Review

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Advances in Target Therapy Research in Osteosarcoma

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Abstract: Osteosarcoma (OS) are the most prevalent malignant bone tumors in adolescents and young adults.. OS cells grow in a permissive local microenvironment which modulates their behavior and facilitates all steps in tumor development (e.g., proliferation/quiescence, invasion/migration, drug resistance) and contributes to their intrinsic heterogeneity. The lung parenchyma is the most common metastatic site in OS, and metastatic foci are frequently associated with a poor clinical outcome. Although multiple factors may be responsible for the disease, including genetic mutations (e.g., Rb, p53), the molecular mechanism of development of OS remains unclear and the conventional treatment for OS is still based on a sequential approach that combines chemotherapy and surgery. Also, despite the increase in clinical trials, the survival rates for OS have not improved. Non-specific targeting therapies thus show poor therapeutic effects with side effects at high doses. For these reasons, many efforts have been done to characterize the complex genome of osteosarcoma thanks to the whole exome analysis with the aim to identify predictive biomarkers to give to these patients a better therapeutic option. This review aims to summarize and discuss the main recent advances in OS molecular research for precision medicine.

Keywords: osteosarcoma; next generation Sequencing; tumor targeted therapy; immunotherapy

Introduction

Osteosarcoma is the most common nonhaematological primary malignant tumor of the bone, it arises from mesenchymal cells that produce osteoid and immature bone and affects mainly the extremities of adolescents and young adults [1,2]. Its biologic behavior involves various factors such as: Aggressiveness: Osteosarcoma is highly aggressive and tends to grow rapidly. It can metastasize to other organs, commonly to the lungs. Local Invasion: It has the capacity to invade nearby tissues and structures, causing bone destruction and potential fractures. High Recurrence Risk: Even after treatment, there's a risk of recurrence in osteosarcoma patients, particularly if the tumor wasn't completely removed or if cells have spread to other areas of the body [3]. Osteosarcoma is a type of bone tumor that has a complex and multifactorial pathogenesis [4,5]. Although it is not fully understood, there are some key factors that contribute to its formation:

- Pre-existing bone lesions: Osteosarcoma can develop following pre-existing lesions to the bones, such as trauma or bone pathologies.
- Hereditary factors: In some cases, osteosarcoma may be associated with rare hereditary conditions that increase the risk of developing this type of tumor, such as hereditary retinoblastoma. Indeed, mutations in this gene have been commonly associated with hereditary osteosarcoma. The RB1 protein plays a crucial role in cell cycle control, and its dysfunction can lead to uncontrolled cell proliferation, a common trait in tumors [6]. For example, Li-Fraumeni syndrome is linked to mutations in the TP53 gene, which is involved in

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tumor suppression. People with this syndrome have a higher risk of developing several types of tumors, including osteosarcoma [7]. Other genetic conditions such as Rothmund-Thomson syndrome, Bloom syndrome, and Werner syndrome can increase the risk of developing osteosarcoma [8].

- Radiation Exposure: Ionizing exposure to high doses of radiation may increase the risk of developing osteosarcoma.
- Rapid growth and development: Because osteosarcoma often affects growing young people, it is thought that rapid bone growth and development may play a role in its formation.
- Genetic mutations: Genetic alterations, such as mutations in the TP53, RB1 genes and Wnt signaling pathways, are associated with the development of osteosarcoma. These mutations can alter the control of cell growth, favoring the formation of tumor cells [4,5].

Treatment of high grade osteosarcoma is based on a multidisciplinary approach that includes neoadjuvant chemotherapy, surgical excision of the primary tumor and metastasis excision; evaluation of response to therapy in the surgical specimen is crucial to eventually schedule a postoperative chemotherapy [9]. Patients' survival is related to the development of metastasis and the response to chemotherapy. Standard therapy regimens often involve the use of high-dose methotrexate, doxorubicin, cisplatin and ifosfamide [10,11]. Moreover, osteosarcoma cases are commonly resistant to traditional chemotherapies, and high-dose chemotherapy results in severe side effects [12].

