International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Primary Bone Cancers: Latest Trends in Pathogenesis, Diagnosis and Management

Nursuffi Rathiyah binti Mohd Hizam[a], Kaswinna Malar A/P Ananthan[b], Nur Zahrin Syahirah binti Raman[c], Kishore Gopal Banerjee[d]*

[a,b,c]*International Medical School, Management and Science University (MSU), University Drive, Off Persiaran Olahraga, 40100 Shah Alam, Selangor, Malaysia*

[d]*Corresponding author: Senior Lecturer, Department of Pathology, International Medical School, Management & Science University, 40100 Shah Alam, Selangor, Malaysia*

[a]*Email: nursuffihizam@gmail.com,* [b]*Email: kaswinnamalar@yahoo.com*

[c]*Email: zahrinsyahirah@gmail.com,* [d]*Email: kishore@msu.edu.my*

Abstract

Primary bone tumours arising from the bony tissue are further classified into three groups; bone-producing, cartilage-producing, and tumours of unknown origin. In this review article, we have analyzed the latest trends in the pathogenesis, diagnosis, and management of the key primary malignant tumours (osteosarcoma, chondrosarcoma, and Ewing sarcoma) of bony origin. The Hippo-Yap signaling pathway is a newly discovered pathogenesis for osteosarcoma, and the inhibition contributes to the treatment of the malignancy. Moreover, carbon-ion particle therapy is proven to be a remedy for radioresistant tumours. The advancement of diagnosis methods for osteosarcoma is hastened with the identification of biomarker microRNA, liquid biopsy, and 'Omic' technology. Chondrosarcoma has a different and tailored approach to manage patients. Due to the un-known pathogenesis, treatments are being considered holistically depending on the patients' age, overall health, medical history, type and stages of chondrosarcoma, the severity of the carcinoma, and patients' preference or opinion on the treatment plans. It has no specific treatment, and the ideal goal is to remove the mass and reduce the likelihood of relapse.

---

* Corresponding author.

Lastly, for Ewing sarcoma, there is a variety of new targeted therapy such as Lysine-specific demethylase-1 (LSD-1), PRKCB, Poly (ADP-Ribose) polymerase1 inhibitors (PARBP1) have been developed to treat recurrent ES, and a large dose of chemotherapy with autologous hematopoietic stem cell has been identified to cause toxicity in Ewing sarcoma. The origin of Ewing sarcoma remained un-known, but the rearrangement of the gene is thought to be the cause of tumorigenesis of Ewing sarcoma.

Keywords: Primary bone tumour; osteosarcoma; Ewing sarcoma; chondrosarcoma; pathogenesis; treatment; diagnosis.

1. Introduction

Primary bone cancers are mostly idiopathic and account for almost 0.2% of all carcinoma, originating from mesenchymal cells. Osteosarcoma, chondrosarcoma, and Ewing sarcoma are the most common types. They are aggressive and vary in demographics, imaging presentation, biological behaviour, thus require early diagnosis [49]. Osteosarcoma has a bimodal distribution. The first peak arises in the long bones of pubescents and the second peak mainly affects the axial skeleton [1,3-5,8]. Chondrosarcoma starts in cartilaginous tissues and commonly forms in the pelvis, femur, and humerus and grows slowly, although sometimes it can proliferate and metastasize. Chondrosarcoma occurs primarily in adults over the age of 40. Ewing sarcoma usually occurs in the diaphysis of bone but rarely occurs in the soft tissues. The risk of Ewing sarcoma is higher in children younger than 19 years of age, and boys are more likely to suffer from this than girls [46,49]. Primary bone cancers account for less than 1% of cancers diagnosed each year and are associated with substantial morbidity and mortality. Timely diagnosis is difficult due to late patient appearance, non-specific signs that resemble typical musculoskeletal conditions, and low medical suspicion. Plain X-ray is the ideal screening test. Radiographic suspicion of bone malignancy should trigger a fast referral to a cancer center for multidisciplinary care. Surgical excision remains the mainstay of curative treatment, with chemotherapy and radiotherapy used in conjunction [35]. Clinical experts these days are tirelessly finding new ways to strategize the treatment of malignant growth comprehensively.

2. Methodology

This study aimed to review and describe the recent trends in pathogenesis, diagnosis and management of Primary Bone Cancer. The publish paper were reviewed using electronic searches such as PubMed, Google Scholar, Mendeley, UpToDate, and Clinical Key. The search strategy includes article published in the last decade (2010-2020) in the reputed journals. The keywords used by electronic search are primary bone cancer, pathogenesis, diagnosis, management, Osteosarcoma, Chondrosarcoma, and Ewing sarcoma.

3. Discussion

3.1. Osteosarcoma

Osteosarcoma is a chemo-resistant bone tumour [10] with an aggressive proliferation rate [1]. One-fourth of the cases are diagnosed with pulmonary metastasis [3,10]. The malignancy usually surfaces at the growth plate in the metaphysis [2,10,13,17], and the femur is the frequently affected bone (40-43%), followed by the tibia (19-

23%), humerus (10%), facial bones (8%) and, the pelvis (8%) [4,5,10]. It is an uncommon disease that accounts for 4.4 cases/million children/annum [2], albeit it is the most common type of primary bone tumour [1,8]. It regularly affects males in the pediatric age range [1,3-5,8]. Patients with low socioeconomic statuses fail to recognize the grave situation and favors other alternatives like traditional healers and medication [71-73]. Quality of life (QoL) is a vital aspect that must be assessed continuously. According to European American Osteosarcoma Study-1 (EURAMOS-1), patients have substandard communicative skills [6], and amputated patients have a lower health-related QoL [2]. A key point that contributes to the genesis of a tumour is the dysfunctional tumour suppressor genes. The mutation of p53 (product of TP53) impedes the response of DNA repair, thus activates the oncogenesis of osteosarcoma [7,13,17,18]. No dissociation of p53 from MDM2, G1 phase not halted, inactivity of BAX and P21 (proapoptotic factors), and promotes angiogenesis [13,17,19]. The mutation of the Rb gene inhibits the Rb/E2F pathway, thus regulating cell proliferation without apoptosis [8,13,18]. Transcription factors like Activator protein-1 complex (AP-1) and MYC are upregulated, thus stimulating proliferation, differentiation, and metabolism of cells [13,14] Abnormal regulation of Transforming Growth Factor Beta (TGF-β), Insulin-like Growth Factor (IGF), and Connective Tissue Growth Factor (CTGF) amplifies proliferation with unfettering apoptosis of cells and promotes metastasis [7,8,13,16,17] by activating Phosphoinositide 3-Kinase PI3/AKT and Microtubule-Associated Protein Kinase (MAPK) signaling pathways. IGF-I and IGF-II will activate the PI3K and MAPK pathways. Both promote uncontrolled proliferation without restriction while TGF-β blocks apoptosis (Figure 1) [13,16-18]. Angiogenesis in osteosarcoma is triggered when there is a hypoxic condition (Figure 2). [12,13,15]

