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Ewing sarcoma – pathomechanisms, standard treatment and new therapeutic perspectives

- **Wojciech Zezuliński**
  Student Scientific Club at the Department of Orthopedics and Traumatology Medical University of Lublin
  https://orcid.org/0009-0005-6395-3217

- **Filip Woliński**
  Student Scientific Group, Department of Forensic Medicine, Medical University of Lublin, ul. Jaczewskiego 8b
  https://orcid.org/0000-0002-6444-5645

- **Julia Zyśk**
  Military Teaching Hospital with Polyclinic of Independent Public Health Care Unit in Lublin, Poland
  https://orcid.org/0009-0000-4316-4839

- **Mateusz Korga**
  Student Scientific Club at the Department of Orthopedics and Traumatology Medical University of Lublin
  https://orcid.org/0000-0002-3317-5726

- **Jakub Klas**
  Student Scientific Club at the Department of Radiotherapy, Medical University of Lublin
  https://orcid.org/0000-0002-4795-1909

- **Jacek Baj**
  Department of Human Anatomy, Medical University of Lublin, 20-090 Lublin, Poland.
  https://orcid.org/0000-0002-1372-8987

- **Eliasz Dzierżyński**
  St. John's Cancer Center, Department of Plastic Surgery, 20-090 Lublin, Poland
  https://orcid.org/0009-0005-4181-7556

Abstract

Introduction: Ewing sarcoma is the second most common bone tumor among children, and due to its high malignancy 5-year survival rate for patients with primary lesions is around 70%. This number drops to merely 30% if metastases are present. Despite combined modality treatment, including radiotherapy, surgery, pre- and post-surgery chemotherapy, the mortality of patients is still too high. This shows a great need to look for new therapeutic options.

Methodology: Comprehensive literature review was conducted across databases, including PubMed and Google Scholar for studies published between 2000 and 2023. This review presents factors that play a key role in pathogenesis and are potential points of targeted therapy. The paper discusses, among other things, treatment attempts based on the role of the EWS-FLI1 protein, epigenetics, tyrosine kinase inhibitors, immunotherapies and the use of nanomedicine and viruses, as well as the difficulties associated with their application.

Findings: The studies cited vary depending on the phase of the clinical trial they are on, of which teprotumumab, robatumumab, and a combination of cixutumumab/temsyrolimus, ivodesinib, Nivolumab (a PD-1 inhibitor), and Ipilimumab (a CTLA-4 inhibitor) are quite advanced as well as those conducted only on animals and in vitro like YK-4-279 molecule, mithramycin 2'-oxime, NK cells, siRNAs with cationic detonation nanodiamonds (DNDs) and Il-12 by means of lentiviruses.

Conclusions: They are new and promising approaches in cases where standard treatments fail, yet they still require further study. Knowledge of the mechanisms of Ewing's sarcoma formation and its metastases, currently accepted treatment standards, critical points in the pathomechanism and current attempts at treating Ewing's sarcoma is essential for choosing the best treatment and effectively reducing its mortality rate.

Keywords

Ewing sarcoma, EWS-FLI1, Epigenetics, Pathomechanisms, Metastasis

1. Introduction

Sarcomas are solid tumors developing from connective tissue. They are classified into three categories: 1) soft tissue and visceral sarcomas, 2) bone sarcomas, and 3) undifferentiated small round cell sarcomas of bone and soft tissue - which include Ewing Sarcoma (ES).

According to the latest 2020 World Health Organization (WHO), classification of soft tissue tumors Ewing's sarcomas is identified as undifferentiated small round cell sarcomas of the bone and kept separate from other similar neoplasms that have different underlying mutations and thus present separate molecular and clinical features (Table 1) [1], [2], [3].

Table 1. 2020 WHO classification of small round cell sarcomas of bone and soft tissue, their typical molecular alternations, and immunochemistry markers.

Type of the tumor	**Molecular alternation**	**Gene fusion**	**Immunochemistry**
Ewing sarcoma	t(11;22)(q24;q12) t(21;22)(q22;q12)	EWSR1-FLI1 EWSR1-ERG EWSR1-ETS gene family FUS-ETS gene family	CD99 NKX2.2 PAX7
Round cell sarcoma with EWSR1-non-ETS fusions	t(20;22)(q13.2;q12) t(20;16)(q13.2;p11.2) inv(22)(q12; q12)	EWSR1-NFATC2 FUS-NFATC2 EWSR1-PATZ1	NKX2.2 PAX7 NKX3.1
CIC-rearranged sarcomas	t(4;19)(q35;q13) t(10;19)(q26;q13) t(x;19)(q13;q13.3) t(;19)() t(15;19)(q14;q13.2) t(10;19)(q23.3;q13)	CIC-DUX4 CIC-DUX4 CIC-FOXO4 CIC-LEUTX CIC-NUTM1 CIC-NUTM2B	ETV4 WT1 NUT
Sarcoma with BCOR genetic alternations	inv(x)(p11;p11) BCOR-ITD T(10;17)(q23.3;p13.3) t(4;x)(p11;q31) t(x;22;)(p11;q13.2)	BCOR-CCNB3 BCOR-ITD YWHAE1-NUTM2B BCOR-MAML3 ZC3H7B-BCOR	BCOR SATB2 PAX7 CCNB3

First described by James Ewing in 1921 as diffuse bone endothelioma [4] , ES is a malignant tumor accounting for 3% of all adolescent cancers. Typically, ES affects patients younger than 20 years old, with the highest incidence at the age of 15, in Europe affecting 7.5 children per one million. It is more common in males, with a 3:2 ratio. Moreover, ES is

observed less often in Asian and African populations, possibly because of genetic differences [5].

Ewing sarcomas can be divided by the primary lesion location into osseous (70-80%) and extraosseous (20-30%). ES commonly develops in the pelvis, ribs, and diaphysis of long bones (femur, tibia, humerus). Extrasceletal localization such as soft tissues of the prevertebral region and proximal portions of upper limbs is more common for older patients. Moreover, the literature describes primary lesion occurrence in viscera (kidney, pancreas, meninges) and skin [3], [6].

