature data are described in detail—the results of treatment of children with osteosarcoma with various courses of chemotherapy, as well as new approaches in treatment, including personalized therapy. But the results of treatment of children with primary metastatic osteosarcoma, relapse, and refractory course of the disease remain unsatisfactory.

Keywords: pediatric oncology, osteosarcoma, chemotherapy, personalized therapy, combination treatment

1. Introduction

Osteosarcoma is a primary malignant bone tumor that develops from primitive mesenchymal stem cells capable of differentiating into bone, cartilage, or fibrous tissue [1].

Osteosarcoma accounts for 3% of all malignant tumors, 35–50% of all malignant bone tumors in pediatric patients. The frequency of occurrence is 4 cases per 1 million children and adolescents per year. About 60% of cases of osteosarcoma detection are recorded at the age of 10–20 years (mainly in the prepubertal and pubertal periods). The gender ratio (boys/girls) is 1.3–1.6:1 [2].In 50% of cases, the tumor is located in the projection of the knee joint (distal femur, proximal tibial bone). The third place in terms of frequency of occurrence is the lesion of the proximal metadiaphysis of the humerus. The defeat of the axial skeleton (pelvis, spinal column) is detected in 12% of cases [3–5].

In the treatment of children with osteosarcoma, chemotherapy is the main method. Nonadjuvant and adjuvant chemotherapy courses are important. In the middle of the twentieth century, when the main treatment was surgical, the frequency of relapse and metastasis was extremely high. Increased patient survival is due precisely to the intensification of chemotherapeutic treatment, which has reduced the frequency of relapses and metastasis.

2. Classification and staging

2.1 WHO classification of soft tissue and bone tumors of 2013 (fourth revision)

A localized (locally advanced) variant of osteosarcoma, which occurs in 80% of cases and a disseminated (primary metastatic) variant, which occurs in 20% of cases, are distinguished [3, 6].

2.2 Histological classification

- low grade, central osteosarcoma
- classic (conventional) version of osteosarcoma:
  - chondroblastic osteosarcoma
  - fibroblastic osteosarcoma
  - osteoblastic osteosarcoma
  - osteosarcoma, unspecified accuracy
- telangiectatic osteosarcoma
- small cell osteosarcoma
- high degree of malignancy, superficial osteosarcoma.

2.3 TNM classification 2018:

2.3.1 TNM classification 2018 for the extremities

T—primary tumor
Tx—the primary tumor cannot be determined [7].
T0—no signs of primary tumor.
T1—the largest tumor size ≤8 cm.
T2—the largest tumor size>8 cm.
T3—several unrelated tumors in the primary zone of bone damage.
N—regional lymph nodes:
Nx—the presence of metastatic lesions in the regional lymph nodes cannot be determined.
N0—no regional metastases in the lymph nodes.
N1—regional lymph node metastases.
M—distant metastases:
Mx—the presence of distant metastases could not be determined or the study was not conducted.
M0—distant metastases are absent.
M1—there are distant metastases.
M1a—in the lungs.
M1b—another localization.
G—degree of differentiation:
Gx—the degree of differentiation could not be determined or the study was not conducted.

G1—well differentiated.
G2—moderately differentiated.
G3—poorly differentiated.
G4—undifferentiated.
G1–2—low degree of malignancy.
G3–4—a high degree of malignancy.

2.3.2 TNM classification 2018 for the spine

TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Tumor confined to one vertebral segment or two adjacent vertebral segments
T2	Tumor confined to three adjacent vertebral segments
T3	Tumor confined to four or more adjacent vertebral segments, or any nonadjacent vertebral segments
T4	Extension into the spinal canal or great vessels
T4a	Extension into the spinal canal
T4b	Evidence of gross vascular invasion or tumor thrombus in the great vessels
NX	Regional lymph nodes cannot be assessed. Because of the rarity of lymph node involvement in bone sarcomas, the designation NX may not be appropriate, and cases should be considered N0 unless clinical node involvement clearly is evident
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis
cM0	No distant metastasis
cM1	Distant metastasis
cM1a	Lung
cM1b	Bone or other distant sites
pM1	Distant metastasis, microscopically confirmed
cM1a	Lung, microscopically confirmed
cM1b	Bone or other distant sites. Microscopically confirmed

2.3.3 TNM classification 2018 for the pelvis

TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Tumor confined to one pelvic segment with no extraosseous extension
T1a	Tumor ≤8 cm in greatest dimension
T1b	Tumor >8 cm in greatest dimension
T2	Tumor confined to one pelvic segment with extraosseous extension or two segments without extraosseous extension
T2a	Tumor ≤8 cm in greatest dimension
T2b	Tumor >8 cm in greatest dimension
T3	Tumor spanning two pelvic segments with extraosseous extension
T3a	Tumor ≤8 cm in greatest dimension
T3b	Tumor >8 cm in greatest dimension

T4	Tumor spanning three pelvic segments or crossing the sacroiliac joint
T4a	Tumor involves sacroiliac joint and extends medial to the sacral neuroforamen
T4b	Tumor encasement of external iliac vessels or presence of gross tumor thrombus in major pelvic vessels
NX	Regional lymph nodes cannot be assessed. Because of the rarity of lymph node involvement in bone sarcomas, the designation NX may not be appropriate, and cases should be considered N0 unless clinical node involvement clearly is evident
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis
cM0	No distant metastasis
cM1	Distant metastasis
cM1a	Lung
cM1b	Bone or other distant sites
pM1	Distant metastasis, microscopically confirmed
cM1a	Lung, microscopically confirmed
cM1b	Bone or other distant sites. Microscopically confirmed

Stage	TNM	Degree of malignancy
IA	T1 N0 M0	Low
IB	T2 N0 M0	Low
IIA	T1 N0 M0	High
IIB	T2 N0 M0	High
III	T3 N0 M0	Any
IVA	Any T N0 M1a	Any
IVB	Any T N1 Any M Any T Any N M1b	Any Any

Table 1.
Staging by TNM.