Some of the predictive markers and prognostic factors that are considered include:

- Tumor grade: Classifying the tumor based on the degree of aggressiveness can provide information on the growth rate and potential of the cancer to spread.
- Extension of the tumor: The size of the tumor and whether it has spread to surrounding tissues can influence treatment and prognosis.
- Metastasis: The presence or absence of metastases, particularly in the lungs, is an important prognostic factor for osteosarcoma. Indeed, in patients with metastatic osteosarcoma treated with neoadjuvant therapy, the "Responder" status shows improved survival (82% at 5-years) compared to "Non-Responder" (70% at 5-years) [13,14].
- Response to neoadjuvant chemotherapy: The response of the tumor to chemotherapy administered before surgery can be a prognostic indicator. A good response may indicate a better prognosis.
- Age: The age of the patient at the time of diagnosis can influence the prognosis. For example, younger patients tend to respond better to treatment.
- Tumor location: The specific location of the tumor within the bone may have prognostic implications [15].

Moreover, some genetic mutations can influence tumor behavior and response to treatment. For example, the presence of mutations in the TP53 or RB1 genes may correlate with less favorable prognoses [4,5]. However, the somatic genome of the osteosarcoma is considered complex and characterized by tumor heterogeneity [16,17]; indeed, increased number of mutations, not only in TP53 or RB1 genes, but also in genes that are part of the Wnt signaling pathway, such as APC (adenomatous polyposis coli) and β-catenin, have been associated with osteosarcoma. This signaling pathway regulates cell growth and differentiation [18]. Mutations in genes involved in the MAP kinase signaling pathway, such as BRAF, may be present in some subtypes of osteosarcoma, affecting the growth and survival of tumor cells [19] and the mutation rate of osteosarcoma is the highest among all pediatric tumors [16,17]. In addition to point mutations, osteosarcoma can present structural genomic changes, such as copy number variations, chromosomal rearrangements, deletions, or amplifications that can affect the function of key genes in the control of cell growth [20].

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Also, the gene expression analysis can identify genes that are overexpressed or downregulated in tumor cells [5] and alterations in cellular signaling pathways, such as the insulin-like growth factor (IGF) pathway or the epidermal growth factor (EGF) pathway, may be involved in the growth and survival of tumor cells [21]. Furthermore, phenomena of chromothripsis (massive genomic rearrangement that occurs in a single event) and kataegis (localized hypermutation) have been demonstrated in osteosarcomas. Kataegis was found in over 50% of osteosarcomas analyzed with whole genome sequencing [22]. Nonetheless survival rates of patients have not greatly improved [23], because these alterations can vary from patient to patient, contributing to the diversity of the disease and making it difficult to identify a single cause or pathogenetic pathway. Identification of these genetic mutations in osteosarcoma is essential to better understand tumor biology and develop targeted therapies that can stop specific molecular pathways involved in its growth and spread resulting in a lack of more effective and tailored chemotherapy drug regimens [24].

So, identifying potential prognostic and predictive markers in osteosarcoma involves exploring various avenues of research and analysis and different areas of investigation are explored [25]:

- Clinical data analysis: analyzing large datasets of clinical information from osteosarcoma patients to identify patterns or correlations between demographic factors, treatment protocols, and patient outcomes. This could involve retrospective studies or meta-analyses of existing clinical data [26].
- Radiological and imaging markers: using advanced imaging techniques like MRI, PET-CT scans, or other imaging modalities to identify specific radiological markers associated with tumor aggressiveness, response to treatment, or recurrence. Changes in tumor characteristics visible on imaging might provide insights into prognosis [27].
- Immunohistochemistry studies: examining tissue samples from osteosarcoma patients to identify specific protein markers or antigen expressions associated with disease behavior or response to treatment. Immunohistochemistry studies can reveal valuable information about the tumor immune microenvironment, and it has become a recent research hot spot providing valuable insight into tumor heterogeneity that could influence disease progression [28].
- Molecular biomarkers: investigating specific genetic mutations or molecular markers associated with osteosarcoma progression, response to treatment, or recurrence. This also involves analyzing gene expression profiles, identifying oncogenes or tumor suppressor genes, or exploring epigenetic modifications [25,29]. Also, searching for circulating biomarkers in blood, urine, or other bodily fluids that can indicate disease progression, treatment response, or recurrence. This involves analyzing proteins, circulating tumor cells, circulating tumor DNA (ctDNA), or microRNAs [30].
- Drug sensitivity and resistance studies: investigating factors that contribute to drug resistance or sensitivity in osteosarcoma treatments. Understanding why certain tumors respond differently to therapies can lead to the identification of predictive markers [31].
- Multi-Omics approaches: integrating data from genomics, proteomics, metabolomics, and other omics fields to comprehensively understand the complex molecular landscape of osteosarcoma. This holistic approach might unveil novel markers or pathways relevant to prognosis and treatment response [32].
- Machine learning and artificial intelligence: employing computational methods to analyze complex datasets and identify potential prognostic or predictive markers. Machine learning algorithms can help in discovering patterns and associations that might not be immediately apparent through traditional analysis methods [33].