The clinical presentation is not specific as a fast-growing tumor Ewing sarcoma presents with mass effect and pain – lesions located intraosseous lead to osteolysis and pathologic fractures. Other common symptoms are malaise, fevers, and weight loss. The mean duration between the onset of symptoms and diagnosis is 5 months. Moreover, 20-25% of patients have metastases present at diagnosis, which can lead to a variety of organ symptoms [3], [6], [7].

2. Etiology and basic therapy resistance mechanisms

The exact cause of genetic changes leading to the development of ES remains elusive. Contrary to osteosarcoma, ES is not very heterogeneous. Characteristic changes are recurrent translocations between two gene families:

- FET protein family encoded on 16,17, and 22 chromosomes which includes FUS, EWSR, and TAF15. In nonpathological settings, they are responsible for controlling transcription, RNA processing, and the fate of mRNA in metazoa. FET proteins can bind with DNA, possibly having a role in transcription and damage responses [8].
- One of the ETS family of transcription factors – mainly FLI1, ERG, and ETS.

In 85% of cases, we can observe classic translocation – t(11; 22) (q24; q12) with a fusion of EWSR1 and FLI1 genes, thus creating fusion protein EWSR1-FLI1. According to Smith et al., it acts as a transcription factor up-regulating 320 other genes and down-regulating 1151, with the most crucial downstream target being NKX2.2 – part of the NK2 homeobox family genes, which is mostly expressed during neuronal development [9].

Other important targets include transcription factors (FOXM1, DAX-1), secreted proteins such as LOX and cholecystokinin, neuronal crest development proteins (MAPT), cell cycle regulators (p21), kinases (PIM3, AURKA, AURKB) [10]

Moreover, EWSR1-FLT1 affects transcript degeneration, and alternative splicing and induces genome instability facilitating tumorigenesis. Thus, through dysregulation of the cell cycle and enhancement of cell growth Ewing sarcoma is born.

The remaining 15% is characterized by other translocations between the EWS gene and ETS family members. The second most common is t(21;22):(q22;q12) merging ERG and EWS genes. It has been shown that patient survival rate doesn't depend on the translocation type [5], [11].

Acquiring therapy resistance is an important part of ES pathophysiology. Below we discuss some of the key mechanisms involved in this process.

As with any other type of neoplasm – Ewing sarcoma consists of many cell populations with various degrees of differentiation, which can respond distinctly to treatment. In the case of ES, even high-risk patients may show improvement after initial chemotherapy

before relapsing. This sequence is often attributed to the presence of chemotherapy-resistant cancer stem cells (CSC).

Chemotherapy-resistant cancer stem cells (CSC) are a small population of cells within ES. They are intrinsically resistant to many toxic agents and thus play a pivotal role in repopulating the tumor post-treatment [11], [12].

Ewing sarcoma cells hijack physiological antioxidation and detoxification mechanisms to prevent chemotherapy damage. One of them is nonprotein thiol named glutathione (GHS). Glutathione S-transferases (GST) catalyze the conjugation of the GSH sulfhydryl group as a first step in the mercapturic acid pathway that leads to the neutralization of toxic compounds. The previously mentioned EWS/FLI fusion protein directly binds to the promoter of glutathione S-transferase M4 (GSTM4) increasing its expression. Higher levels of GSH and GSTM4 correlate with worse prognosis in ES patients. Moreover, the reduction of GSTM4 resulted in increased sensitivity of ES cells to chemotherapy further establishing its role in drug resistance [13], [14], [15].

The abundance of proliferative pathways such as MAP kinase/Erk, PI3K/mTOR/Akt, NF-kappa B, and the VEGF pathway enhance carcinogenesis and interfere with targeted inhibitory therapy. Especially constant activation of MAP kinase/Erk and PI3K/mTOR/Akt pathways have been shown to occur in resistant ES. Another mechanism regulating the proliferation and migration of ES cells is the Insulin-like growth factor pathway. Being one of the new treatment targets it will be described in the next chapters.

3. Metastases

The presence of metastases is the most important prognostic factor for ES patients. The five-year survival rate is only 30% for patients with metastases, while if metastases are absent it increases to 70%. In the vast majority of cases ES metastases to the lungs (50%), bones (25%), and bone marrow (20%). Although other sites are plausible such as viscera or central nervous system they remain relatively rare [5], [16].

The development of metastases is a gradual multistep process, involving the release of malignant cells into the bloodstream, extravasation of the tumor cells, and colonization of microenvironmental conditions at the ectopic site.

In this process, fluctuating EWSR1-FLI1 expression has been shown as the most important for ES metastatic capacity. Cells that have high EWSR1-FLI1 expression tend to keep typical round cell morphology, and proliferate uncontrollably, yet preserve cell-to-cell interactions and adhesion capabilities, which makes them grow fast, but unlikely to start to metastasize. In the case of low EWSR1-FLI1 expression, the opposite is true.

The Actin cytoskeleton becomes less organized, there is less E-cadherin which results in losing its typical round shape and weaker cell adhesion. Thus, metastases are more probable.

In short, high expression of EWSR-FLI1 is typical for primary lesions and keeps ES cells relatively close to one another. Low EWSR-FLI1 expression is more typical for metastatic disease, because of loss of cell-to-cell interactions.

Other factors influencing metastases development can be divided into extracellular and intracellular signals.

The distancing of tumor cells from the blood vessels as their mass grows bigger causes increasing hypoxia. This induces the production of HIF-1 and HIF-2 which enhance metastatic abilities by up-regulating genes connected with invasion and energy-producing metabolic pathways. Another extracellular mechanism involves FGF produced by bone marrow stromal cells. This protein by activating the FGFR1 signaling pathway changes ES cell morphology making them more likely to spread beyond the primary lesion site. What is more - according to studies localized bone marrow T regulatory lymphocyte accumulation creates a supportive environment for developing ES metastases [17].

Main intracellular signals involved in the development of ES metastases can be grouped into five categories: 1) chromatin modifiers, 2) Wnt/β-catenin signaling, 3) hippo/YAP/TAZ/TEAD axis, 4) receptor tyrosine kinases (RTKs), and 5) cytochrome P450 isoforms.