Staging according to the TNM classification is presented in **Table 1**.

3. Treatment

The methods of treatment of osteosarcoma over the past 30 years have not changed. There are five main drugs (cisplatin, adriamycin, methotrexate, ifosfamide, and etoposide) that have been used in various combinations and doses [8–13].

The rates of treatment outcome in the world remain at about the same level. In patients with a localized variant of osteosarcoma, 5-year overall survival (OS) does not exceed 75% and 5-year event-free survival (EFS)—62% (**Table 2**).

In patients with primary metastatic osteosarcoma, the results are much worse, despite attempts to use high doses of drugs, including high-dose polychemotherapy with transplantation of autologous hematopoietic stem cells. At the same time, the 5-year OS does not exceed 35% on average and the 5-year EFS–25% (**Table 2**).

Therapy program	5-year overall survival, %	5-year event-free survival, %
IOR/OS2 the Istituto Ortopedico Rizzoli [14]	75	63
ISG/OS1 (Italian Sarcoma Group) [15]	74	64
ISG/SSG1 (Italian and Scandinavian Sarcoma Group) [16]	77	64
COSS 88/96 (Cooperative Osteosarcoma Study Group) [17]	79	
SSG XIV (Scandinavian Sarcoma Group) [18]		65
NECO93J/95J (Neoadjuvant Chemotherapy for Osteosarcoma) [19]	78	65
BOTG III/IV (Brazilian Osteosarcoma Treatment Group) [20]	61	45
POG8651 (Pediatric Oncology Group) [21]	78	65
SFOP94 (Société Française d'Oncologie Pédiatrique) [22]	76	62
St.Jude CRH OS91 (Children Research Hospital) [23]	74	65
St.Jude CRH OS99 (Children Research Hospital) [24]	79	67
INT0133-COG (+MTP/-MTP) Children's Oncology Group [25]	78/70	67/61
MSKC NY (+PAM) Memorial Sloan-Kettering Cancer Center, NY [26]	94	72
COG INT0133, CCG7943, AOST0121 [27]	47	22
ISG/SSG II (Italian and Scandinavian Sarcoma Group) [28]	55	46
EURAMOS1 [29, 30]	75	59

Table 2.
The results of the treatment of pediatric patients with localized osteosarcoma.

3.1 Traditional treatment

The most significant interest in the treatment of children with a localized version of osteosarcoma are the studies of the Italian and Scandinavian groups (Italian and Scandinavian sarcoma group–ISG/SSGI, SSG XIV), the French Pediatric Oncological Group (Societe Francaise d'Oncologie Pediatrique–SFOP OS94), and EURAMOS1.

Ferrari et al. showed the data of the joint study of the Italian and Scandinavian groups (ISG/SSG I), which was conducted from 1997 to 2000. The study included 182 patients.

A special feature of neoadjuvant chemotherapy was the use of two courses of monotherapy with high-dose ifosfamide (in a course dose of 15 g/m^2) and two courses of MAR (methotrexate (M) 12 g/m^2, adriamycin (A) 75 mg/m^2, and cisplatin (P) 120 mg/m^2) in alternating mode. Adjuvant chemotherapy started at week 14. In this case, the course dose of adriamycin was increased to 90 mg/m^2, the dose of cisplatin to 150 mg/m^2, and a high-dose ifosfamide was administered in PIM chemotherapy courses (cisplatin, ifosfamide, and methotrexate) and PAI (cisplatin, adriamycin, and ifosfamide).

After removal of the primary tumor focus, a good histological response (therapeutic pathomorphism of grade 3–4) was achieved in 63% of patients, a poor histological response (treatment pathomorphism of grade 1–2) in 37%. At the same time, the 5-year OV and EFS accounted for 77 and 64%. Consequently, the use of high-dose ifosfamide in an alternating mode with the MAP scheme led to an increase in the frequency of achieving a good histological response, but did not affect the rates of OS and EFS [15, 16, 31].

Smeland et al. presented the data of the study of the Scandinavian Group (SSG XIV), which was conducted from 2001 to 2005. The study included 63 patients.

Neoadjuvant chemotherapy consisted of two courses of IDA. High-dose ifosfamide (in the course dose of 10 g/m^2) was used in monotherapy in patients with a poor histological response to treatment, only after five courses of MAP.

After removal of the primary tumor lesion, a good histological response was achieved in 45% of patients and a poor histological response in 55%. At the same time, the 5-year OV and BSV accounted for 76–65% and the 5-year EFS in the group with a good histological response for 89%, with a poor histological response 48%. Consequently, the use of ifosfamide after MAP courses in the adjuvant mode did not lead to an increase in OS and EFS, and the frequency of achieving a good histological response was lower than in studies in which the MAP scheme was used in alternating mode with ifosfamide [18].

Le Deley et al. presented the results of the randomized SFOP OS94 study, which was conducted from 1994 to 2001. The study included 239 patients (120 in group A and 119 in group B).

Neoadjuvant therapy included seven courses of high-dose methotrexate and two courses of monotherapy with adriamycin (in a course dose of 70 mg/m^2) in group A or seven courses of high-dose methotrexate and two courses of IE (ifosfamide (I) 12 g/m^2 and etoposide (E) 300 mg/m^2) in group B. In the adjuvant mode, chemotherapy was replaced with IE courses in group A, and AP in group B for patients with a poor histological response detected after removal of the primary focus. The operative stage of treatment was carried out at 12 and 14 weeks in groups A and B, respectively.