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Relevant Sections

Precision medicine may involve analyzing the tumor's genetic profile to identify specific mutations or molecular alterations present in tumor cells. This information can be used to identify specific therapeutic targets within tumor cells, enabling the targeted use of drugs or therapies that may be most effective against those specific mutations or markers, to allow the choice of treatments best suited to the molecular profile of a patient's tumor, avoiding treatments that may not be effective or potentially harmful and to use molecular testing to monitor the tumor's response to treatment over time and make any therapeutic changes or adjustments based on the patient's response. So, precision medicine, thanks to developments in genomics and the understanding of the molecular mechanisms of tumors, is helping to improve therapeutic options and the management of patients with osteosarcoma [34]. The increasing use of Next Generation Sequencing (NGS) has completely revolutionized clinical research over the last decade [35]; particularly, exome sequencing can be used to better understand the genetic causes underlying the development of osteosarcoma [36] thanks to the integration of molecular and clinical data to obtain a more complete and accurate view of the disease and develop personalized therapeutic approaches.

There are many examples of "molecular targeted therapy", where tailored therapeutic agents have been selected to aim against specific molecules and their downstream effector pathways in each patient [37]. Tyrosine kinase inhibitors (TKIs) have been explored in the context of osteosarcoma treatment due to their ability to target specific pathways involved in cancer growth and progression [38]. TKIs are a class of drugs that work by blocking the activity of specific enzymes called tyrosine kinases. These enzymes are involved in various cellular processes, including cell growth and proliferation. By inhibiting these kinases, TKIs can potentially impede the growth and spread of cancer cells. Several TKIs have been studied in the context of osteosarcoma, either as standalone treatments or in combination with other therapies. Drugs like sorafenib, sunitinib, and dasatinib are examples of TKIs that have shown some promise in preclinical studies or early-phase clinical trials for osteosarcoma [38]. However, while there have been some encouraging results in laboratory studies and early trials, the effectiveness of TKIs in treating osteosarcoma in larger clinical settings is still under investigation. Challenges remain, including issues related to drug resistance, side effects, and the need for more extensive clinical data to establish their efficacy and safety [39]. As research advances and more clinical data becomes available, the role of TKIs in osteosarcoma treatment may become clearer, potentially offering new avenues for improved therapies and outcomes for patients.

Anti-angiogenesis agents are drugs that inhibit the formation of new blood vessels. In the context of cancer, these drugs aim to prevent the growth of new blood vessels that tumors need to thrive and spread [40]. Osteosarcoma is known for its highly vascularized nature, meaning it has a significant network of blood vessels supplying it with nutrients and oxygen [41]. Anti-angiogenesis therapy might be a promising approach in treating osteosarcoma because these agents can potentially hinder its growth and metastasis but the efficacy of anti-angiogenesis agents alone in treating osteosarcoma might be limited. They are often used in combination with other treatments like chemotherapy or targeted therapies for more effective results. Drugs like bevacizumab and sorafenib are among those that have been studied for their anti-angiogenic properties in osteosarcoma. Clinical trials are ongoing to evaluate their effectiveness, both alone and in combination with other therapies, in improving outcomes for patients with osteosarcoma. While there's promise in utilizing anti-angiogenesis agents, further research is needed to determine their optimal use, potential side effects, and long-term benefits in treating osteosarcoma [42].

The mTOR pathway plays a significant role in the osteosarcoma disease. mTOR (mammalian target of rapamycin) is a protein that regulates various cellular processes, including cell growth, proliferation, and survival. In osteosarcoma, there's often dysregulation or overactivity in the mTOR pathway, contributing to the uncontrolled growth of cancer cells [43]. The abnormal activation of mTOR signaling can lead to increased cell proliferation, resistance to cell death, and the promotion of tumor growth. As a result, targeting the mTOR pathway has been a subject of interest in the development of potential treatments for osteosarcoma [44]. mTOR inhibitors, such as everolimus and sirolimus, aim to block or reduce the activity of mTOR, thereby potentially slowing down the growth

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and spread of osteosarcoma cells [43]. However, the effectiveness of mTOR inhibitors in treating osteosarcoma is an active area of research and often involves combination therapies or clinical trials to assess their efficacy [45].