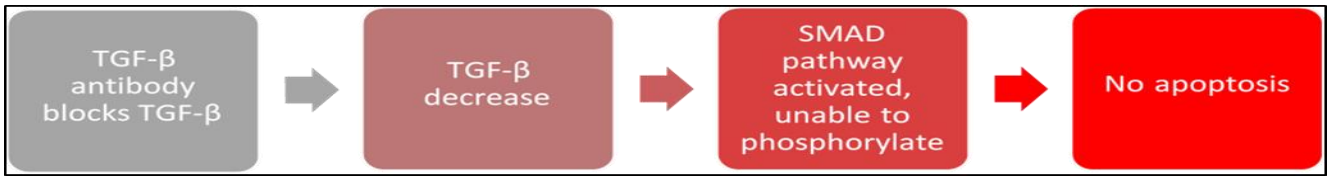

Figure 1: TGF-B blocking pathway. Author in [13] hypothesizes that osteosarcoma produces TGF-B antigen and proves the pathway above.

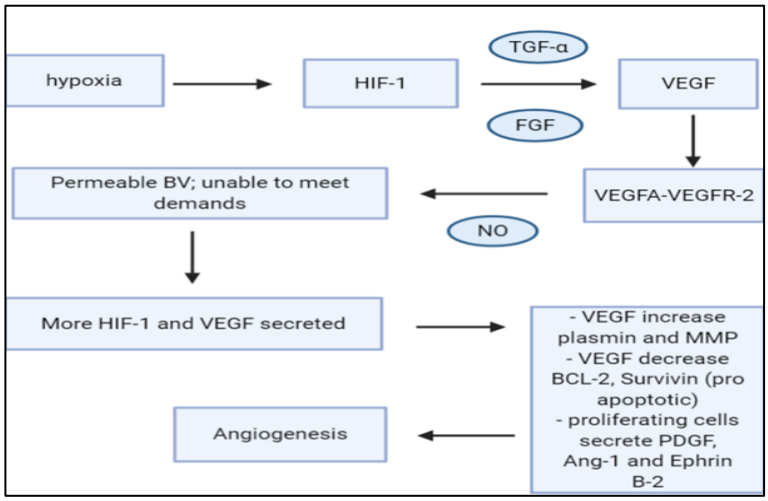

Figure 2: Angiogenesis in hypoxic condition

HIF-1: Hypoxia induce factor -1; TGF- α: Transforming Growth Factor- α; FGF: Fibroblast Growth Factor; VEGF: Vascular Endothelial Growth Factor; VEGFR-2: Vascular Endothelial Growth Factor Receptor-2; NO: Nitric Oxide; BV: Blood Vessel; MMP: Matrix Metalloproteinase; PDGF - Platelet-Derived Growth Factor; Ang-1 – Angiopoietin-1 When osteosarcoma cells remove themselves from the matrix to metastasize, anoikis is usually activated. However, they are Anchorage Independent Growth (AIG) that makes them unsusceptible to anoikis [13]. The metastasis of osteosarcoma starts with adhesion and migration of cells. The extracellular membrane (ECM) sticks to fibronectin, and a cascade of cell signaling occurs. RhoA GTPase will hyper-stimulate RhoA and subsequently brings Rac1 down to promote cell migration. Cells migrate from ligand-dense stroma to tumour stroma (rigid). Ezrin proteins are also hyper-expressed that promotes metastasis [18,19]. Additionally the breakdown of ECM by Matrix Metalloproteinase (MMP) (Figure 3) and Urokinase Plasminogen Activator (uPA) system (Figure 4) also contributes to tumour invasion. [12,13].

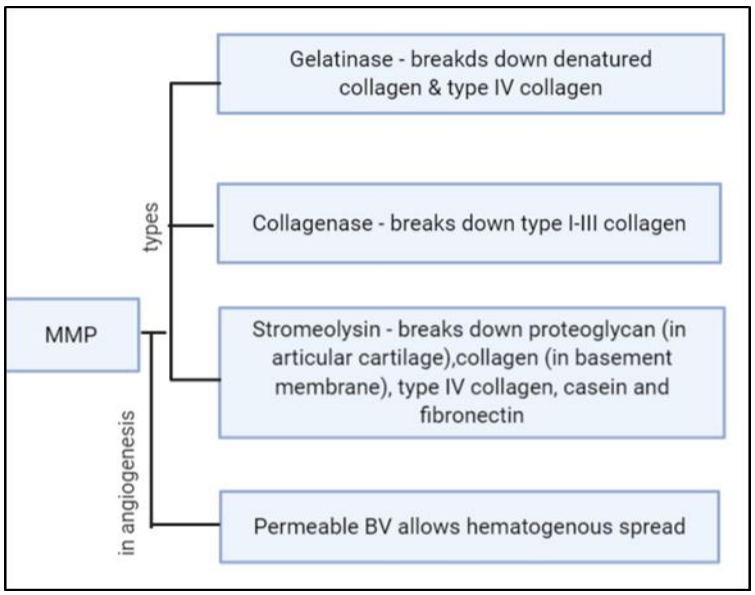

Figure 3: How MMP helps in tumour invasion

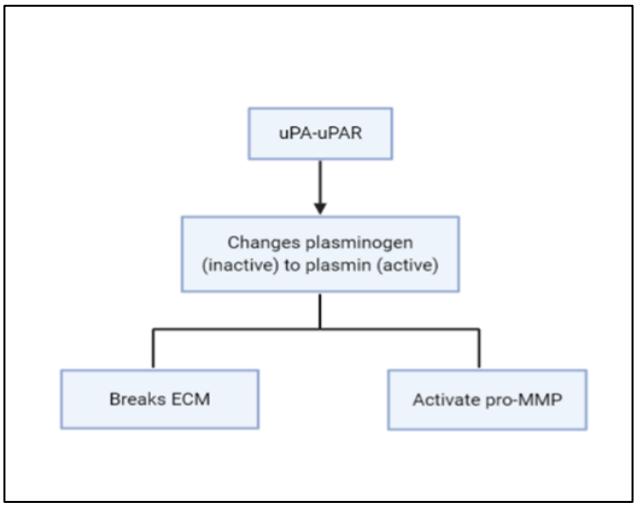

Figure 4: uPA system contributes to tumour invasion

uPAR: Urokinase Plasminogen Activator Receptor; ECM: Extracellular Membrane;

Nowadays, there is a newly discovered tumour-signaling pathway called the Hippo-Yap pathway. It includes a cascade of events similar to the pathogenesis discussed above with different integral components. In the Hippo pathway, it correlates with the inhibition of Hippo activators, merlin (Nf-2), and kibra (WWC1) by SOX-2. The downregulation by the activators promotes the Yes-Associated Protein (YAP) pathway, thus amplifying the proliferation of malignant cells. Yes-Associated Protein-1-Transcriptional Enhanced Associate Domain-1 (YAP-1-TEAD-1) complex impairs the Hippo pathway. The hippo-Yap pathway associates with TGF-β at transcriptional levels where it stimulates TBR-I by phosphorylation of TBR-II. Activated TBR-I triggers SMAD 3 to accumulate SMAD 4, thus promoting the hyperexpression of targeted genes associated with its co-factors and promote metastasis [11] (Figure 5).