A good histological response was achieved in group A in 43% of patients, in group B in 64%, poor histological response in group A in 57%, and in group B in 36% ($p = 0.009$). The 5-year OS in group A was 75%, in group B—76%, the 5-year EFS in group A—58%, and in group B—66%. A 3-year EFS in group A in patients showed a good histological response for 82%, with a poor histological response for 49%, in group B—77 and 60%, respectively.

Consequently, the use of methotrexate, ifosfamide and etoposide in neoadjuvant chemotherapy led to a statistically significant increase in the frequency of achieving a good histological response, but not to an increase in OS and EFS [22].

Of particular interest in the treatment of children with primary metastatic osteosarcoma are the Pediatric Oncology Group (POG) IE and ISG/SSG II studies.

Goorin et al. presented the results of a phase II/III nonrandomized clinical trial of high-dose ifosfamide and etoposide in patients with primary metastatic osteosarcoma. The study included 43 patients.

Neoadjuvant chemotherapy was represented by two courses of IE (ifosfamide (I) 17.5 g/m^2 and etoposide (E) 500 mg/m^2). Removal of the primary tumor lesion was performed after two courses of IE at 7–8 weeks of therapy. The timing of the removal of metastatic foci was chosen individually during adjuvant chemotherapy, which included four courses of MAP chemotherapy and three courses of iE (with a course dose of ifosfamide (i) 12 g/m^2) in an alternating mode.

A good histological response was achieved in 65% of patients and poor in 35%. However, the 2-year OS and EFS were 55 and 45%, respectively. Consequently, the use of high-dose ifosfamide in combination with etoposide therapy led to an increase in the frequency of achieving a good histological response, but not indicators of OS and EFS [32].

Boye et al. showed the results of the nonrandomized study ISG/SSG II, which was conducted from 1996 to 2004. The study included 57 patients with primary metastatic osteosarcoma.

Neoadjuvant chemotherapy included two courses of MAPI. Surgical removal of the primary tumor lesion was performed at week 14.

In the adjuvant mode, two courses of ACyVP (adriamycin (A) 90 mg/m^2, cyclophosphamide (Cy) 4 g/m^2, and vepesid (VP) 600 mg/m^2) and two courses of high-dose chemotherapy VPCarbo (vepesid (VP) 600 mg/m^2 and carboplatin (Carbo) 1.5 g/m^2) with the support of autologous hematopoietic stem cells. Surgical removal of the primary tumor lesion was performed at week 14.

A good histological response was achieved in 29% of patients and poor in 71%. The 5-year OM and BSV were 31 and 27%, respectively [28].

Marina et al. presented the results of the EURAMOS1 study in patients with a poor histological response after neoadjuvant MAP chemotherapy. Within the protocol, patients are randomly assigned to the MAP treatment lines (methotrexate (M) 12 g/m^2, adriamycin (A) 75 mg/m^2, and cisplatin (P) 120 mg/m^2) and MAPIE (ifosfamide (I) 14 g/m^2 and etoposide 500 mg/m^2). In the age group up to 30 years, the MAPIE line of therapy was carried out in 310 patients, the MAPIE line in 308 patients, in the age group up to 20 years—259 (84%) and 271 (88%) patients. Groups of patients are statistically significantly comparable by sex, age, localization of the primary tumor lesion, the presence of metastatic lesions, and the histological variant of the tumor.

In the group of 541 patients with a localized version of osteosarcoma, 247 events were identified, 118 in patients who received the MAP therapy line and 129 in patients who received the MAPIE therapy line. At the same time, the 3-year EFS was 60 and 57%. In the group of patients with primary metastatic osteosarcoma, 3-year EFS was 24 and 18%, for MAP and MAPIE, respectively. Therefore, this study showed that the use of alternating chemotherapy courses for MAP, IE, and Ai in an adjuvant regimen did not lead to an increase in EFS indices [33].

3.2 Experimental treatment

Treatment outcomes for children with primary metastatic osteosarcoma remain extremely low and the optimal therapeutic strategy is unknown.

New programs are being developed around the world taking into account the molecular biological features of tumor cells that determine sensitivity to chemotherapy (ERCC1 to cisplatin, TOPO2α to anthracyclines and etoposide, MGMT to epigenetic therapy and cisplatin, RFC1 to methotrexate) [34–39] and invasive and metastatic potential of a tumor (stem cell markers—CD133, OCT4; transcription factors—p-STAT3, C-MYC; cytokine-associated signaling pathways—ErbB2, VEGFR1, VEGFR2, PDGFRα, and PDGFRβ) [40–43].

Cui et al. presented the results of a study to determine the expression of MGMT protein (methylguanine–DNA–methyltransferase) and MGMT gene methylation in patients with osteosarcoma in the age group up to 40 years (mean age 17 years) who were treated with cisplatin in single mode, in a course dose of 120 mg/m^2 Determination of MGMT protein expression in immunohistochemical (IHC) study was performed in biopsy tumor material in 76 patients and MGMT gene methylation in 51 patients. The result of IHC was considered positive with a high level of expression—more than 30% (3+), with an average level of expression—20–30% (2+), and with a low level of expression—10–20% (1+). MGMT protein expression was detected in 52 (68%) patients, low expression level in 27 (35%), medium level in 18 (24%), and high level in 7 (9%).

A statistically significant relationship was established between the presence of MGMT protein expression and an increase in the frequency of a poor histological response ($p = 0.004$). The expression level above 20% was detected in 22 out of 43 (51%) patients in the group of patients with 1–2°of therapeutic pathomorphosis and only in 3 out of 33 (9%) patients in the group with 3–4° of therapeutic pathomorphosis.