Moreover, immunotherapy has been an area of interest in the treatment of osteosarcoma [46]. Checkpoint Inhibitors help the immune system recognize and attack cancer cells by targeting proteins that inhibit immune responses; drugs like pembrolizumab and nivolumab have been investigated in clinical trials for osteosarcoma [47]. Vaccine therapies aim to stimulate the body's immune system to recognize and attack cancer cells and some studies have explored vaccine-based approaches for osteosarcoma [48]. Chimeric Antigen Receptor (CAR) T-cell Therapy is a type of adoptive cell therapy where a patient's own immune cells are modified to better recognize and attack cancer cells; while this has seen success in some blood cancers, its efficacy in solid tumors like osteosarcoma is an area of ongoing research [49]. Immunotherapy for osteosarcoma is still in its early stages compared to its use in other cancers, and while there have been some promising results, more research is needed to understand its effectiveness, potential side effects, and the best ways to combine it with existing treatments. Clinical trials continue to explore these avenues to improve outcomes for individuals with osteosarcoma [48].

Discussion

Besides the advance in the field of predictive biomarkers for cancer therapy in the last decades, osteosarcoma is a so-called "orphan cancer" with no known driver oncogenes [50]. Some studies based on NGS approach were performed to better understand the complex biology of this tumor and the molecular pathways that lead to the development of metastases and resistance to therapy [51]. Surely, studies on exome sequencing in osteosarcoma have significantly contributed to our understanding of the genetic landscape and molecular mechanisms underlying this bone cancer, nevertheless, exome sequencing studies have revealed a complex mutational landscape in osteosarcoma, characterized by a wide range of genetic alterations [52]. In our previous studies [36,53], we performed a WES analysis on high osteosarcoma biopsies obtained before neoadjuvant therapy confirming the complexity of osteosarcoma karyotype [52]. We found that the KMT2C gene, a key component of histone H3 lysine 4 methyltransferase complexes [54], showed the highest number of variations in most of the samples being analyzed. KMT2C is mutated in a wide spectrum of neoplasms, it has been linked to tumorigenesis [54] and some studies suggest that variations in coding sequences of regulating elements, which act on enhancers to recognize specific transcriptional factors, may be the cause of tumor development [55]. Indeed, these modifications can activate or repress gene expression, influencing cell behavior. Alterations in KMT2C can disrupt this regulation, leading to abnormal expression of genes involved in cell growth, division, and differentiation, which can contribute to cancer development. Moreover, a recent study of Gaeta et al. [56], showed a prevalence of mutations in some genes, in particular in genes involved in homologous recombination repair process by opening doors to the possibility to use the parp inhibitors as a potential therapeutic option in osteosarcoma patients; indeed, another previous study that analyzed all bone tumors, showed a high frequency of HRD related mutations through germline mutation analysis [57].

Not only HRD but also other biomarkers for immune checkpoint inhibitor response were studied in osteosarcoma through WES. Indeed, a recent review [58] about sarcomas highlights how specific signatures as TMB (Tumor Mutational Burden), MSI (MicroSatellite Instability) and deficiency in BRCA1/2 genes could be useful biomarkers in this type of tumor. Particularly, in some studies was observed that osteosarcomas that showed deficiency in BRCA1/2 genes also presented specific genomic alterations such as those seen in other parp-sensitive tumors [59,60].

Regarding TMB, defined as total numer of non-synonymous somatic mutations per megabase (muts/Mb) in coding areas harbored by tumor cells in a given neoplasm, another study [61] about the analysis of this biomarker through WES in osteosarcoma supports the use of TMB to predict the prognosis in osteosarcoma and so to be relevant in the treatment decision making process.

Also, the most common genetic alterations involved in cell cycle were frequently found in osteosarcoma after WES analysis [57,61]. Despite some of the genes harboring genetic alterations are

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the same in adult and pediatric osteosarcomas, it is showed that pediatric tumors presented more CNV (Copy Number Variation) and genetic fusion than SNVs (Single Nucleotide Variations) and insertions and deletions. Indeed, a study focalized on the use of NGS for pediatric osteosarcoma, showed an association between specific genetic alterations and the patient's age at time of diagnosis [62].

Moreover, a recent study showed how the WES analysis in osteosarcoma is able to identify alteration in genes involved in the protein-protein networking on specific pathways implicated in skeletal system development [63].

So, it is clear that WES analysis plays a crucial role in understanding the genetic landscape of osteosarcoma and there are several reasons highlighting the importance of this approach in this type of cancer such as the discovery of driver mutations for developing personalized treatment approaches, the identification of prognostic markers and a better understanding of tumor heterogeneity. So, WES analysis in osteosarcoma is instrumental for unraveling the complex genetic basis of the disease, guiding treatment decisions, and advancing our understanding of the underlying molecular mechanisms. This knowledge is essential for the development of more effective and personalized therapeutic approaches for patients with osteosarcoma.