4. Current treatment regimen for Ewing's sarcoma

Current treatment schema for Ewing's sarcoma includes surgery, radiotherapy and chemotherapy (Figure 1). Many authors agree that patients should have a chance to explore local treatment options as soon as possible after diagnosis and decisions about local therapy should be made in collaboration with patients and their families [25], [26] . For localized osteosarcoma and Ewing's sarcoma, local control measures generally consist of surgery and radiotherapy. Usually, when the lesion is accessible (e.g., in the limbs) and with a small mass, the preferred method is surgery, while for less accessible sarcomas, radiotherapy is an effective alternative [27].

Surgery and radiation therapy are also commonly applied in treating metastatic and recurrent sarcoma, but in most cases, chemotherapy is combined as adjuvant or induction therapy, especially for patients with high-grade tumors [27]. Surgery offers some benefits; it provides the opportunity to assess the response to neoadjuvant chemotherapy, reassess the disease status, and reduces the risk of secondary malignancies in connection with RT. Therefore, the increasing use of surgery as a local control modality has led to a re-evaluation of the indications for radiotherapy. It is important to remember that surgery and RT are complementary modalities in the management of Ewing sarcoma, not competitive.

Euro-Ewing-2012 radiotherapy guidelines recommend postoperative RT in the following clinical situations: if all tissues involved by the pre-chemotherapy tumor volume have not been surgically excised (as often seen in pelvic and sacral sarcomas) or if the histological response to preoperative chemotherapy is inappropriate (<90% necrosis) despite the presence of negative surgical margins [28].

Multi-drug chemotherapies are standard methods in treating sarcoma, and mono-drug chemotherapy is rarely observed [27].

In the past few years, there were trials notarizing that when ifosfamide (IFO) alone or IFO-etoposide combination were included in traditional chemotherapies improved crucially the survival rate, and the quality of life of patients with sarcoma [27] . Double agents combined with IFO and etoposide, multiple agents with IFO alone, and multiple agents with IFO and etoposide had advantages over traditional multi-drug therapy [27]. When compared with multi-chemotherapy, double chemotherapy, double chemotherapy with IFO-etoposide combination, and multiple chemotherapy with IFO-etoposide combination had insignificantly

lower HR in all three periods. In 5-year OS, dual-agent with IFO also seemed to perform better than the multi-agent group. However, multi-drug strategy had significant superiority over placebo in both 5-year OS (HR = 1.58, 95%CI, 1.06–2.35) and 10-year OS (HR = 1.91, 95%CI, 1.26–2.89). Dual- and multi-drug with IFO-etoposide had significantly better efficiency in treating osteosarcoma over placebo (HR = 0.59, 95%CI, 0.36–0.96, and HR = 0.5, 95%CI, 0.29–0.87, respectively) in 5-year OS. Similar results were also observed in the 10-year OS, the HR of the dual-drug group was 0.49 (95%CI, 0.30–0.79) and the HR of the multiple-drug with IFO-etoposide combination group was 0.42 (95%CI, 0.24–0.71). Multi-drug with IFO also had good performance with HR = 0.57 (95%CI, 0.35–0.95). Overall, multiple chemotherapeutic combinations with IFO-etoposide combination was the optimal choice in prolonging the life-span of patients, with the highest surface under the cumulative ranking curve (SUCRA) values of 0.753, 0.781, and 0.822 for 3-, 5-, and 10-year OS, respectively [27] . The first treatment regimens that demonstrated a statistically significant improvement in survival in ET patients included vincristine, dactinomycin, and cyclophosphamide (VAC). Later studies showed an increase in recurrence-free survival from 24% to 60% when doxorubicin was added to the VAC regimen (VACD). Moreover, a beneficial effect on the survival of patients was found after aggressive initial cytoreductive treatment with alkylating drugs (cyclophosphamide at a dose of over 1.4 g/m2). Adding ifosfamide and etoposide to standard treatment (VCD) in patients without metastases prolongs the recurrence-free period and overall survival [31].

The Euro EWING 2012 protocol lists several of the most important treatment regimens for Ewing's sarcoma.

Currently, the basis of treatment for patients with Ewing's sarcoma are programs containing doxorubicin, vincristine, cyclophosphamide, ifosfamide, etoposide, and dactinomycin [32], [33], [34], [35], [36], [37] . As indicated in Euro EWING 2012 VDC/IE chemotherapy is superior to VIDE for both event-free survival and overall survival, with no excess toxicity. This benefit is consistent across all baseline stratification parameters [32], [38] . Multiple chemotherapeutic combinations with IFO-etoposide combination were the optimal choice in prolonging the life-span of patients for 3-, 5-, and 10-year over survival [27].

As for relapse, the secondary outcome, only multi-drug with IFO and etoposide performed better than traditional multi-drug treatment without IFO and etoposide, though no statistical significance was revealed. However, both multi-agents with or without IFO and etoposide were proven to reduce the incidence of relapse [27] . Multi-drugs with IFO and etoposide had the overall best outcomes, with SUCRA values [27].

In patients with localized form, in whom no metastatic foci are detected in the initial examination, it is necessary to use combined treatment: induction chemotherapy (12–18 weeks) + local treatment (surgery ± radiotherapy or radiotherapy), followed by adjuvant chemotherapy - consolidation together. up to approximately 48 – 52 weeks [31], [32], [33], [34], [35], [36], [37] . The only exception may be life-threatening situations requiring urgent surgical intervention or radiotherapy, e.g., spinal cord compression by an intracanal tumor or pericardial tamponade caused by cancer effusion. In these situations, after decompression, it is necessary to diligently implement chemotherapy [31] . In the local treatment of the primary lesion, surgical treatment is recommended first [31], [32], [33], [34], [35], [36], [37], [39],

[40] . Radiotherapy is reserved for inoperable cases or as an adjuvant treatment after non-radical procedures. Local treatment also plays an important role in patients with primary generalized Ewing's sarcoma. If radical local resection of the sarcoma is not possible, radical radiotherapy should be used [27], [32], [33], [34], [35], [36], [37] . In the case of extensive cancer lesions that cannot be radically resected - infiltration of the pelvic bones, retroperitoneal space or spine - radiotherapy is preferred as a local treatment, providing local control in these locations in approximately 25%. Radical radiotherapy should begin between the 12th and 18th week of combined treatment [31].