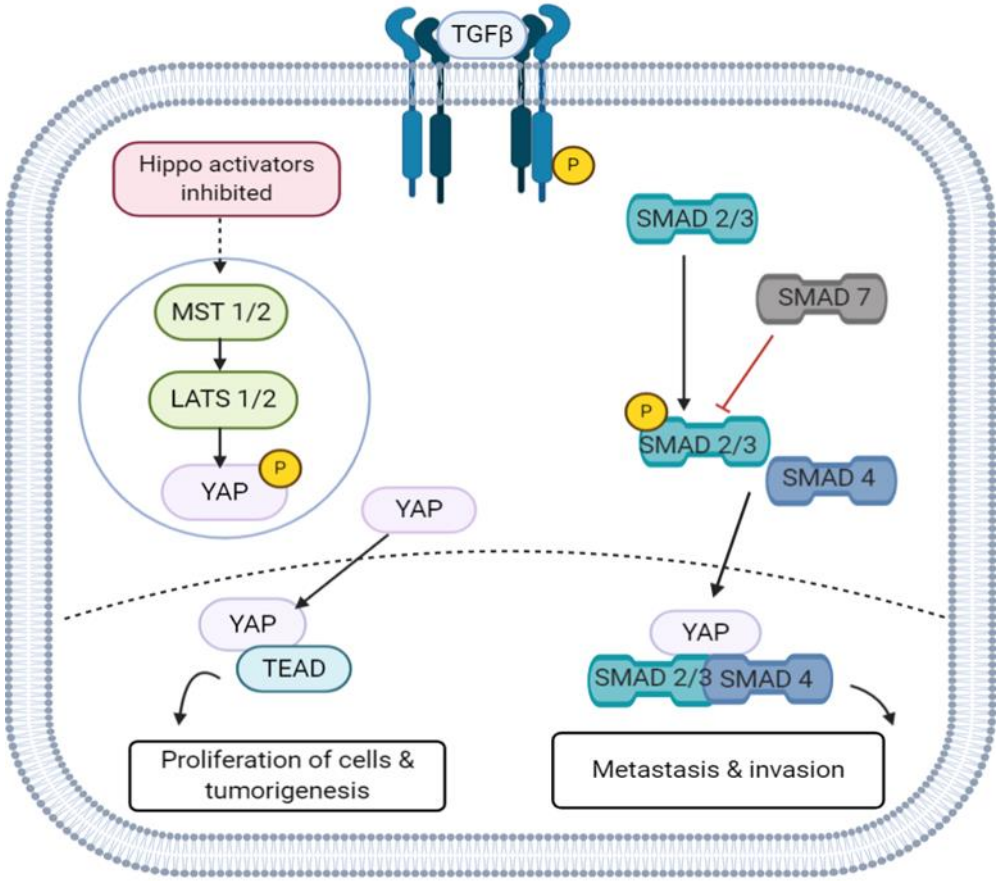

Figure 5: Hippo-Yap pathway

The inhibition of the Hippo pathway activates MST1/2 protein kinases (mammalian STE20-like kinase 1/2) which incites the activation of LATS1/2 (large tumor suppressor 1/2). LATS 1/2 phosphorylates YAP (Yes associated protein) and will either be confined in the cytoplasm or lysed by the proteasome. Unphosphorylated YAP translocates to the nucleus where it binds with TEAD to promote tumorigenesis Osteosarcoma is diagnosed with radiological imaging to view the bone and adjacent structures [4,23]. Bone marrow changes, skip metastases and monitor chemotherapy response done with MRI [20,23]. CT scan aids in showing

irregularities of the cortex and the neurovascular system. Angiography is essential for patients who have sarcomas on the appendicular skeleton. 6-12 weeks after the first scan, patients are scheduled for another scan to detect small tumours (< 5 mm) [23]. Core biopsy is preferred because it reduces the risk of malignancy infiltration and is vital for limb-salvaging patients. Moreover, CT-guided biopsy is more precise than fine-needle aspiration (Hau and his colleagues) but, a closed needle biopsy is the most beneficial (Skrzynski and his colleagues) [23]. In 2018, authors in [21] finds that increased levels of Lactate Dehydrogenase (LDH) are associated with increased mortality percentage, whereas Alkaline Phosphate (ALP) levels are increased parallelly with distal metastasis. A newly discovered biomarker, microRNA (miRNA), originates from B cells and are extracted by Real Time Polymerase Chain Reaction (RT-PCR). Although it is not a specific biomarker for osteosarcoma, it can detect most of the components discussed in the pathogenesis (Table 1) [9,10,17,22]. Additionally, liquid biopsy is a minimally invasive and precise method of diagnosis by measuring and examining the cells of osteosarcoma. It is still under trial, but it detects nucleic acids from malignant tumours in the blood [10].

Table 1: miRNA detects components of pathogenesis.

miRNA	Detects
miR-374b (decrease)	VEGF (Hyper-stimulated)
miR-183 (decrease)	Ezrin protein (Hyper-stimulated)
miR-150 (decrease)	
miR-23a	Cytotoxic T Lymphocytes (CTL)
miR-329 (decrease)	CD146 (hyper-expressed)
miR-766-3p (increase)	B-Cell Lymphoma 9-Like Protein (BCL9L) and β-catenin (down regulated)
miR-27a (increase)	Lung metastasis
miR-95-3p (decreased)	
miR-195 (decreased)	
miR-140 (increase)	Induces p53 to block G1/G2 cycle

Omic technology is a technology that detects a set of genes, mRNA, proteins, and metabolites. Whole-Genome/Whole-Exome Sequencing (WES/WGS) is used to analyze osteosarcoma samples. However, there is a flaw in the studies due to the uncommonness of the disease (Table 2) [19].