Methylation of the promoter portion of the MGMT gene was observed in 12 of 51 (23.5%) patients and the lack of expression of MGMT protein in 14 of 51 (27.5%) patients. A statistically significant relationship between the absence of methylation and the presence of MGMT protein expression ($p < 0.001$) was established. In the group of patients with 1–2 degrees of therapeutic pathomorphosis, the absence of MGMT gene methylation was detected in 36 of 38 (94.7%) patients and with 3–4 degrees of therapeutic pathomorphosis in 3 of 13 (23%) patients ($p < 0.001$).

Consequently, the data obtained indicate the formation of tumor resistance to treatment with an alkylating agent—cisplatin in patients whose biopsy material revealed the absence of methylation of the promoter portion of the MGMT gene and the presence of expression of the MGMT protein [34, 35].

Pitano-Garcia et al. (Spain sarcoma group) conducted a study to determine the expression of RFC1 micro-RNA (reduced folate carrier 1, a transmembrane protein that provides folate and methotrexate transport to the cell) by real-time polymerase chain reaction (PCR) in a tumor substrate in children with osteosarcoma.

In 34 samples, biopsy tumor material in 14 children and metastatic foci tumor material in 20 children were analyzed. In 13 of 14 (92.9%) biopsy specimens and in 11 of 20 (68.8%) metastatic specimens, a low level of RFC1 expression was detected.

A poor histological response after neoadjuvant chemotherapy (three courses of intravenous administration of doxorubicin at a dose of 75 mg/m^2, three courses of intraarterial administration of cisplatin at a dose of 105 mg/m^2, four courses of intravenous administration of methotrexate at a dose of 14 g/m^2) in 45% of cases. The biopsy tumor substrate in this group of patients was characterized by a low level of expression of RFC1 micro-RNA in 90% of cases compared to 60% in patients with a good histological response ($p = 0.053$). The average level of expression was statistically significantly lower in the biopsy material than in the metastatic tumor foci ($p = 0.024$) [38, 44].

Therefore, in this study, there was a tendency to an increase in the frequency of detection of low expression levels of RFC1 micro-RNA in patients with a poor histological response.

Hattinger et al. (Italian sarcoma group) presented the results of the study, the purpose of which was to determine the prognostic significance of ERCC1 protein expression (excision repair crosscomplementation group 1) in biopsy tumor material in patients with localized osteosarcoma, who underwent programmed treatment of ISG/OS-oss and ISG/SSG1. A tumor sample was considered positive in the presence of a score of 2–3: score 1 (1–10% of positive nuclei), score 2 (11–50% of positive nuclei), and score 3 (more than 50% of positive nuclei).

ERCC1-positive tumor (score 2–3) was detected in 30 patients (30%). During the ISG/OS-oss program in groups of patients with ERCC1-negative/score 1 and ERCC1-positive (score 2–3), the 5-year-old OS and BSV tumor variants were 91, 38, and 57, 25% ($p = 0.001$; $p = 0.042$), with the ISG/SSG1 program–82, 64, and 69, 36% ($p = 0.022$; $p = 0.028$), and with both therapy programs–82, 50 and 62, 34% ($p < 0.001$; $p = 0.006$). Consequently, a statistically significant relationship has been established between the ERCC1-positive variant of the tumor and lower rates of 5-year OS and BSV [36].

Nguyen et al. (SFOP) presented the results of a study to determine the prognostic significance of TOP2A protein expression (topoisomerase DNA 2 alfa) and the presence of rearrangement of the TOP2A gene in biopsy tumor material in 105 children with osteosarcoma treated with the SFOP protocol OS94. Patients with a primary metastatic osteosarcoma variant accounted for 17%. After neoadjuvant chemotherapy, a good histological response was detected in 56 patients (53%) and a poor histological response in 49 (47%). Real-time PCR amplification of the

TOP2A gene and the TOP2A gene deletion were detected in 21 (21.2%) and 25 (25.3%) patients. In 53 children (53.5%), rearrangements of the TOP2A gene were not detected. A statistically significant relationship was established between the presence of the TOP2A gene rearrangement (amplification and deletion) and the presence of a good histological response after neoadjuvant polychemotherapy ($p = 0.004$). There was also a tendency to achieve lower rates of 5-year OM and BSV in patients whose tumor cells had amplified the TOP2A gene ($p = 0.09$ and 0.06). The expression of the TOR2A protein was determined in 17 patients by immunohistochemistry. Medium (2+) and high (3+) levels of expression were detected in all patients; expression was above 30% in 12 of 17 children (70.5%). There is no statistically significant relationship between the expression of the TOR2A protein above 30% and the presence of amplification or deletion of the TOP2A gene ($p > 0.05$) due to an insufficient number of observations [37].

Xiao et al. presented the results of a study of a personalized approach to the prescription of chemotherapy depending on the presence or absence of markers of drug resistance in 28 patients with localized osteosarcoma. The average age in the patient group was 20.1 g. To determine the sensitivity to chemotherapy, the following markers were used: for doxorubicin–expression of TOP2A micro-RNA, mutation of the ABCB1 gene, and mutation of the GSTP1 gene; for cisplatin–expression of microcryptal ERCC1, BRCA1, and mutation of genes XRCC1-exon6 and XRCC1-exon10, and for ifosfamide–mutation of CYP2C9 * 3.

At the same time, a high level of sensitivity to ifosfamide was detected in all patients (100%), to cisplatin in 11 out of 28 (39.2%), to doxorubicin in 6 out of 28 (21.4%); medium and high levels of sensitivity to cisplatin in 17 of 28 (60.7%), to doxorubicin in 20 of 28 (71.4%). Chemotherapy, taking into account the sensitivity of the tumor to drugs, was performed in 8 of 28 patients (28.5%). In this group, only one relapse of the disease was detected, while in the rest of the 20 patients, four relapses of the disease were detected: in one case, progression during neoadjuvant chemotherapy and in another case, fatal outcome from toxicity of therapy. The average duration of observation for groups was not indicated, and no statistically significant difference was obtained due to the insufficient number of observations [39].