5. Treatment effectiveness

The implementation of combined treatment with neo- and adjuvant chemotherapy and the postponement of local treatment significantly improved the long-term results of treatment of Ewing sarcomas in adult patients. Five-year survival increased from 5–10% to approximately 40% in adults. The worse prognosis concerns locations within the pelvis and spine, as well as extraosseous forms. Among patients experiencing distant recurrence, pulmonary metastases were present in 82% and were the only identifiable site of disease in 53% [41] . Of the patients treated with focal therapy, 47.1% recurred [42] . The presence of distant metastases at the beginning of intensive combined treatment reduces the percentage of cures to 30%. In the case of bone metastases, <20% of patients survive 5 years, while in the case of lung metastases - 20–40% [31]. Despite more intensive chemotherapy, 30% to 40% of young people with Ewing sarcoma will have a recurrence of the disease. Less than 30% of young people with a recurrence of Ewing sarcoma are alive at 24 months, and less than 10% are alive at 48 months [28].

6. Treatment difficulties

Part of what makes Ewing sarcoma so difficult to treat is its ability to rapidly spread and its resistance to chemotherapy treatments. Despite multimodal treatment, survival of metastatic disease occurring in 20–25% of patients, mainly in the lungs (70–80%) and bone/marrow bone (40–45%), is still associated with a poor prognosis. During radiotherapy in adults with large bone areas (pelvic Ewing's sarcoma), it is difficult to conduct intensive systemic treatment at the same time. Sometimes it is necessary to interrupt chemotherapy during radiotherapy or to administer less intensive two-drug programs containing vincristine and dactinomycin[31].

7. New treatments for Ewing's sarcoma

Unfortunately, more than 50% of patients at the time of Ewing's sarcoma diagnosis have metastases - most often located in the lungs. The presence of metastatic lesions at the time of diagnosis significantly worsens the prognosis - reducing the cure rate to 30% [36].

Another treatment problem is the frequent recurrence. On the other hand, the therapeutic plan is burdensome for the patient - it contains neoadjuvant chemotherapy, surgery, radiation therapy and postoperative chemotherapy. This raises the need to search for new therapeutic approaches. A great opportunity lies in therapeutic options that take advantage of ever-evolving knowledge of genetics, immunology, and metabolic pathways. The following section collects information on new treatments for Ewing's sarcoma. They are based on

different mechanisms and are currently in various phases of clinical trials. In this part, we present some new options for treatment.

7.1. Blockade of activity of protein EWS-FLI1

In the pathogenesis of Ewing's sarcoma, at (11,22) (q24;q12) translocation - EWS-FLI1 - is crucial. It occurs in 85% of patients. Expression of the mutated gene results in a fusion protein that acts as an abnormal transcription factor. It also affects pre-mRNA splicing [43], [44] . EWS-FLI1 is characterized by the presence of intrinsically disordered regions - those that show a lack of stable structure when isolated. They enable the formation of a protein-protein structure - that is, for example, the binding of EWS-FLI1 to transcription regulators. This process leads to oncogenesis [45]. Inhibition of EWS-FLI1 activity provides a potential site of action for targeted therapy for Ewing's sarcoma. However, EWS - FLI1 is a rather difficult target, as it lacks enzymatic activity and has no enzyme domain [45], [46] . Another factor that hinders the creation of a drug targeting this protein is the aforementioned lack of an ordered structure of EWS-FLI1 [47].

However, the possibility of targeting the entire EWS-FLI1 protein complex and thus inhibiting its activity is being explored. One of the new therapeutic possibilities is the molecule YK-4-279 and its analog TK-216. YK-4-279 blocks the interaction of EWS-FLI1 with RNA helicase A - RHA. (a coactivator of transcription). By blocking it, transcription activation is reversed [45], [48] . In one study, YK-4-279 was shown to induce apoptotic cell death in EWS lines and cause cell cycle arrest. In studies on Ewing's sarcoma cells and xenografts of the tested tumor in mice, YK-4-279 was shown to have no toxicity and to induce apoptosis. It acts synergistically with vincristine [47] . In another study, the molecule was shown to act more on alternative splicing, conditioned by EWS-FLI1 [49].

Regardless of the mechanism, YK-4-279 reduces the oncogenic effects of the mutant gene. It is a promising therapeutic option for Ewing's sarcoma.

Another drug that blocks EWS-FLI1 gene expression is mithramycin. This is a natural product that binds to DNA. It disrupts the connection between EWS-FLI1 and promotor NR0B1, which inhibits transcription activation. Unfortunately, a phase I trial using it was discontinued due to the substance's hepatotoxicity. Therefore, the search began for a mithramycin derivative with other properties that could be a safe drug. In vitro and in vivo studies in mice were conducted, in which mithramycin 2'-oxime was also shown to block the activity of EWS-FLI1. In vivo studies established a dose that does not cause hepatotoxic and hematological complications. The efficacy of the drug was then evaluated in a comparison between the group given the vehicle and the group treated with mithramycin. There was an increase in survival among mice given mithramycin 2'-oxime. In addition, it was shown that EWS-FLI1 mRNA levels decreased. However, there was no significant effect on the expression of genes controlled by this transcription factor.

The research team suspects that this effect is related to the poorly chosen time point at which this parameter was measured. 2'-oxime therefore requires further study, but is a promising direction for the development of Ewing's sarcoma therapy [50].

7.2. Epigenetic treatment

Epigenetics is a branch that describes changes in gene expression that are unrelated to changes in DNA. Epigenetic processes include DNA methylation, nucleosome remodeling, histone protein modifications, and remodeling of higher chromatin structure [51]. Disruption of these processes can lead to a halt in cancer cell proliferation. DNA methylation is an essential process in cell differentiation. Disruption of this process occurs in many cancers - including Ewing's sarcoma. Gene hypermethylation is associated with increased tumor aggressiveness. The therapeutic target of the new group of drugs is DNA methyltransferase (DNMT) and ten-eleven translocation (TET) methylcytosine dioxygenase. These are responsible for DNA demethylation. Unfortunately, DNMT inhibitors (DNMTi), such as azacitidine and decitabine, have shown high toxicity in phase I clinical trials. Combinations of them with other drugs are being tested. There have also been reports of non-epigenetic drugs that inhibit TET by indirectly inhibiting its hypermethylation. Mutations that disrupt isocitrate dehydrogenase IDH1/2 enzymatic function, cause an increase of 2-hydroxyglutarate (2HG). That inhibits TET, which leads to hypermethylation. Inhibitors of mutant IDH 1/ 2 restore the proper level of methylation. In this regard, ivodesinib is being tested for the treatment of recurrent solid tumors in children - including Ewing's sarcoma [52]

Histones are responsible for the first step of chromatin packing. They are subject to numerous post-translational modifications. The modifications form a code that affects the availability of DNA for transcription factors and thus regulates gene expression. Enzymatic activities behind this histone code include authors that establish these modifications (including histone acetyltransferases (HATs) or histone methyltransferases (HMTs)), erasers that eliminate them (including histone demethylases (HDMs) or HDACs), and readers that recognize and mediate epigenetic signaling. Authors include Polycomb proteins, which divide into PRC1 and PRC2 complexes. PRC1 contains the E3 ubiquitin ligase RING1A or RING1B.