Table 2: Dysfunctional genes/mRNA/protein detected by Omic Technology

	Description
RUNX2 (Hyper-expressed)	• Uncontrolled proliferation of cells without apoptosis • Poor prognosis due to a lack of response towards chemotherapy
Ezrin protein (high)	Lung metastasis
PTEN (low)	Cell activity
mTOR	Prognosis

RUNX2: Runt-Related Transcription Factor 2; PTEN: Phosphatase and tensin homolog; mTOR: Mammalian Target of Rapamycin

Osteosarcoma is treated with a combination of high-dosage chemotherapy. Methotrexate with leucovorin rescue, doxorubicin (Adriamycin), cisplatin (MAP) is the first-line drugs [2,4,5,8,9,23-25]. The addition of ifosfamide gives a better histological result [2]. Furthermore, patients who are unable to tolerate the MAP is given a different combination such as doxorubicin-carboplatin-ifosfamide (API-AI) and other radiotherapy agents. [9] Surgical treatment is fundamental. The resection of tumours is vital in patients who have a poor response or resistant to chemotherapy. Limb-salvaging and amputation depend on the surgical margins [2,4,8,23,25] Nonetheless, there are various reconstruction alternatives available to improve their QoL [2,9,23-25]. Radiotherapy manages the tumour metastasis up to 78% and stagnates the progression at 75%. According to the COG AOST0331/EURAMOS study, 60-66 Gy is the recommended dose for patients with bad margins and 70Gy for metastatic control. Proton therapy is used with a dose of 68-70 Gy and has 72% metastatic management for five years. Radioresistant patients are given Carbon-ion-particle therapy [2]. Also, there are new radioactive therapies currently under trial; the beta-emitting Samarium-153 Ethylenediamine Tetramethylene Phosphonate (Sm-153-EDTMP) [2] and alpha-emitting Radium-223 dichloride (Ra-223) [26,27]. Ra-223 is the favored choice because of its efficacy [28]. Next, Multi-kinase inhibitors (MKIs) targets various kinases (Table 3) [8-10,12,23] and the inhibition of the Hippo-Yap pathway. The hyper-expression of the YAP pathway promotes the burgeoning of malignant cells (Table 4). Unfortunately, there is still no known drug to inhibit the Hippo pathway [11]. Cyclooxygenase-2 (COX-2) Inhibitors can be given to reduce pain and block the PI3/AKT pathway [18]. Based on a case report, a patient who has pulmonary metastasis and resistance to MAP regimen is treated with celecoxib and thalidomide [9].

Table 3: MKI drugs and its description

MKI drugs	Description
Sorafenib	Targets angiogenic kinases
Regorafenib	Targets pro-angiogenesis kinases, kinases on connective tissues, and pro-oncogenic kinases
Pazopanib	Given in osteosarcoma with metastasis and relapsed patients • Inhibits VEFR, PDGFR
Cabozantinib	• Advanced and relapsed phases • Shows no progression of malignancy in 6 months
Lenvatinib	Under trial
Everolimus	mTOR inhibitor

Table 4: Hippo-Yap pathway inhibitors

Hippo-Yap pathway inhibitors	Description
Verteporfin	• Blocking YAP- TEAD activity • Reduces cell proliferation • Increases apoptosis • Downregulates genes in the Hippo pathway like Cyr61 and CTGF
Dasatinib (TKI)	Inhibits the nuclear translocation in the YAP pathway
A35 (topoisomerase)	Impedes the nuclear translocation on serine 127
JQ1 (chemotherapy agent)	Competitive inhibitor of bromodomain-containing protein-4 (BRD4).

3.2. Chondrosarcoma

Chondrosarcoma is a rare cartilage matrix-producing malignant tumour. The estimated total incidence of chondrosarcoma is 1 in 200,000 per year, which makes it the third most common malignant bone tumour after multiple myeloma [48]. Chondrosarcoma that arises de novo are called primary chondrosarcoma, whereas chondrosarcoma developing superimposed on pre-existing benign cartilage tumours such as an enchondroma or osteochondroma are referred to as secondary chondrosarcoma [29,31,48]. Chondrosarcoma is a heterogeneous group of tumours that can be categorised as central by anatomical location when they occur within the

medullary canal or periphery when they occur in the exostosis cartilage cap [48]. In addition to conventional chondrosarcoma, which shows hyaline cartilage differentiation, there are other types of chondrosarcoma, such as dedifferentiated, mesenchymal or clear cell, which have distinct genetic and clinicalopathological characteristics [31,48]. Most chondrosarcomas, however are conventional chondrosarcomas, with the majority occurring in the long bone medullary cavity [48]. The minority of conventional chondrosarcoma is secondary peripheral chondrosarcoma that develops from the surface of the bone as a result of malignant transformation within the cartilage cap of a pre-existing benign cartilage tumour such as osteochondrome or de novo on the bone surface, and is not rarely referred to as juxtacortical or periosteal chondrosarcoma [29,31,48]. The exact pathogenesis of chondrosarcoma is not well known [49]. Many genes have been implicated in pathogenesis of chondrosarcoma. Structural differences in chromosomes 1, 6, 9, 12 and 15 has been revealed from the cytogenetic study of chondrosarcoma. Chondrosarcoma is frequently associated with numerical abnormalities in chromosomes 5, 7, 8 and 18. Chromosome 9(9p12-22) associated defects are most generally found in central chondrosarcomas. Patients with several osteochondromas tend to have germline exostoses (EXT1 or EXT2) chromosomes. This results in reduced EXT expression and biosynthesis and release of heparan sulphate proteoglycans (HSPGs), which is crucial for cell signalling through IHH/PTHLH pathways [49]. This in essence, reduces normal chondrocyte proliferation and differentiation within the normal human growth plate. In addition, the malignant transformation of osteochondrome to secondary peripheral chondrosarcoma has implicate us the genetic mutations in the TP53 or pRb pathway. Point mutations in isocitrate dehydrogenase-1 and isocitrate dehydrogenase - 2 genes (IDH1 and IDH2) have been suggested in enchondromas and central chondrosarcomas. Besides, the Ollier disease and Maffucci syndrome are also result of somatic mosaic mutations in IDH1 and IDH2. The conversion of isocitrate to alpha-ketoglutarate in the tricarboxylic acid cycle requires the necessary enzyme, isocitrate dehydrogenase [49]. Elevated levels of D-2-hydroxyglutarate (D-2-HG) oncometabolite due to IDH1 and IDH2 mutation, which induces chondrogenesis and prevents osteogenic differentiation of mesenchymal stem cells as well as induces DNA hypermethylation and histone alteration, both of which result in decreased differentiation. Missense mutation (R150C) in the PTHRP receptor encoding gene (PTH-1 or PTH1R receptor) has been associated with enchondromatosis in patients with Ollier disease and reduced receptor activity. Low-grade chondrosarcomas are near-diploid and have relatively few caryotypic abnormalities. On the other hand high-grade chondrosarcomas are aneuploid and have diverse caryotypes. The development of chondrosarcoma was related to the CDKN2A (p16) tumour suppressor gene present at 9p21 and the mutation at p53.In chondrosarcoma pathogenesis, the mutation in COL2A1 have also been hypothesised [49]. In addition, the amplification of c-myc and fos/jun proteins has also been involved in chondrosarcoma pathogenesis. Mesenchymal chondrosarcoma resulted in specific HEY1-NCOA2 fusion product due to intrachromosomal rearrangement of the chromosome arm 8q. The translocation of t(9;22)(q22;q12) is normal with extraskeletal myxoid chondrosarcomas [49]. Chondrosarcomas can be categorised into the following three histological grades based on the cellularity, atypia and pleomorphism findings. Grade I (low grade) which is cytologically similar to enchondrome cellularity is greater, with occasional plump nuclei having an open chromatin structure. A definite and increased cellularity, distinct nucleoli are found in most cells, and the concentration of myxoid modification can be observed in Grade II (intermediate grade) whereas in grade III (high grade) is distinguished by high cellularity, prominent nuclear atypia and mitosis. The higher the rating, the more likely it is to propagate and metastasize. For instance, grade III lesions metastasize more than 50%. Grade