In addition, the study of markers of stem tumor cells CD133 (Prominin 1) and Octamer-binding transcription factor 4 (OCT4), as well as the transcription factors signal transducer and activator of transcription 3 (STAT3), and myelocytomatosis viral oncogene homolog (C-MYC), which determines the invasive and metastatic potential of a tumor [45–47].

So in the work of He et al., there was a significant correlation between the expression of CD133 in tumor cells and a higher frequency of metastatic lesions, a lower median of overall survival. A CD133-positive variant was detected in 46 of 70 (65.7%) patients, in 6 out of 16 (37.5%) in the group with a localized osteosarcoma variant, and in 40 out of 54 (74%) in the group with the primary metastatic osteosarcoma ($p = 0.002$). The median overall survival rate was statistically significantly lower in the group with CD133-positive tumor ($p = 0.000$). When conducting the study "Transwell invasion," a significantly higher invasive potential of the CD133-positive variant of the tumor was established ($p < 0.05$). Real-time PCR established a higher level of expression of micro-RNA OCT4 in a CD133-positive variant of the tumor ($p < 0.05$) [41].

Li et al. in an experimental model on cell lines showed that about 80% of cells in a CD133-positive variant of the tumor are in the G0/G1 phases of the cell cycle ($p < 0.01$). Also, real-time PCR revealed a significantly higher level of expression of the multidrug-resistant gene (MDR1) in the CD133-positive variant of the tumor ($p < 0.05$) [48].

In the studies presented, He and Li et al., the mechanisms of drug resistance, invasion, and metastasis in case of CD133-positive variant of the tumor were established.

In the works of Tu et al., the significance of activation of the IL6R/STAT3/p-STAT3tyr705 mesenchymal stem cell signaling pathway to increase the metastatic potential of tumor cells was exemplified by the example of cell lines (Saos 2 and U2-OS). The relationship between the increased expression of p-STAT3tyr705 and increased expression of the drug resistance markers multidrug resistance protein (MRP) and MDR1 has been established. An increase in sensitivity to doxorubicin, but not to cisplatin, was also noted with inhibition of this signaling pathway [43, 49].

Han et al. using cell lines (MG63 and SAOS2) as an example showed that an increase in C-MYC expression leads to activation of the MEK–ERK signaling pathway and an increase in the expression of MMP2 and MMP9, which enhance the invasive and metastatic potential of a tumor [50].

Wu et al. investigated the prognostic significance of C-MYC expression in biopsy tumor material in 56 children with osteosarcoma who were treated with methotrexate, cisplatin, and adriamycin. Expression of the C-MYC protein was detected in 48 of 56 (85.7%) patients. A statistically significant relationship was established between the presence of C-MYC expression and a decrease in the apoptotic index ($p < 0.05$). In addition, in the group of patients with C-MYC-positive variant of the tumor and the intensity of expression, at 2+ and 3+, a significantly lower 3-year-old OM was established ($p < 0.05$) [51].

Consequently, in the works of Tu, Han, and Wu et al., the significance of transcription factors in the development of drug resistance, invasion, and metastasis of the tumor has been established.

3.3 Theoretical treatment

Innovative therapeutic approaches are used mainly in patients with metastatic osteosarcoma, relapse, and refractory course of the disease. Currently, the following key areas are distinguished: (1) the use of monoclonal antibody preparations, (2) tumor-modifying therapy using nitrogen-containing bisphosphonates, (3) the use of chemotherapeutic drugs that affect various cellular signaling pathways (multikinase inhibitors and mTOR inhibitors), and (4) the use of drugs that promote the activation of tumor-associated macrophages.

Rossi et al. presented the results of a study aimed at determining the expression of vascular endothelial growth factor (VEGF) in a biopsy tumor substrate and in tumor material after neoadvanting chemotherapy (two courses of MAP) in 16 patients with localized osteosarcoma, who received programmed treatment using the SSG XIV protocol. Four levels of expression were evaluated: negative and low–at an expression level <25%, medium—at 25–50% (1+), high—at 50–75% (2+), and very high—at>75% (3+). Medium and high levels of VEGF expression in biopsy tumor material were detected in 11 (6 in medium and 5 in high) out of 16 patients (68.7%). After neoadjuvant chemotherapy and the removal of the primary tumor site, VEGF expression was established in all samples, and there was an increase in expression in samples that were positive in the initial study.

High and very high levels of expression, increased expression after neoadjuvant chemotherapy was statistically significantly correlated with the localization of the primary tumor lesion in the femur ($p = 0.02$), with the appearance of local recurrence ($p = 0.04$) and/or early metastatic lesions in the lungs ($p = 0.04$), with a fatal outcome from the refractory course of the disease ($p = 0.04$).

Therefore, the presence of VEGF expression in the biopsy material and an increase in the expression of VEGF after neoadjuvant chemotherapy are factors for

poor prognosis of the disease [42]. But this study requires the continuation of the fact that it includes a small number of patients.

In addition, Ohba et al. showed in an in vivo experiment using human osteosarcoma cell lines (TE85 and 143B) the mechanism of autocrine stimulation of tumor transformation and proliferation using the example of the VEGF/VEGFR signaling pathway. In this study, the expression of VEGF-A and VEGFR micro-RNA was evaluated [52].

Currently, little experience has been gained with the use of the drug bevacizumab in children with osteosarcoma.