In Ewing's sarcoma, RING1B facilitates the recruitment of oncogenic factors. Aurora kinase (AURK) has been shown to modulate the activity of RING1B. This makes it an interesting target of Ewing’s sarcoma therapy. AZD1152 is an AURKB inhibitor. A study conducted by Sanchez Molina et al. showed that AZD1152 stimulates the process of apoptosis [53]. The PRC2 complex contains the EZH2 subunit. It is overexpressed in Ewing's sarcoma and silencing it may therefore be a method of inhibiting tumor growth. Inhibitors of EZH2 include the nonspecific inhibitor 3-deazaneplanocin A (DZNep) and the specific inhibitor tazemetostat and GSK126. DZNep showed an anti-tumor effect in in vitro and in vivo studies. Tamezostat is undergoing clinical trials. GSK126 and tamezostat also could sensitize Ewing's sarcoma to cellular immunotherapy. Ganglioside GD2 is the potential target for CAR-T cells. Blocking EZH2 by GSK126 or tamezostat causes an increase in ganglioside GD2 level, which could make cells more available to CAR-T cells. [54]. Another drug that affects one of the methyltransferases-G9A-is BIX01294. It reduces metastasis and tumor growth. The role of histone erasers is played by HDAC deacetylases and demethylases.

Treatment targeting them is described in a paragraph above. Histone readers are BETs (the bromodomain and extra-terminal). They interact with the deacetylated lysine residues of histones. BET inhibitors include JQ1. It suppresses EWS-FLI1 activity in in vitro and in vivo studies [55]. It also suppresses cell proliferation, angiogenesis and tumor growth, as tested on

Ewing's sarcoma xenografts. Studies of further BET inhibitors - BMS-986158 and BMS-986378 - are underway [52].

7.3. Tyrosine kinase inhibitors

More than 90% of ES show increased expression of the insulin-like growth factor I receptor (IGF-1R), which appears to promote transcriptional expression of EWS fusion genes and facilitate several oncogenic pathways. Its expression is associated with poorer clinical prognosis and worse response to chemotherapy. This is because IGF-1 activates the PI3K/AKT pathway, which is responsible for the inhibition of apoptosis and possibly induction of VEGFR and FGFR expression. PDGFR-α AND PDGFR-β are also expressed in Ewing's sarcoma cells and its microenvironment, affecting tumor progression. The above receptors belong to the group of tyrosine kinase receptors. This knowledge allows us to predict that tyrosine kinase inhibitors may be useful in the therapy of the described tumor [56].

Atii et al. conducted an efficacy study of regorafenib – a multikinase inhibitor - on 30 patients. During the study, the most common side effect was hypophosphatemia. Sixteen patients required a reduction in drug dosage. Two of these patients discontinued treatment due to toxicity. One death unrelated to the study occurred during the trial. The percentage of patients free of progression after 8 weeks was 63%, thus reaching the study endpoint. The median overall survival in the group of 30 patients was 53 weeks. The RECIST 1.1 response rate was 10%. The median progression-free time was 14,8 weeks. The median OS was 53 weeks. Given these results, the study authors conclude that regorafenib alone has little efficacy against Ewing's sarcoma [57].

Another study of regorafenib in combination with chemotherapy examined 21 pediatric patients with solid tumors, including Ewing's sarcoma (five patients). Eight (38%) patients experienced serious adverse events. The overall response rate was 48% (10/21), and the disease control rate was 86%. Two of the five patients with Ewing's sarcoma experienced partial remission. The study concluded that regorafenib can be safely used in pediatric patients and determined its safe dose. The study suggests that it may have clinical activity in Ewing's sarcoma [58].

The efficacy of IGF-1R inhibitors has also been investigated. A meta-analysis by Amin et al. analyzed five clinical trials using drugs targeting the growth factor receptor. The study contains 56 patients in total. Two evaluated the single drug teprotumumab, one evaluated the single drug robatumumab, and two evaluated the combination cixutumumab/temsyrolimus. The comparison shows that the best clinical response can be achieved with the combination of an IGF-1R inhibitor (cixutumumab) and an mTOR kinase inhibitor (temsyrolimus) [59].

DuBois et al. also conducted a randomized trial of ganitumab, another anti-IGF -1R monoclonal antibody, in combination with chemotherapy. The study included 298 patients - 148 in the standard arm; and 150 in the experimental arm. Patients in the standard arm received chemotherapy in a VDC/IE regimen (vincristine/doxorubicin/cyclophosphamide in compressed intervals alternating once every 2 weeks with ifosfamide/etoposide = VDC/IE).

Patients in the experimental arm received VDC/IE chemotherapy with ganitumab at the beginning of the cycle and as monotherapy once every 3 weeks for 6 months after conventional therapy. The addition of ganitumab did not prolong 3-year survival among patients or event-free time - the results of patients in the experimental group were comparable

to those of patients in the study group. The experimental group reported more cases of pneumonia after chest field irradiation and a nominally higher incidence of neutropenia with fever and increased ALT activity. It can therefore be concluded that ganitumab therapy is associated with increased toxicity [60].

7.4. Immunotherapy

Immunotherapy is a treatment that boosts the immune system's response to cancer or blocks mechanisms that prevent anti-tumor immunity. Ewing's sarcoma is one of the cold tumors - this means that it elicits a weak immune response. Immunotherapy in Ewing's sarcoma includes for example the use of checkpoint inhibitors and adoptive cell therapy.