Conclusions and Future Directions

It is important to note that research in this field is constantly evolving, however osteosarcoma is a complex tumor and treatment varies depending on the individual case. New therapies are emerging thanks to a better understanding of tumor biology and its molecular characteristics, paving the way for more targeted and effective treatment options. Participation in clinical trials can be an opportunity to access promising experimental treatments. Clinical trials of osteosarcoma treatment are crucial to develop new therapies and improve current treatment options. These studies involve volunteer patients and are conducted to evaluate the effectiveness and safety of new drugs, targeted therapies, surgical approaches or combined treatment protocols. Also, successful identification of prognostic and predictive markers in osteosarcoma often requires collaboration between clinicians, pathologists, geneticists, bioinformaticians, and researchers from various disciplines. This interdisciplinary approach allows for a more comprehensive exploration of the disease and increases the likelihood of discovering clinically relevant markers.

References

1. Raymond A.K.; Ayala A.G.; Knuutila S. Conventional osteosarcoma. In: Fletcher CDM, Unni KK, Mertens F, eds. World Health Organization Classification of Tumors Pathology and Genetics of Tumors of Soft Tissue and Bone. Lyon: IARC Press; 2002; pp. 264–270.
2. Damron T.A.; Ward W.G.; Stewart A. Osteosarcoma chondrosarcoma and Ewing's sarcoma: National Cancer Data Base report. *Clin. Orthop. Relat. Res*. **2007**, *459*, 40–47.
3. Jiang W.G; Sanders A.J.; Katoh M.; Ungefroren H.; Gieseler F.; Prince M.; Thompson S.K.; Zollo M.; Spano D.; Dhawan P.; et al. Tissue invasion and metastasis: Molecular, biological and clinical perspectives. *Semin. Cancer Biol*. **2015**, *35*, S244–S275.
4. Durfee R.A.; Mohammed M.; Luu H.H. Review of Osteosarcoma and Current Management. *Rheumatol. Ther*. **2016**, *3*, 221–243.
5. Morrowa J. J.; Khanna C. Osteosarcoma Genetics and Epigenetics: Emerging Biology and Candidate Therapies. *Crit. Rev. Oncog*. **2015**, *20*, 173–197.
6. Yun J.; Li Y.; Xu CT.; Pan BR. Epidemiology and Rb1 gene of retinoblastoma. *Int. J. Ophthalmol*. **2011**, *4*, 103–109.
7. Rocca V.; Blandino G.; D'Antona L.; Iuliano R.; Di Agostino S. Li–Fraumeni Syndrome: Mutation of TP53 Is a Biomarker of Hereditary Predisposition to Tumor: New Insights and Advances in the Treatment. *Cancers (Basel)*. **2022**, *14*, 3664.
8. Hameed M.; Mandelker D. Tumor Syndromes Predisposing to Osteosarcoma. *Adv. Anat. Pathol*. **2018**, *25*, 217–222.
9. Marina N.M.; Smeland S.; Bielack S.S.; Bernstein M.; Jovic G.; Krailo M.D.; Hook J.M.; Arndt C.; van den Berg H.; Brennan B.; et al. Comparison of MAPIE versus MAP in patients with a poor response to preoperative chemotherapy for newly diagnosed high-grade osteosarcoma (EURAMOS-1): An open-label, international, randomized controlled trial. *Lancet Oncol*. **2016**, *17*, 1396–1408.