I lesions seldom metastasize, while grade II lesions metastasize about 10-15%. Low-grade chondrosarcomas mimic benign cartilaginous tumours, although it is impossible to discriminate between the two lesions on the basis of histological features alone. The slow growth capacity of low-grade chondrosarcomas and the minimal growth potential of benign cartilaginous tumours are the key variations in these. Grade III conventional chondrosarcomas is less aggressive thus, has a better prognosis than dedifferentiated chondrosarcomas. For first evaluation plain radiography is used. Identification of the cartilaginous nature and the aggressiveness of the lesion are seen. Plain x-rays may reveal the following findings which are lytic lesions in 50% of the cases, intralesional calcifications, in about 70% of the cases, endosteal scalloping, permeative or moth-eaten appearance in high-grade chondrosarcomas. Cortical remodeling, thickening, and periosteal reaction could also be identified [33,45,46]. Besides that, we can evaluate chondrosarcoma through CT-scans where following findings like matrix calcification in 94% of the cases, endosteal scalloping, a cortical breach in about 90% of long bone chondrosarcoma and heterogenous contrast enhancement can be seen [45,46]. MRI can also be an investigation method where lobulated lesions are commonly seen. Tissue biopsy is very crucial to diagnose chondrosarcoma and differentiate it from other malignant or benign bone tumours. A portion from the most aggressive part of cancer should be taken as a biopsy [45,46]. Targeted therapy for bone cancers include Imatinib and Denosumab, however there are no medications directly related to treating chondrosarcoma specifically, beyond chemotherapy. Hydrocodone can be prescribed as a pain reliever in post-surgery patients [33]. Following a biopsy, surgery is the common procedure to remove the tumour [33]. Even for higher grade tumours, limb-sparing curettage is common, with amputation occurring in rare instances. Chondrosarcomas does not respond to radio or chemo therapy. Different forms of chondrosarcoma including dedifferentiated and mesenchymal maybe on trial chemotherapy before or after surgery. Depending on the grading of the sarcoma, proton-beam radiation has higher sucess rate, but is considered less frequent compared to surgical approaches [39,41]. Currently, there is no specific treatment for chondrosarcomas. Treatment will be determined based on age of patient, overall health, medical history, type and stage of the carcinoma and patients preferance or opinion on the treatment plans. The ideal goal here is to remove the mass and reduce the likelihood of it to return [31].

3.3. Ewing Sarcoma

Ewing sarcoma is one of the primary bone cancer. It rarely occurs tumour among the population. It usually occurs at the diaphysis of the long bone [57] but it also can occur in any bone and soft tissue of the bone [58,60-62,66,70]. Young children and adolescents are the most common group to have ES. The pathogenesis of ES is of unknown origin [59] but it might be due to translocation of chromosomes 11 and 22. This is the most frequent gene rearrangement: t(11;22)(q24;q12) which is seen in about 80% cases [56,58,59,62,66-68]. This gene rearrangement later forms a new hybrid gene which is known as a fusion gene. The fusion gene is EWS-FLI1 and will act as an atypical transcription factor [58,59,66]. Approximately 15% of EWS-ETS gene family rearrangement is the translocation of chromosomes 21 and 22 [56,58], resulting in the fusion of EWS with the ERG gene [58,59,68] such as E1AF, ETV1, and FEV [56,58,59,62]. ES is presumed due to EWS-ETS fusions which acts as proliferation and tumourigenesis [59,62]. Half of EWSR1 is fused with FLI1 by the translocation of chromosome 11 and 22, t(11;22)(q24;q12) [56,58,62,68,70]. Hence, replacement of its transcription activation domain by EWSR1 sequence occurs and later will drive the process of oncogenesis and result in

increased production of chimeric proteins involved in tumourigenesis [59]. There is various cell origin of ES that has been proposed. In 2005, Castillero-Trejo and his colleagues proposed Mesenchymal progenitor which is the stem cells is the origin of Ewing sarcoma [54]. Neural derived mesenchymal cells or neural crest cells were recently suggested in 2010 by Elizabeth C. Toomey.