One of the immunotherapy treatments is checkpoint blockade. PD-1 receptors are found on T lymphocytes, B lymphocytes, monocytes, and macrophages, and their combination with PD-L1 or PD-L2 ligands results in the activation of suppression and apoptosis in cells. Checkpoint inhibitors block the ligand or receptor. As a result, their combination does not occur. This leads to an increased anti-tumor immune response. CTLA-4 is a protein that inhibits T-cell activation. CTLA-4 begins to act when it binds to the ligands CD80 and CD86, located on the antigen-presenting cell. Nivolumab belongs to the PD-1 inhibitors, while ipilimumab is a CTLA-4 inhibitor. Davis et al. conducted a study evaluating the safety and efficacy of the combination of nivolumab and ipilimumab in the treatment of Ewing's sarcoma in pediatric patients. The treatment was administered to 55 patients who had Ewing's sarcoma, striated cell sarcoma, and osteosarcoma. Patients were divided into groups that received the treatment at different doses.

The study found that nivolumab at a dose of 3 mg/kg in combination with ipilimumab 1 mg/kg was well tolerated by children and young adults with solid tumors and showed some clinical activity. In contrast, an increased dose of ipilimumab 3 mg/kg) in combination with nivolumab 1 mg/kg has been associated with increased toxicity without clinical benefit [61].

Another immunotherapy treatment option is NK cell therapy. NK cells are an important player in the anti-tumor response - they exhibit cytolytic activity, ADCC, and cytokine release. Research is underway to administer them to patients with Ewing's sarcoma. NK cells used for therapy can be autologous - taken from the patient, activated, and given back to the patient - and allogeneic - taken from a donor. The activation process involves adding cytokines to the NK cell culture. Autologous cells are safer, but do not elicit such a strong anti-tumor response. For adoptive cell therapy, NK cells can be obtained from several sources, such as peripheral blood, hematopoietic stem cells, induced pluripotent stem cells (iPSCs), and umbilical cord, with each source having its advantages and disadvantages [62]. One study also produced CAR-NK cells - NK cells modified with recombinant antigen receptors. These cells showed activity in vitro, but not in vivo. The research team suspects that this is related to the activation of immunosuppressive HLA-G antigen by tumor cells in response to the presence of NK cells. These results indicate the need for further research into the use of NK cells in the treatment of Ewing's sarcoma [63].

7.5. Nanomedicine and viruses - new drug delivery systems

Nanomedicine is a new therapeutic strategy based on the application of nanotechnology to medicine through the development and use of nanoparticles (NPS). NPS

range from 1 to 100 nm in size. One possibility for their use is more precise delivery of drugs to tissues [52].

An example of the potential use of nanomedicine to treat Ewing's sarcoma is the delivery of siRNAs using cationic detonation nanodiamonds (DNDs). siRNAs are used to control gene expression by silencing target genes. They have low stability and penetrate poorly into cells. A study conducted by Clevau et al. showed that EWS-FLI1 antisense siRNA complexed with DND inhibits EWS-FLI1 expression by about 50%. Research was made on xenografts in mice [64].

Studies have also been conducted on the drug ML111, delivered to the body via nanosystems. ML111 is characterized by anticancer activity. It also enhances the efficacy of chemotherapeutics and shows protective activity in healthy cells. Specifically, ML111 and vincristine exhibit a synergistic effect on Ewing’s sarcoma cells, while this drug pair exhibits antagonistic effects toward non-malignant cells. Unfortunately, it is very poorly soluble in water. This can be remedied by encapsulating it in a hydrophobic core of PEG-PCL-based polymeric nanoparticles. Combining the nanoparticle with the drug improved its uptake by tissues and anti-tumor activity. In a study by Sabei et al. ML111 administered as described inhibited the growth of Ewing's sarcoma in xenografts in mice. It was more effective when combined with vincristine. Importantly, the treatment was well tolerated by the animals [52], [65].

Inorganic NPS are particles based on metals (gold, iron, lead, silver) and metal oxides (aluminum oxide, zinc oxide, etc.) Neumann et al. conducted a study on the delivery of the drug topoisomerase inhibitor SN 38 using a gold nanoparticle, Au-NP. It is activated by EWS-FLI1 or survivin mRNA. EWS-FLI1 is characteristic for Ewing’s sarcoma cells and survivin is overexpressed in that tumor. SN-38 has activity against Ewing's sarcoma but has high toxicity and poor solubility. Au-NP delivers it to the tumor through the presence of mRNA unique to the tumor cell. Au-NP was taken up by Ewing's sarcoma cells with high efficiency. AuNP in combination with SN 38 showed high anti-tumor activity, both in vitro and in vivo [52], [66].

Another promising direction in the development of drug delivery technology is the use of lentiviruses for this purpose. Their mechanism of action is to be absorbed into specific cells, penetrate their interior, integrate with the host's DNA, and produce their proteins. The method of drug delivery by viruses is based on replacing the genetic material of the virus with another element - shRNA interacting with tumor DNA or a functional protein [67] . By being encapsulated in the viral capsid, the substance can directly reach a specific group of cells. This allows the drug to restrict its action elsewhere in the body, thereby reducing the toxicity of the therapy. It also prevents the breakdown of non-persistent particles in the blood.

shRNA - short haircut RNA is a small RNA molecule capable of silencing the expression of specific genes. It is transcripted by reverse transcriptase and then integrates with host nuclear DNA and produces pre-shRNAs that are exported to the cytoplasm, and this shRNA in turn cleaves the target mRNA with the help of a dicer ribonuclease complex [68]. Schafner et al. conducted a screening study to find shRNAs that interact with the Ewing’s sarcoma cell line. During the study, shRNA was delivered to the sarcoma cell line using lentiviruses [69].

8. New attempts in the treatment of metastases of Ewing's Sarcoma

As mentioned earlier, more than 50% of patients have metastases at the time of Ewing's sarcoma diagnosis. In the vast majority of cases, ES metastasizes to the lung (50%), bone (25%), and bone marrow (20%). Although other locations are likely, such as the viscera or central nervous system, they remain relatively rare tan [5], [16]. The presence of metastatic lesions at the time of diagnosis significantly worsens the prognosis. The five-year survival rate is only 30% for patients with metastases, while this rate rises to 70% in the absence of metastases. [5], [16], [36].