Bevacizumab (Avastin) is a partially humanized monoclonal antibody to VEGF-A, IgG1, which realizes its activity through a second type of immunopathological reaction (antibody-mediated complement-dependent cytotoxicity and antibody-mediated cell-dependent cytotoxicity) [53].

Turner et al. (St. Jude Children's research hospital) presented preliminary results of using bevacizumab in combination with neoadjuvant chemotherapy (two courses of IDA) in 27 children with osteosarcoma. The drug was used at a dose of 15 mg/kg. There are three introductions for neoadjuvant chemotherapy. A satisfactory toxicity profile has been established. The study NCT00667342 continues [54, 55].

Back in 1999, employees of the Memorial Sloan-Kettering Cancer Center presented the results of a study assessing the effect of ErbB2 expression (Erb-B2 receptor tyrosine kinase 2) on the nature of the histological response after neoadjuvant polychemotherapy and on the rates of OS and BSV. The study included 53 patients. ErbB2 overexpression was detected in 42% of patients in the entire study group, in 50% with metastatic variant and in 76% at the time of detection of relapse or refractory course of the disease, and also statistically significantly correlated with poor histological response ($p = 0.02$) and BSV ($p = 0.05$). The 5-year BSV in patients with a localized version of osteosarcoma and ErbB2-positive status was 47%, with ErbB2-negative status—79% [40].

Conflicting data on the prognostic significance of ErbB2-positive status in patients with localized osteosarcoma were obtained.

In 2002, the Japanese Osteosarcoma Group (Japanese Osteosarcoma Group) published the results of a study that included 155 patients with localized osteosarcoma from 1984 to 1995. At the same time, 5-year BSV in patients with ErbB2-positive status was 45%, with ErbB2-negative status—72% [56].

In 2014, the Children Oncology Group (COG) presented completely different results of the study, which from 1999 to 2002 included 135 patients with localized osteosarcoma. Only 13% of patients showed ErbB2-positive status. The 5-year RR in patients with ErbB2-positive status was 73%, and with the ErbB2-negative status—72%, the 5-year RR was 59 and 69%, respectively. No statistically significant difference in survival was observed [57].

Thus, it was confirmed that ErbB2 can be considered as a potential target for targeted therapy in metastatic variant, relapse, and refractory course of the disease.

COG presented the results of a phase 2 clinical trial of the drug Trastuzumab (Herceptin) in combination with MAPIE polychemotherapy in 96 patients with primary metastatic osteosarcoma. This study was conducted from 2001 to 2005.

Trastuzumab is a partially humanized IgG1κ monoclonal antibody to ErbB2, which also realizes its activity through a second type of immunopathological reaction (antibody-mediated complement-dependent cytotoxicity and antibody-mediated cell-dependent cytotoxicity). The drug was administered at a dose of 4 mg/kg in the first week, and then 2 mg/kg 1 time per week (34 in total) only in patients in whose tumor substrate ErbB2 expression was detected.

Surgical removal of the primary tumor lesion was performed at week 11. Adjuvant chemotherapy began at week 13.

In the group with trastuzumab, a good histological response was detected in 56% of patients and without trastuzumab, it was 40%, a poor histological response of 44–60%, respectively. At the same time, the 3-year OS and BSV in the group of patients who received treatment with trastuzumab accounted for 59 and 32%, and in the group of patients who received treatment without trastuzumab for 50 and 32%. Consequently, the use of trastuzumab with polychemotherapy MAPIE led to an increase in the frequency of achieving a good histological response, but not to an increase in the rates of OS and EFS [58].

Of particular interest is tumor-modifying therapy using nitrogen-containing bisphosphonates. Currently, the following mechanisms of action of nitrogen-containing bisphosphonates have been identified, which are represented by the activation of tumor cell apoptosis by the caspase mechanism (indirectly through protein Rb and P53) and without the participation of the caspase mechanism (an increase in AIF—apoptosis of the inducing factor); increased expression of TNF-related apoptosis-inducing ligand–death receptor 5 (TRAIL-DR5, TRAIL-induced apoptosis); reduction of receptor activator of nuclear factor kappa-B ligand (RANKL) expression–ligand of nuclear factor activation receptor kB in osteosarcoma cells, which leads to suppression of tumor cell proliferation, osteoclast activity, changes in the tumor microenvironment, bone resorption, and risk of metastasis; γδT activation of cellular cytotoxicity; and tumor activation of associated macrophages [59–62].

In addition, the potentiating effect of nitrogen-containing bisphosphonates on cisplatin and adriamycin has been confirmed [63].

Currently, a rather small experience has been gained in using these drugs in children with osteosarcoma.

Meyers et al. published the results of a study on the combined use of pamidronate with MAP chemotherapy. The study included 40 patients, 32 in the age group under 18, 29 with a localized version of osteosarcoma, and 11 with a primary metastatic option of osteosarcoma.

In accordance with the program, pamidronate was administered once a month at a dose of 2 mg/kg 48–72 h after adriamycin, methotrexate, a total of 12 administrations.

Surgical removal of the primary tumor lesion was performed at week 11. Adjuvant chemotherapy began at week 13. Removal of metastatic foci was carried out individually at the stage of adjuvant therapy.

The frequency of achieving a good and poor histological response is not indicated. However, relatively high rates of 5-year OS and EFS were obtained: 93 and 72% in patients with localized osteosarcoma and 64 and 45% in patients with metastatic osteosarcoma [26].

COG presented the results of the pilot protocol AOST06P1 aimed at studying the combined use of zoledronic acid with MAPIE polychemotherapy in children with the primary metastatic osteosarcoma. This study included 24 patients. Zoledronic acid was administered at a dose of 1.2–3.5 mg/m^2 in each course of chemotherapy.