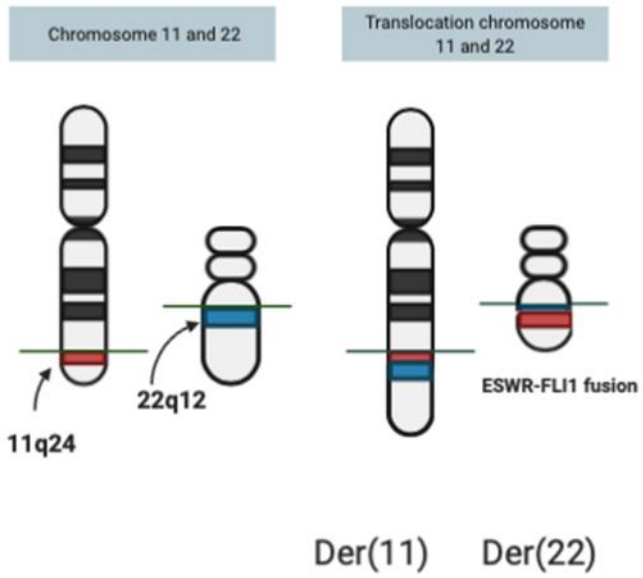

Figure 16: Translocation of chromosome 11 and 22

Immunohistochemically, CD99 is positive in most cases of ES, in approximately 90% cases [55,58,66,68]. However, CD99 not specific only for ES [55]. ES can be diagnosed using a plain radiograph. The appearance of this tumour is very variable. Common appearance includes “onion skin” [57,61,70] and “Hair on end” appearance is forming along the periosteal vessels. Codman triangle and sunburst appearance have rarely seen when imaging of the bone is done [61]. Computerized tomography scan is useful to determine the tumour outside the bone such as extraskeletal soft tissue and pulmonary metastasis involvement [55,58]. Magnetic Resonance Imaging can detect the arising of tumour outside the bone [58,70]. Therefore, it is an advanced technique to establish the diagnosis. For the past year till now, the diagnosis of ES is commonly by molecular technique because of its chromosomal translocation. The main goal is to detect the fusion gene. Radiotherapy alone has a higher chance of local recurrence [54,55] and has increased the risk of growth impairment, especially in young children. Definitive radiotherapy is only advised for large lesions [54,63], the involvement of the lymph node, presence of multifocal disease, or blood vessel invasion. The recommendation of the dose is between 54 to 55 Gy [54,65], depending on the site of the tumour. A higher dose is required for large tumours [54]. Currently, postoperative radiation therapy (PRT) is still not clear and debate among researchers is continues. PRT is indicated for incomplete surgical resection tumour [63]. Preoperative radiation therapy has been introduced to patients [54,55]. The indication is resection of the margin is expected to be close and this approach is better to reduce the size of the tumour before undergoing surgery [55]. Histological investigations are not done yet. Newly, resection of ES tumour is only performed when a wide resection or marginal of the

bones is possible due to the superiority for local control. The latest treatment for ES is large dose chemotherapy with suchlike hematopoietic stem cell rescue [54,60,65]. It has higher toxicity to the patients therefore this approach is only for very high-risk patients such as metastatic involvement [54,65]. COG-AEWS1031 has tried the addition of cyclophosphamide plus topotecan in VDC-IE and it has been observed recurrent ES occur with these trials [60]. Zoledronate is added in VIDE for a localised tumour in standard-risk patients to see the possible benefits of it. Good histology response was observed. For high-risk patients, busulfan or melphalan is added to VIDE and poor histologic response is observed [60]. Presently, a variety of new target therapies for drug development has been identified to treat relapsed ES. EWS-FLI1 signature-based approaches have been used to make a new drug. Agents and RNA helicase A is a protein used to target the EWS-FLI1 interaction in the transcriptional complexes and can disturb the protein interactions [60,62]. Lysine-specific demethylase 1 (LSD-1) is also expressed in ES [60]. However, these are still in clinical trials. IGF1R is expressed on ES tumour cells too and acts as a receptor [62]. The functions of IGF1R is driving the process of oncogenesis of ES [59,60]. No mutations are described in IGF-1 but a highly expressed effect is frequently seen in ES [62] and it has been identified to store in the bone matrix. It also will induce the process of osteolysis. Therefore, it has been seen as involved in the process of malignant transformation of ES when the body cell is transfected. There are therapeutic drugs that are on trials against IGF1R like antibodies IgG1, R1507, and AMG479 while IgG2 is figitumumab [60,66,68]. IGF-IR cannot be used alone as no clinical benefits have been identified. IGF-1R with monoclonal antibodies produce a good response in an early trial of ES [59]. There is currently a new study for patients with newly diagnosed metastatic ES that has been identified. Combination of IGF-1R antibody gantinumab with conventional chemotherapy. Insulin-like growth factor-binding protein 3 (IGFBP-3) has been identified to be a potential anticancer molecule for ES [59,60,66,68]. It has been found to involve in the mechanism pathway of IGF-1 [59,68]. It inhibited EWS growth. However, the administration of IGFBP-3 systemically induced toxicities like osteoporosis. The next target gene is PRKCB. In ES models, if the PRKCB gene is lost, tumour growth can be prevented in vivo and it will induce apoptosis in vitro. VEGF, bevacizumab, and immunoglobulin G1 monoclonal antibody is also a target gene for new development therapy. It will inhibit VEGF receptor -1, VEGFR receptor-2, and VEGF-dependent angiogenesis [60]. A trial of a combination of vincristine, topotecan, and cyclophosphamide with bevacizumab in recurrent ES shows good tolerance. (COG AEWS0521) Another targeted therapy is the DNA repair protein. Poly (ADP-ribose) polymerase 1 (PARP1) is a cofactor for EWS-FLI1 DNA binding and is a key enzyme involved in the single-strand repair of DNA [60]. This enzyme is seen to elevate in ES. However, clinical studies show PARP inhibitors cannot be used as a single agent [60]. Therefore, it can be combined with temozolomide or irinotecan which is one of the DNA damaging agents [60]. The results from these studies are still under development. Olaparib is the PARP1 inhibitor and it is highly sensitive in ES models in vitro and in vivo.

4. Conclusion

Primary bone cancer is widespread in the population. Bone sarcomas derive from Mesenchymal Stem Cells and share a similar trait in their pathogenesis with a pronounced effect on the local environment. However, the ES cell origin is unidentified and the precise pathogenesis of Chondrosarcoma is unknown, causing a challenge to treatment. Specific miRNA should be recognised for the commencement of treatment for osteosarcoma. Mutations that cause primary bone cancer should be identified to create new technologies so that increase 5

years-survival rates can be achieved. Further advanced research should recognize any alternative pathways that may prevent widespread proliferation and perhaps other tumour phenotypes. A much stronger and tailored therapy in the treatment of this disease can be facilitated by strengthened molecular insight into this disease. To further enhance prognosis, the use of multidisciplinary care teams consisting of experts from different fields is important. More studies on pathogenesis of primary bone tumour should be investigated for better management in the future.

5. Limitations/Constraints

. This study reviews articles that were published from the year 2010 to 2020. More recent studies are not being covered in this article. Furthermore, primary bone tumor is a rare disease and there are not many information and resources that are available. This study mainly focuses on the current trends in pathogenesis, diagnosis and treatment of primary bone tumors.