Various attempts have been made to expand therapeutic options for patients with distant metastases. The R2Pulm trial compared the effect of high-dose chemotherapy with busulfan and melphalan in combination with autologous stem cell rescue (BuMel) without whole lung irradiation (WLI) on event-free survival (primary endpoint) and overall survival compared with a standard chemotherapy regimen of vincristine, dactinomycin and ifosfamide (VAI) with WLI for Ewing's sarcoma (ES) with lung and/or pleural metastases. The study showed no clear benefit with BuMel compared to conventional VAI and WLI (Event-free survival was 50.6% VAI plus WLI compared to 52.9% in patients following BuMel treatment at 8 years of follow-up) [70].

In another study, patients with Ewing's sarcoma and lung metastases were treated with busulfan and melphalan (BU-MEL) with autologous stem cell transplantation, followed by whole lung irradiation (WLI) (at a dose of 12 Gy for patients aged <14 years and 15 Gy for patients aged ≥14 years administered at least eight weeks after BU-MEL). Five-year OS, EFS, and PRFS with 95% confidence intervals (CI) were 69.8% (57.1-79.3), 61.2% (48.4-71.7), and 70.5% (56.3-80.8), respectively. Patients with good histologic necrosis of the primary tumor after neoadjuvant chemotherapy showed a significantly reduced risk of disease recurrence or death compared to patients with poor histologic necrosis [71].

The Ewing 2008R3 trial was conducted in 12 countries and evaluated the effect of high-dose treosulfan and melphalan (TreoMel-HDT) chemotherapy followed by autologous hematopoietic stem cell reinfusion on event-free survival (EFS) and overall survival in patients with Ewing sarcoma with distant metastases, excluding patients with lung metastases. All patients initially received six cycles of induction therapy with vincristine, ifosfamide, doxorubicin, and etoposide and eight cycles of consolidation therapy with vincristine, actinomycin D and cyclophosphamide, and were then randomly assigned to a group receiving additional TreoMel-HDT or to a group that received no further treatment (control).
The three-year EFS was 20.9% (95% CI, 11.5 to 37.9) in the TreoMel-HDT group and 19.2% (95% CI, 10.8 to 34.4) in the control group. Patients aged <14 years benefited from TreoMel-HDT treatment with a 3-year EFS of 39.3% (95% CI, 20.4 to 75.8%) versus 9% (95% CI, 2.4 to 34); P = 0.016; HR 0.40 (0.19 to 0.87) [72].

Another study compared the clinical outcomes of patients with metastatic ES after single and combined radiotherapy and surgery. The 5-year overall survival (OS) and cancer-specific survival (CSS) rates were 33.1% and 34.3%, respectively, and the median OS and CSS rates were 29.0±1.9 and 29.0±2.1 months, respectively [74].

In the case of radiotherapy, there have been attempts to use SRS to treat ES in children and young adults with ES with spinal metastases. Of 11 patients with bone malignancies with spinal metastases, the patients tolerated the treatment well, with only one experiencing grade 3 late toxicity [75].

Attempts are being made to analyze the validation of potential drugs, which includes a critical evaluation of each drug using clinical and non-clinical parameters. The study analyzed formulations of eribulin, dinutuximab, cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors, anti-angiogenic tyrosine kinase inhibitors, and poly-ADP-ribose polymerase (PARP) inhibitors. A study by the established Children's Oncology Group (COG) is ongoing at the time of writing [76].

Another study compared the outcomes of patients who achieved post-induction resection of lung metastases after radiation therapy (CR) to patients without complete CR. After a median follow-up period of 3.6 years, the five-year EFS and OS were 30.8% ± 6.4% and 49.6% ± 7.1%, respectively. Post-induction pulmonary CR was associated with prolonged EFS ($p < 0.001$), but did not improve OS ($p = 0.065$) [77].

9. Conclusions

An attempt was made to treat recurrent ES with the drug Lurbinectedin. The phase Ib/II trial showed an ORR of 14.3% [95% confidence interval (CI), 4.0%-32.7%], and the median duration of response was 4.2 months (95% CI, 2.9-5.5 months). The median progression-free survival time was 2.7 months (95% CI, 1.4-4.3 months), the clinical benefit rate was 39.3%, and the disease control rate was 57.1%. The median overall survival was 12.0 months (95% CI, 8.5-18.5 months). The most common grade 3/4 adverse events were neutropenia (57%), anemia, thrombocytopenia, and neutropenia with treatment-related fever (14% each) [78]. For the primary lesion of Ewing's sarcoma, we use consolidated treatment. Depending on the patient, this may consist of neoadjuvant and adjuvant chemotherapy, resection with surgical margins, which is crucial, and post- or total radiotherapy. Multi-drug chemotherapies are standard methods and usually consist of doxorubicin, vincristine, cyclophosphamide, ifosfamide, etoposide, and dactinomycin. Based on Euro EWING 2012 VDC/IE chemotherapy consisting of vincristine, doxorubicin, cyclophosphamide, and ifosfamide with etoposide is superior to VIDE - vincristine, ifosfamide, doxorubicin with etoposide, and it should be a standard treatment [31] [27]. As mentioned at the outset, Ewing's sarcoma exhibits complex pathophysiology, and more than that, it has multiple types of resistance, which significantly complicates treatment but offers the possibility of potentially new targeted therapies. There are many new, rapidly developing methods of treatment for Ewing’s sarcoma, including blocking the activity of the EWS-FLI1 protein, modifications of epigenetics processes in tumors, inhibiting metabolic patterns, and stimulating the immune system to overcome the disease. Researchers also discovered interesting ways to deliver drugs directly to mutated cells. Studies about the blocking activity of the EWS-FLI1 protein by the YK-4-279 molecule, mithramycin 2'-oxime and inhibitors of dihydroorotate dehydrogenase (DHODH) like K-234 were conducted. Researchers have noted inhibiting growth, inducing apoptosis or increasing the survival of mice or mice tissue.