The maximum tolerated dose of zoledronic acid was established, which was 2.3 mg/m^2. Indicators of a 2-year OV and EFS were 60 and 32%, respectively [63].

Piperno-Neumann et al. presented the results of a phase 3 randomized study OS 2006, the purpose of which was to identify the potentiating effect of zoledronic acid when used together with polychemotherapy MIE and MAP.

The study included 217 children, 107 in the control group, and 110 in the group with zoledronic acid. Groups of patients were statistically significantly comparable by sex, age, foci of primary and metastatic lesions, and histological variant of the tumor.

Zoledronic acid was administered at a dose of 0.05 mg/kg (maximum dose of 4 mg) with each course of chemotherapy (IE and AP).

Neoadjuvant chemotherapy consisted of two courses of IE (ifosfamide (I) 12 g/m^2, etoposide 300 mg/m^2) and seven administrations of high-dose methotrexate ((M) 12 g/m^2). Surgical removal of the primary tumor lesion was performed at week 14. Adjuvant chemotherapy included two courses of MIE in the group with a good histological response and five courses of MAP in the group with a poor histological response. A good histological response after neoadjuvant polychemotherapy was achieved in 73% of patients. However, there was no statistically significant difference in achieving a good histological response, in terms of OS and BSV in groups of patients who received programmed treatment with or without zoledronic acid. The number of events in the group with zoledronic acid was 42% (47/110) and in the group without zoledronic acid was 31% (34/107). Consequently, this study shows the high effectiveness of chemotherapy courses with IE in combination with methotrexate in the neoadjuvant regimen. The presence of the potentiating effect of zoledronic acid has not been proven [64].

In the treatment of refractory forms of osteosarcoma, drugs are also used that affect various cellular signaling pathways. Understanding the mechanisms of tumor activation opens up the possibility of using multikinase and mammalian target of rapamycin complex (mTOR) inhibitors.

Takagi and Peng et al. in an in vitro experiment on cell lines (SaOS2, MG63, HOS), pathogenetic mechanisms of cytokine-induced tumor transformation and proliferation were shown through the activation of VEGF/VEGFR/PI3K (phosphatidylinositol-4,5-bisphosphate 3-kinase)/AKT (protein kinase B) and the platelet-derived growth factor receptor (PDGFR)/PI3K/AKT signaling pathways [65, 66]. The most studied drugs from this group are currently sorafenib (nexavar) and everolimus (afinitor) [67]. Sorafenib is a nonselective multikinase inhibitor that inhibits the activity of various cellular signaling pathways, in particular VEGFR1, VEGFR2, PDGFRα, and PDGFRβ, while everolimus is an mTOR inhibitor [68].

Ymera et al. of the Italian Sarcoma Group published the results of a preclinical study (in vitro and in vivo), which noted the mutually potentiating antitumor effect of everolimus and sorafenib on osteosarcoma cell lines (KHOS, MNNG-HOS, and U2OS). The effect of everolimus and sorafenib on mTORC1/mTORC2 is manifested in a decrease in the expression of mTORC1 and an increase in the expression of mTORC2, which provides proapoptotic and antiproliferative effects. With the combined use of everolimus and sorafenib, there is a decrease in the expression of both mTORC1 and mTORC2 [69].

From 2008 to 2009, Grignani et al. of the Italian Sarcoma Group conducted a second phase of clinical trials of the drug sorafenib in patients with relapse and refractory osteosarcoma. The study included 35 patients with osteosarcoma in the age group over 14 years. Partial response was achieved in 5 (14%) patients and stabilization of the disease in 12 (34%) patients. The overall response rate was 48%. At the same time, 4-month progression-free survival was 45% (15 out of 35) [70].

From 2011 to 2013, Grignani et al. conducted a second phase of clinical trials of a combination of drugs of everolimus and sorafenib in patients with relapse and refractory osteosarcoma after performing standard polychemotherapy MAP (study NCT01804374). The study included 38 patients over the age of 18 years. Everolimus was administered in a dose of 5 mg once a day and sorafenib 400 mg two times a day. The duration of chemotherapy was 28 days. Partial response was achieved in 4 (10%) patients and stabilization of the disease in 20 (53%) patients. The overall response rate is 63%. This figure is 15% more than in the study, where sorafenib was used in monomode. A 4-month progression-free survival was 58% and for 6-month, it was 45% (17 out of 38) [71].

Thus, taking into account the data of studies in 2008 (application of sorafenib in mono mode) and 2011 (using a combination of sorafenib with everolimus), it can

be said that the combination of sorafenib with everolimus leads to an increase in the overall response rate and an increase in survival rate without disease progression within 6 months. However, by the year, this difference disappears.

At ASCO 2016, preliminary results were presented in a pilot study of the use of everolimus/sorafenib in children with relapse and refractory osteosarcoma, which was carried out at the Institute of Pediatric Oncology and Hematology N.N. Blokhin Medical Research Center of Oncology from 2013 to 2016. This protocol included 14 patients. The first line of therapy is represented by the program "Osteosarcoma 2006" in seven patients and "Osteosarcoma 2014" in seven patients. All patients underwent therapy, which included doxorubicin, cisplatin, high-dose methotrexate, high-dose ifosfamide, and gemcitabine and docetaxel.

The number of courses of therapy for everolimus/sorafenib ranged from 2 to 18. The toxicity of therapy was erythema cutaneous in all patients (100%), palmar and plantar syndrome in 1 (7%), and mucositis 1–2 in 4 (28.5%). Hematologic toxicity did not exceed 1–2 degree in all patients. A transient increase in transaminases up to five norms in all patients (100%) was also noted.