6. Recommendations

Osteosarcoma is the most common primary bone tumor thus it is highly recommended to meticulously study the Hippo-Yap pathway to create better drugs to further ameliorate the treatment plan. Other pathways should also be recognized to prevent other tumorigenesis courses. Furthermore, the mechanism of action of osteosarcoma drugs that are specifically tailored to target the microenvironment of the tumor should also be continued to reduce the over-expression of tumorigenesis factors and decrease the rate of metastasis. Other than that, further studies on miRNA, 'Omic' technology, and liquid biopsy can be commenced to make it more specific for the early detection of osteosarcoma for prompt treatment for a better prognosis and salvaging rate in 5 years. Although chondrosarcoma is responding poorly to chemotherapy and radiation, health professionals are still working in an interprofessional team that consists of orthopedists, radiologists, surgical oncologists, pathologists, and specialty care nurses to come up with the best treatment for these patients. Promising findings have been reported from current research targeting the platelet-derived growth factor receptor to inhibit these cancer cells. Chemotherapy should not be an option anymore in the future due to the poor outcomes, higher chances of side effects, and low survival rates. Hopefully, these findings will give us a hand on how to deal with this disease process and in improving the treatment of this carcinoma. On top of that, every patient and their family members need to be educated on how to recognize the early signs and symptoms to prevent late diagnosis and for a better prognosis.The quality of care for patients with Ewing Sarcoma, which consists mainly of polychemotherapy and local treatment with surgery and/or radiotherapy, has not significantly advanced in the past decades. Cancer vaccinations or CAR-T therapy may target fusion-derived antigens, as well as CD 99 or IGF1R expression. Nonetheless, research into the potential role of such drugs, as well as the use of immune checkpoint inhibitors, has yet to yield positive results. Therefore, the study of new treatment is vital for the treatment of ES. It is critical to strengthen ES management and sustainability.

Acknowledgement

The authors would like to offer their utmost gratitude to International Medical School(IMS), Management and

Science University (MSU) for the support provided.

References

[1]. S. Xin and G. Wei, "Prognostic factors in osteosarcoma: A study level meta-analysis and systematic review of current practice," J. Bone Oncol., vol. 21, Apr. 2020, doi: 10.1016/j.jbo.2020.100281.

[2]. B. R. Eaton et al., "Osteosarcoma," Pediatr. Blood Cancer, no. January, pp. 1–7, 2020, doi: 10.1002/pbc.28352.

[3]. D. A. Müller and U. Silvan, "On the biomechanical properties of osteosarcoma cells and their environment," Int. J. Dev. Biol., vol. 63, no. 1–2, pp. 1–8, 2019, doi: 10.1387/ijdb.190019us.

[4]. M. Mustafa, H. M. Iftikhar, E. M. IIIzam, M. K. Nang, and A. M. Sharifa, "Osteosarcoma: Current treatment trends and outcome," no. November, 2018, doi: 10.9790/0853-1711023238.

[5]. M. S. Isakoff, S. S. Bielack, P. Meltzer, and R. Gorlick, "Osteosarcoma: Current treatment and a collaborative pathway to success," J. Clin. Oncol., vol. 33, no. 27, pp. 3029–3035, 2015, doi: 10.1200/JCO.2014.59.4895.

[6]. G. Calaminus et al., "Quality of life of patients with osteosarcoma in the european american osteosarcoma study-1 (EURAMOS-1): Development and implementation of a questionnaire substudy," JMIR Res. Protoc., vol. 8, no. 9, pp. 1–16, 2019, doi: 10.2196/14406.

[7]. C. C. Wu and J. A. Livingston, "Genomics and the immune landscape of osteosarcoma," Adv. Exp. Med. Biol., vol. 1258, pp. 21–36, 2020, doi: 10.1007/978-3-030-43085-6_2.

[8]. R. A. Durfee, M. Mohammed, and H. H. Luu, "Review of Osteosarcoma and Current Management," Rheumatol. Ther., vol. 3, no. 2, pp. 221–243, 2016, doi: 10.1007/s40744-016-0046-y.

[9]. I. Lilienthal and N. Herold, "Targeting molecular mechanisms underlying treatment efficacy and resistance in osteosarcoma: A review of current and future strategies," Int. J. Mol. Sci., vol. 21, no. 18, pp. 1–56, 2020, doi: 10.3390/ijms21186885

[10]. M. H. Osteosarcoma, "MicroRNA-Based Diagnosis and Treatment of," pp. 1–14, 2019.

[11]. S. Morice, G. Danieau, F. Rédini, B. Brounais-Le-royer, and F. Verrecchia, "Hippo/yap signaling pathway: A promising therapeutic target in bone paediatric cancers?," Cancers (Basel)., vol. 12, no. 3, 2020, doi: 10.3390/cancers12030645.

[12]. I. Corre, F. Verrecchia, V. Crenn, F. Redini, and V. Trichet, "The Osteosarcoma Microenvironment: A Complex But Targetable Ecosystem," Cells, vol. 9, no. 4, pp. 1–25, 2020, doi: 10.3390/cells9040976.

[13]. P. F. M. Choong, M. L. Broadhead, J. C. M. Clark, D. E. Myers, and C. R. Dass, "The molecular pathogenesis of osteosarcoma: A review," Sarcoma, vol. 2011, 2011, doi: 10.1155/2011/959248.

[14]. S. Gambera et al., "Role of Activator Protein-1 Complex on the Phenotype of Human Osteosarcomas Generated from Mesenchymal Stem Cells," Stem Cells, vol. 36, no. 10, pp. 1487–1500, 2018, doi: 10.1002/stem.2869.

[15]. Y. sheng Li, Q. Liu, J. Tian, H. bo He, and W. Luo, "Angiogenesis Process in Osteosarcoma: An Updated Perspective of Pathophysiology and Therapeutics," Am. J. Med. Sci., vol. 357, no. 4, pp. 280–288, 2019, doi: 10.1016/j.amjms.2018.12.004.

[16]. M. Cheng et al., "Growth hormone receptor promotes osteosarcoma cell growth and metastases," FEBS Open Bio, vol. 10, no. 1, pp. 127–134, 2020, doi: 10.1002/2211-

[17]. J. W. V. de Azevedo et al., "Biology and pathogenesis of human osteosarcoma (Review)," Oncol. Lett., vol. 19, no. 2, pp. 1099–1116, 2020, doi: 10.3892/ol.2019.11229.

[18]. J. Zhang, X. H. Yu, Y. G. Yan, C. Wang, and W. J. Wang, "PI3K/Akt signaling in osteosarcoma," Clin. Chim. Acta, vol. 444, pp. 182–192, 2015, doi: 10.1016/j.cca.2014.12.041.

[19]. E. Tirtei, M. Cereda, E. De Luna, P. Quarello, S. D. Asaftei, and F. Fagioli, "Omic approaches to pediatric bone sarcomas," Pediatr. Blood Cancer, vol. 67, no. 2, pp. 1–7, 2020, doi: 10.1002/pbc.28072.

[20]. J. R. Barnett, P. Gikas, C. Gerrand, T. W. Briggs, and A. Saifuddin, "The sensitivity, specificity, and diagnostic accuracy of whole-bone MRI for identifying skip metastases in appendicular osteosarcoma and Ewing sarcoma," Skeletal Radiol., vol. 49, no. 6, pp. 913–919, 2020, doi: 10.1007/s00256-019-03364-0.