Epigenetic treatments like azacitidine and decitabine, which are inhibitors of DNA methyltransferase (DNMT) were a potential therapeutic option due to the reduction of gene hypermethylation, but unfortunately, they have shown high toxicity in phase I clinical trials. Ten-eleven translocation (TET) methylcytosine dioxygenase is still a potential therapeutic option, and its blocking by ivodesinib is being tested for the treatment of recurrent solid tumors in children - including Ewing's sarcoma [52]. Competitive HDAC inhibitors, i.e., hydroxamic acid suberoylanilide (SAHA) and reversible LSD1 inhibitors, i.e., HCl-2509, can inhibit subunits of the nucleosome remodeling deacetylase (NuRD) complex, which is stimulated by EWS-FLI1. According to the study by Dominiques et al. both SAHA and HCI stop tumor growth in vitro, promote cell cycle arrest, and induce apoptosis in ES cell lines. A study by Atii et al. on regorafenib, which is a multikinase inhibitor, on 30 patients has shown no effect against Ewing's sarcoma, [57] yet a second study suggests that it may have clinical activity in Ewing's sarcoma [58] although it is based only on a group of 5 patients. A meta-analysis by Amin et al. analyzed five clinical trials using drugs targeting the growth factor receptor. The study contains 56 patients in total and evaluated teprotumumab and robatumumab in monotherapy and combination with cixutumumab/temsyrolimus. The comparison shows that the best clinical response can be achieved with the combination of an IGF-1R inhibitor (cixutumumab) and an mTOR kinase inhibitor (temsyrolimus) [59].

DuBois et al. also conducted a randomized trial of ganitumab, another anti-IGF-1R monoclonal antibody, in combination with chemotherapy in a VDC/IE regimen. The addition of ganitumab did not prolong 3-year survival among patients or event-free time, and its addition was associated with increased toxicity [60]. Ganitumab was also tested in combination with palbociclib on 10 patients by Shulman et al. but was terminated prematurely, not for medical reasons. Ewing sarcoma elicits a weak immune response, which makes immunotherapy harder but not impossible due to the use of checkpoint inhibitors and adoptive cell therapy. Nivolumab belongs to the PD-1 inhibitors, while ipilimumab is a CTLA-4 inhibitor. Davis et al. conducted a study evaluating the safety and efficacy of the combination of nivolumab (a PD-1 inhibitor) and ipilimumab (a CTLA-4 inhibitor) in the treatment of Ewing's sarcoma in 55 pediatric patients. The study found that nivolumab at a dose of 3 mg/kg in combination with ipilimumab 1 mg/kg was well tolerated by children and young adults with solid tumors and showed some clinical activity. In contrast, an increased dose of ipilimumab (3 mg/kg) in combination with nivolumab (1 mg/kg) has been associated with increased toxicity without clinical benefit [61]. NK cells are promising new immunotherapy options for Ewing sarcoma thanks to their cytolytic activity, ADCC, and cytokine release. For now, research is underway to administer them to patients with Ewing's sarcoma. The key role of nanomedicine lies in its ability to precise delivery of drugs to tissues [52] like it is with siRNAs using cationic detonation nanodiamonds (DNDs), which can inhibit EWS-FLI1 expression by about 50% in mice's xenografts [64]. Delivery by nanoparticles of the ML111 drug, which is very poorly soluble in water, can improve its uptake by tissues and anti-tumor activity. In a study by Sabei et al. ML111 administered this way inhibited the growth of Ewing's sarcoma in xenografts in mice. The treatment was well tolerated by the animals [65]. The next example is SN-38, which is highly toxic and has poor solubility. Its connection with inorganic NPS particles based on gold Au-NP enabled its administration into Ewing sarcoma cells, showing high anti-tumor activity, both in vitro and in vivo [52], [66].

The last-mentioned promising method is the use of lentiviruses with genetic material replaced by shRNA interacting with tumor DNA or a functional protein [67]. Il-12 systemic treatment shows high toxicity. In a study conducted by Rademacher, Ewing's sarcoma cell lines in mice were transduced with a lentivirus containing IL-12, and the animals showed enhanced IL-12 secretion with significant control of tumor growth [69]. In ES with lung or pleural metastases, there is no clear benefit of BuMel over conventional treatment with VAI and WLI [70]. According to the investigators, WLI at the recommended doses and interval after BU-MEL administration is feasible and may contribute to disease control in Ewing's sarcoma with lung metastases and responsive disease [71]. Trends in PFS and OS improvement at five years were observed among patients receiving CRLT compared to the non-CRLT group, but were not statistically significant [79]. Additional treatment with TreoMel-HDT was shown to have no benefit in the entire patient cohort. TreoMel-HDT may be beneficial for children aged <14 years [72]. Survival of ES patients with extensive metastases remains poor, even if they have CR after systemic treatment [73]. SRS for Ewing's sarcoma and osteosarcoma metastasis to the spine may be considered a therapeutic option in patients with AYA and is associated with acceptable toxicity rates [75]. Multivariate analysis showed that age younger than 20 years and surgical resection of the primary tumor are significantly associated with improved OS in patients with metastatic primary bone ES [74]. Lurbinetedine may be a valuable adjunct therapy for Ewing's sarcoma and is currently being evaluated in combination with irinotecan for the treatment of advanced Ewing's sarcoma, but only in a preliminary trial [78]. In summary, current attempts to increase the treatment efficacy of ES patients with distant metastasis show a high degree of variation with respect to the choice of a new therapeutic modality. The aforementioned treatments have been able to statistically increase OS, and PFS significantly, but despite their diversity, the trials have not produced a breakthrough that significantly improves the life expectancy and quality of life of metastatic ES patients.

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Conceptualization: Wojciech Zezuliński
Methodology: Wojciech Zezuliński
Software: Wojciech Zezuliński, Jacek Baj, Eliasz Dzierżyński
Check: Wojciech Zezuliński, Jacek Baj, Eliasz Dzierżyński
Formal Analysis: Jacek Baj, Eliasz Dzierż
Investigation: Wojciech Zezuliński, Filip Woliński, Julia Zyśk, Mateusz Korga, Jakub Klas
Resources: Filip Woliński, Julia Zyśk, Mateusz Korga, Jakub Klas
Data storage: Filip Woliński, Julia Zyśk, Mateusz Korga, Jakub Klas
Writing-Rought Preparation: Wojciech Zezuliński, Filip Woliński, Julia Zyśk, Mateusz Korga, Jakub Klas
Writing – Review and Editing: Wojciech Zezuliński, Jacek Baj, Eliasz Dzierżyński
Visualization: Wojciech Zezuliński, Jacek Baj
Supervision: Jacek Baj, Eliasz Dzierżyński
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