10. Baldini N. Multidrug resistance a multiplex phenomenon. *Nat. Med*. **1997**, *3*, 1380–1385.
11. Dorfman H.D.; Czerniak B. Bone Tumors. Mosby, 1998.
12. Whelan J.S.; Bielack S.S.; Marina N.; Smeland S.; Jovic G.; Hook J.M.; Krailo M.; Anninga J.; Butterfass-Bahloul T.; Bohling T.; et al. EURAMOS-1, an international randomised study for osteosarcoma: Results from pre-randomisation treatment. *Ann. Oncol*. **2015**, *26*, 407–414.
13. Ren L.; Mendoza A.; Zhu J.; Briggs J.W.; Halsey C.; Hong E.S.; Burkett S.S.; Morrow J.; Lizardo M.M.; Osborne T.; et al. Characterization of the metastatic phenotype of a panel of established osteosarcoma cells. *Oncotarget*. **2015**, *6*, 29469–29481.
14. Wittig J.C.; Bickels J.; Priebat D.; Jelinek J.; KellarGraney K.; Shmookler B.; Malawer M.M. Osteosarcoma: A multidisciplinary approach to diagnosis and treatment. *Am. Fam. Physician*. **2002**, *65*, 1123–1132.
15. Xina S.; Weia G. Prognostic factors in osteosarcoma: A study level meta-analysis and systematic review of current practice. *J. Bone Oncol*. **2020**, *21*, 100281.
16. Perry J.A.; Kiezun A.; Tonzi P.; Van Allen E.M.; Carter S.L.; Baca S.C.; Cowley G.S.; Bhatt A.S.; Rheinbay E.; Pedamallu C.S.; et al. Complementary genomic approaches highlight the PI3K/ mTOR pathway as a common vulnerability in osteosarcoma. *Proc. Natl. Acad. Sci*. **2014**, *111*, E5564–E5573.
17. Rickel K.; Fang F.; Tao J. Molecular genetics of osteosarcoma. *Bone*. **2017**, *102*, 69–79.
18. Fang F.; VanCleave A.; Helmuth R.; Torres H.; Rickel K.; Wollenzien H.; Sun H.; Zeng E.; Zhao J.; Tao J. Targeting the Wnt/β-catenin pathway in human osteosarcoma cells. *Oncotarget*. **2018**, *9*, 36780–36792.
19. Liu H.; Nazmun N.; Hassan S.; Liu X.; Yang J. BRAF mutation and its inhibitors in sarcoma treatment. *Cancer Med*. **2020**, *9*, 4881–4896.
20. Martin J.W.; Squire J.A.; Zielenska M. The Genetics of Osteosarcoma. *Sarcoma*. **2012**, *2012*, 627254.
21. Ji Z.; Shen J.; Lan Y.; Yi Q.; Liu H. Targeting signaling pathways in osteosarcoma: Mechanisms and clinical studies. *MedComm (2020)*. **2023**, *4*, e308.
22. Gianferante D.M.; Mirabello L.; Savage S.A.;. Germline and somatic genetics of osteosarcoma—Connecting aetiology, biology and therapy. *Nat. Rev. Endocrinol*. **2017**, *13*, 480–491.
23. Martin J.W.; Squire J.A.; Zielenska M. The genetics of osteosarcoma. *Sarcoma*. **2012**, *2012*, 627254.
24. Batanian J.R.; Cavalli L.R.; Aldosari N.M.; Ma E.; SoteloAvila C.; Ramos M.B.; Rone J.D.; Thorpe C.M.; Haddad B.R. Evaluation of pediatric osteosarcomas by classic cytogenetic and CGH analyses. *Mol. Pathol*. **2002**, *55*, 389–393.
25. Zamborsky R.; Kokavec M.; Harsanyi S.; Danisovic L. Identification of Prognostic and Predictive Osteosarcoma Biomarkers. *Med. Sci.* **2019**, *7*, 28.