Partial response to treatment was achieved in 5 of 14 (35.7%) patients and stabilization of the disease in 9 (64.3%). The overall response rate was 100%. Survival without disease progression for more than 6 months was detected in 6 out of 14 (43%) patients. The mean follow-up was 7 ± 1.2 months. The maximum period without progression of the disease is 18.4 months.

The findings suggest that everolimus/sorafenib combination resulted in a partial response in 35.7% of cases with a satisfactory toxicity profile [72].

Compared to international data (Italian sarcoma group) in the presented study, the achievement of a partial response, stabilization of the disease, and the overall response rate were significantly higher.

Currently, a number of studies aimed at studying the role of tumor-associated macrophages. Activation of tumor-associated macrophages can be achieved through the use of preparations of liposomal tripeptides (mifamurtide) and interferon preparations (interferon alpha-2A).

Meyers et al. presented the results of the randomized study CCG 7921/POG 9351, which was conducted from 1993 to 1997. The study included 662 patients with a localized version of osteosarcoma.

A feature of line A therapy was the use of two courses of neoadjuvant chemotherapy for MAP, and in line B therapy, two courses of neoadjuvant chemotherapy MAi, alternating courses of MAR and MAi at the stage of adjuvant chemotherapy, was used. Surgical removal of the primary tumor lesion was performed at week 10. Mifamurtide (MTP) was administered at a dose of 2 mg/m^2 two times a week for 12 weeks, and then once a week for 24 weeks in accordance with randomization.

The mechanism of action of mythamurtide (MTP) is to activate monocytes/macrophages with antitumor activity, which is realized by binding to specific receptors toll-like receptor 4 (TLR4) and nucleotide-binding oligomerization domain 2 receptor (NOD2), followed by altering the activity of cellular signal pathways (ERK1/2—extracellular-signal regulated kinase 1/2), NF-kB—nuclear factor kappa-B, and AP1—adapter protein 1 [73].

After removal of the primary tumor focus, a good histological response in group A was achieved in 42% of patients and in group B in 48%, and a poor histological response in group A was 58% and in group B was 52%. At the same time, the 6-year-old RH was 74%, without the use of MTP was 70% and with the MTP was 78%; BSV was 64%, without the use of MTR was 61% and with MTP was 67%. In group A: OS without the use of MTR was 71% and with MTR was 75%; BSV without MTR was 64% and with MTR was 63%. In group B: OS without the use of MTR was 71% and with MTR was 75%; BSV without MTR was 64% and with MTR was 63%.

Authors	Agents
Ferrari S.	Ifosfamide, adriamycin, cisplatin
Le Deley M.C.	Methotrexate, adriamycin, ifosfamide, etoposide
Cui Q.	Cisplatin
Pitano-Garcia A.	Doxorubicin, cisplatin
Wu X.	Methotrexate, cisplatin, adriamycin
Ohba T.	Bevacizumab (Avastin)
Children Oncology Group	Trastuzumab

Table 3.
Trials/authors and agents.

The addition of MTP to polychemotherapy led to a statistically significant increase in the 6-year OS from 70 to 78% ($p = 0.03$), and there was also a tendency to an increase in BSV, mainly in group B ($p = 0.08$) [25].

Kubo et al. published the results of a pilot study that determined the prognostic significance of the expression level of interferon α/β receptors in 40 patients with localized osteosarcoma who received treatment according to the NECO95J program. Expression of interferon α/β receptors was detected in 45% of patients. When conducting multivariate statistical analysis, a significant association was observed between the expression of interferon α/β receptors and 5-year-old OM and survival free of metastatic lesions (VSMP). The 5-year OM, in the presence of expression of the α/β interferon receptor in the tumor substrate, was 81%, with no expression, 47% ($p = 0.043$), and in the 5-year HSMP, it was 75 and 41% ($p = 0.023$). This study confirms the possibility of using interferon preparations in the treatment of osteosarcoma in patients with overexpression of α/β interferon receptors [74].

Bielack et al. presented the results of the EURAMOS1 study in patients with a good histological response after neoadjuvant MAP chemotherapy. In the age group up to 30 years, the MAP line of therapy was carried out to 359 patients, the MAP INF line α–2b—to 357 patients, in the age group up to 20 years—333 (92.7%) and 332 (92.9%) patients. Groups of patients are statistically significantly comparable by sex, age, localization of the primary tumor lesion, the presence of metastatic lesions, and the histological variant of the tumor.

In accordance with the program, pegylated INF–α–2b was administered at a dose of 0.5 mg/kg (at a maximum dose of 50 mg) once a week for 4 weeks, and then 1 mg/kg (at a maximum dose of 100 mg) 1 time per week (from 30 to 104 weeks of programmed treatment).

In a group of 630 patients with a localized version of osteosarcoma, 135 events were detected: 72 in patients who received the MAP therapy line and 63 in patients who received the MAP INF therapy line–2b. At the same time, the 3-year EFS was 77 and 80%, respectively. Therefore, the use of INF–α–2b as maintenance therapy after MAP in patients with a good histological response did not lead to an increase in EFS [75].

The data set out in paragraph 3 are summarized in **Table 3**.

4. Conclusion

Thus, the results of treatment of children with primary metastatic osteosarcoma, relapse, and refractory course of the disease remain unsatisfactory. Molecular biological factors that determine sensitivity to chemotherapy, invasive,

and metastatic potential of the tumor, as well as the prognosis of the disease, among which special attention is deserved are as follows: expression of MGMT protein, methylation of the promoter part of the MGMT gene, expression of ERCC1 proteins, VEGF, CD133, p-STAT3tyr705, C-MYC, expression of RFC1 micro-RNA, and the presence of rearrangement of the TOR2A gene. It is important to note that there was no comprehensive assessment of the value of these markers for the histological response to neoadjuvant chemotherapy and survival rates in patients with osteosarcoma.

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