26. Li W.; Jin G.; Wu H.; Wu R.; Xu C.; Wang B.; Liu Q.; Hu Z.; Wang H.; Dong S.; et al. Interpretable clinical visualization model for prediction of prognosis in osteosarcoma: A large cohort data study. *Front. Oncol*. **2022**, *12*, 945362.
27. Kubo T.; Furuta T.; Johan M.P.; Ochi M.; Adachi N. Value of diffusion-weighted imaging for evaluating chemotherapy response in osteosarcoma: A meta-analysis. *Mol. Clin. Oncol.* **2017**, *7*, 88–92.
28. Zhu T.; Han J.; Yang L.; Cai Z.; Sun W.; Hua Y.; Xu J. Microenvironment in Osteosarcoma: Components, Therapeutic Strategies and Clinical Applications. *Front. Immunol. Immune*. **2022**, *13*, 907550.
29. Sun J.; Xu H.; Qi M.; Zhang C.; Shi J. Identification of key genes in osteosarcoma by meta-analysis of gene expression microarray. *Mol. Med. Rep.* **2019**, *20*, 3075–3084.
30. Raimondi L.; De Luca A.; Costa V.; Amodio N.; Carina V.; Bellavia D.; Tassone P.; Pagani S.; Fini M.; Alessandro R.; et al. Circulating biomarkers in osteosarcoma: New translational tools for diagnosis and treatment. *Oncotarget*. **2017**, *8*, 100831–100851.
31. Hattinger C.M.; Patrizio MP.; Fantoni L.; Casotti C.; Riganti C.; Serra M. Drug Resistance in Osteosarcoma: Emerging Biomarkers, Therapeutic Targets and Treatment Strategies. *Cancers* **2021**, *13*, 2878.
32. Tang S.; Roberts R.D.; Cheng L.; Li L. Osteosarcoma Multi-Omics Landscape and Subtypes. *Cancers* **2023**, *15*, 4970.
33. Vezakis I.A.; Lambrou G.I.;Matsopoulos G.K. Deep Learning Approaches to Osteosarcoma Diagnosis and Classification: A Comparative Methodological Approach. *Cancers* **2023**, *15*, 2290.
34. Tirtei E.; Campello A.; Asaftei S.D.; Mareschi K.; Cereda M.; Fagioli F.; Santucci A. Precision Medicine in Osteosarcoma: MATCH Trial and Beyond. *Cells* **2021**, *10*, 281.
35. Morganti S.; Tarantino P.; Ferraro E.; D'Amico P.; Duso B.A.; Curigliano G. Next Generation Sequencing (NGS): A Revolutionary Technology in Pharmacogenomics and Personalized Medicine in Cancer. *Adv. Exp. Med. Biol*. **2019**, *1168*, 9–30.
36. Chiappetta C.; Mancini M.; Lessi F.; Aretini P.; De Gregorio V.; Puggioni C.; Carletti R.; Petrozza V.; Civita P.; Franceschi S.; et al. Whole-exome analysis in osteosarcoma to identify a personalized therapy. *Oncotarget* **2017**, *8*, 80416–80428.
37. PosthumaDeBoer J.; Witlox M.A.; Kaspers G.J.; van Royen B.J. Molecular alterations as target for therapy in metastatic osteosarcoma: A review of literature. *Clin. Exp. Metastasis* **2011**, *28*, 493–503.
38. Assi A.; Farhat M.; Hachem M.C.R.; Zalaquett Z.; Aoun M.; Daher M.; Sebaaly A.; Kourie HR. Tyrosine kinase inhibitors in osteosarcoma: Adapting treatment strategies. *J. Bone Oncol*. **2023**, *43*, 100511.

 doi:10.20944/preprints202402.1596.v1

39. Chen C.; Shi Q.; Xu J.; Ren T.; Huang Y.; Guocorresponding W. Current progress and open challenges for applying tyrosine kinase inhibitors in osteosarcoma. *Cell Death Discov*. **2022**, *8*, 488.
40. Teleanu R.I.; Chircov C.; Grumezescu A.M; Teleanu D.N. Tumor Angiogenesis and Anti-Angiogenic Strategies for Cancer Treatment. *J. Clin. Med*. **2020**, *9*, 84.
41. Corre I.; Verrecchia F.; Crenn V.; Redini F.; Trichet V. The Osteosarcoma Microenvironment: A Complex but Targetable Ecosystem. *Cells*. **2020**, *9*, 976.
42. Xie L.; Ji T.; Guo W. Anti-angiogenesis target therapy for advanced osteosarcoma. *Oncol. Rep*. **2017**, *38*, 625–636.
43. Ding L.; Congwei L.; Bei Q.; Tao Y.; Ruiguo W.; Heze Y.; Bo D.; Zhihong L. mTOR: An attractive therapeutic target for osteosarcoma? *Oncotarget* **2016**, *7*, 50805–50813.
44. Chamcheu J.C.; Roy T.; Uddin M.B.; Banang-Mbeumi S.; Chamcheu RC. N.;1 Walker A.L.; Liu YY:, Huang S. Role and Therapeutic Targeting of the PI3K/Akt/mTOR Signaling Pathway in Skin Cancer: A Review of Current Status and Future Trends on Natural and Synthetic Agents Therapy. *Cells* **2019**, *8*, 803.
45. Rathore R.; Van Tine B.A. Pathogenesis and Current Treatment of Osteosarcoma: Perspectives for Future Therapies. *J. Clin. Med.* **2021**, *10*, 1182.
46. Yahiro K.; Matsumoto Y. Immunotherapy for osteosarcoma. *Hum. Vaccin. Immunother*. **2021**, *17*, 1294–1295.
47. Zhang Z.; Tan X.; Jiang Z.; Wang H.; Yuan H. Immune checkpoint inhibitors in osteosarcoma: A hopeful and challenging future. *Front. Pharmacol*. **2022**, *13*, 1031527.
48. Supra R.; Agrawal D.K. Immunotherapeutic Strategies in the Management of Osteosarcoma. *J. Orthop. Sports Med*. **2023**, *5*, 32–40.
49. Köksal H.; Müller E.; Inderberg E.M.; Bruland O.; Wälchli S. Treating osteosarcoma with CAR T cells. *Scand. J. Immunol*. **2019**, *89*, e12741.
50. Reed D.E.; Shokat K.M. Targeting osteosarcoma. *Proc. Natl. Acad. Sci .USA* **2014**, *111*, 18100–18101.
51. Shyr D.; Liu Q. Next generation sequencing in cancer research and clinical application. *Biol. Proced. Online* **2013**, *15*, 4.
52. Batanian J.R.; Cavalli L.R.; Aldosari N.M.; Ma E.; SoteloAvila C.; Ramos M.B.; Rone J.D.; Thorpe C.M.; Haddad B.R. Evaluation of pediatric osteosarcomas by classic cytogenetic and CGH analyses. *Mol. Pathol*. **2002**, *55*, 389–393.
53. Chiappetta C.; Puggioni C.; Carletti R; Petrozza V.; Della Rocca C.; Di Cristofano C. The nuclear-cytoplasmic trafficking of a chromatin-modifying and remodelling protein (KMT2C), in osteosarcoma. *Oncotarget*. **2018**, *9*, 30624–30634.
54. Shilatifard A. The COMPASS family of histone H3K4 methylases: Mechanisms of regulation in development and disease pathogenesis. *Annu. Rev. Biochem*. **2012**, *81*, 65–95.
55. Herz H.M. Enhancer deregulation in cancer and other diseases. *Bioessays*. **2016**, *38*, 1003–1015.
56. Gaeta R.; Morelli M.; Lessi F.; Mazzanti C.M.; Menicagli M.; Capanna R.; Andreani L.; Coccoli L.; Aretini P.; Franchi A. Identification of New Potential Prognostic and Predictive Markers in High-Grade Osteosarcoma Using Whole Exome Sequencing. *Int J Mol Sci*. **2023**, *24*, 10086.
57. Xie X., Bian Y., Li H., Yin J., Tian L., Jiang R., Zeng Z., Shi X., Lei Z., Hou C.; et al. A Comprehensive Understanding of the Genomic Bone Tumor Landscape: A Multicenter Prospective Study. *Front. Oncol.* **2022**, *8*, 835004.
58. Vyse S., Thway K., Huang P.H., Jones R.L. Next-generation sequencing for the management of sarcomas with no known driver mutations. *Curr. Opin. Oncol*. **2021**, *33*, 315–322.
59. Chudasama P., Mughal S.S., Sanders M.A., Hübschmann D., Chung I., Deeg K.I., Wong S.H., Rabe S., Hlevnjak M., Zapatka M.; et al. Integrative genomic and transcriptomic analysis of leiomyosarcoma. *Nat. Commun.* **2018**, *9*, 1–15.
60. Kovac M., Blattmann C., Ribi S., Smida J., Mueller N.S., Engert F., Castro-Giner F., Weischenfeldt J., Kovacova M., Krieg A.; et al. Exome sequencing of osteosarcoma reveals mutation signatures reminiscent of BRCA deficiency. *Nat. Commun*. 2015, 6, 8940.
61. Xie L., Yang Y., Guo W., Che D., Xu J., Sun X., Liu K., Ren T., Liu X., Yang Y., Ji et al. The Clinical Implications of Tumor Mutational Burden in Osteosarcoma. *Front. Oncol*. **2021**, *7*, 595527.
62. Guimarães G.M., Tesser-Gambaa F., Petrilli A.S., Donato-Macedoa C.R.P., Alves M.T.S., de Limaa F.T., Garcia-Filhoa R.J., Oliveiraa R., Toledoa S.R.C. Molecular profiling of osteosarcoma in children and adolescents from different age groups using a next-generation sequencing panel. *Cancer Genet*. **2021**, *258-259*, 85-92.
63. Ferreira Pires S., Sobral de Barros J., Souza da Costa S., Bandeira do Carmo G., de Oliveira Scliar M., van Helvoort Lengert A., Boldrini E., Regini Morini da Silva S., Onofre Vidal D., Maschietto M.; et al. Analysis of the Mutational Landscape of Osteosarcomas Identifies Genes Related to Metastasis and Prognosis and Disrupted Biological Pathways of Immune Response and Bone Development. *Int. J. Mol. Sci.* **2023**, *24*, 10463.

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