[21]. A. Kamal and R. Prasetyo, "Association between laboratory markers and oncological outcomes in patients with osteosarcoma-A review of osteosarcoma treatment in Indonesia," Formos. J. Surg., vol. 51, no. 3, pp. 111–117, 2018, doi: 10.4103/fjs.fjs_140_17.

[22]. S. Zhang et al., "miR-766-3p Targeting BCL9L Suppressed Tumorigenesis, Epithelial-Mesenchymal Transition, and Metastasis Through the β-Catenin Signaling Pathway in Osteosarcoma Cells," Front. Cell Dev. Biol., vol. 8, no. October, pp. 1–15, 2020, doi: 10.3389/fcell.2020.594135.

[23]. A. Misaghi, A. Goldin, M. Awad, and A. A. Kulidjian, "Osteosarcoma : a comprehensive review," 2018.

[24]. M. F. Wedekind, L. M. Wagner, and T. P. Cripe, "Immunotherapy for osteosarcoma: Where do we go from here?," Pediatr. Blood Cancer, vol. 65, no. 9, pp. 1–9, 2018, doi: 10.1002/pbc.27227.

[25]. Y. Yang et al., "Advances in limb salvage treatment of osteosarcoma," J. Bone Oncol., vol. 10, no. November 2017, pp. 36–40, 2018, doi: 10.1016/j.jbo.2017.11.005.

[26]. V. Subbiah et al., "Alpha particle radium 223 dichloride in high-risk osteosarcoma: A phase i dose escalation trial," Clin. Cancer Res., vol. 25, no. 13, pp. 3802–3810, 2019, doi: 10.1158/1078-0432.CCR-18-3964.

[27]. P. M. Anderson et al., "223-Radium for metastatic osteosarcoma: Combination therapy with other agents and external beam radiotherapy," ESMO Open, vol. 5, no. 2, pp. 1–10, 2020, doi: 10.1136/esmoopen-2019-000635.

[28]. P. M. Anderson, V. Subbiah, and E. Rohren, "Bone-seeking radiopharmaceuticals as targeted agents of osteosarcoma: Samarium-153- EDTMP and Radium-223," Adv. Exp. Med. Biol., vol. 804, pp. 291–304, 2014, doi: 10.1007/978-3-319-04843-7_16.

[29]. Warren A ChowDepartment of Medical Oncology & Therapeutics Research, City of Hope, 1500 E. Duarte Rd, Duarte, CA, 91010, USA,Version Chondrosarcoma: biology, genetics, and epigenetics, 2018; 1826.

[30]. Faten Limalem, Donald Davis, Kristin Sticco, Chondrosarcoma CME, PubMed-chondrosarcoma,2020,19483.

[31]. Zhan Wang, Genlian Chen, Xiaowei Chen, Xin Huang, Meng Liu, Weibo Pan, Xiaobo Yan, Nong Lin, and Zhaoming Ye, Predictors of the survival of patients with chondrosarcoma of bone and metastatic disease at diagnosis J Cancer. 2019; 10(11): 2457–2463.

[32]. Julian Fromm, Alexander Klein, Andrea Baur-Melnyk, Thomas Knösel, Lars Lindner, Christof Birkenmaier, Falk Roeder, Volkmar Jansson & Hans Roland Dürr, Survival and prognostic factors in conventional central chondrosarcoma, 2018, 849

[33]. JASON L. FERGUSON,SEAN P. TURNER, Bone Cancer: Diagnosis and Treatment Principles,2018 Aug 15;98(4):205-213.

[34]. Jie Xu, Dasen Li, Lu Xie, Shun Tang, Wei Guo, Mesenchymal Chondrosarcoma of Bone and Soft Tissue: A Systematic Review of 107 Patients in the Past 20 Years, 2015 April 7, 10(4).

[35]. Jack E. Pullan; Deepa P. Budh, Primary Bone Cancer, July 2020, SatPear 202.

[36]. Meera Hameed MD, Howard Dorfman MD, Recent Development on Primary Malignant Bone Tumours, Volume 28, Issue 1, February 2011, Pages 86-101

[37]. Annemiek M van Maldegem, Judith VMG Bovée, Hans Gelderblom, Comprehensive analysis of published studies involving systemic treatment for chondrosarcoma of bone between 2000 and 2013, 2014, 4-11.

[38]. Aggerholm-Pedersen N, Maretty-Nielsen K, Baerentzen S, Jørgensen PH, Hansen BH, Baad-Hansen T, Keller J, Safwat A, Chondrosarcoma: the impact of comorbidity – 30 years of experience from a population-based database including 199 consecutive chondrosarcoma patients, 3 September 2019 ,Volume 2019:11 Pages 109—116.

[39]. Anthony A. Catanzano,David L. Kerr, Alexander L. Lazarides, Brian L. Dial, Whitney O. Lane, Dan G. Blazer, Nicole A. Larrier, David G. Kirsch, Brian E, Brigman, William C. Eward, Revisiting the Role of Radiation Therapy in Chondrosarcoma: A National Cancer Database Study, 2019, Open Access-Volume 2019-Article ID 4878512

[40]. Iona J MacDonald, Chih-Yang Lin, Shu-Jui Kuo,Chen-Ming Su ,Chih-Hsin Tang, An update on current and future treatment options for chondrosarcoma, 2019,773-786.

[41]. Abbas, Kiran, Siddiqui, Amber T, Evaluation of different treatment and management options for chondrosarcoma; the prognostic factors determining the outcome of the disease, 2018, 3- 58.

[42]. Johnathan R. Lex, Scott Evans, Jonathan D. Stevenson, Michael Parry, Lee M. Jeys & Robert J. Grimer , Dedifferentiated chondrosarcoma of the pelvis: clinical outcomes and current treatment, 2018, 23

[43]. Dhinsa B.S., DeLisa M, Pollock R, Flanagan A.M, Whelan J.· Gregory J., Dedifferentiated Chondrosarcoma Demonstrating Osteosarcomatous Differentiation, 2018, 456-460.

[44]. Rémy Nicolle, Mira Ayadi, Anne Gomez-Brouchet, Lucile Armenoult, Guillaume Banneau, Nabila Elarouci, Matthias Tallegas, Anne-Valérie Decouvelaere, Sébastien Aubert, Françoise Rédini, Béatrice Marie, Corinne Labit-Bouvier, Nicolas Reina, Marie Karanian, Louis-Romée le Nail, Philippe Anract, François Gouin, Frédérique Larousserie, Aurélien de Reyniès ,Gonzague de Pinieux, Integrated molecular characterization of chondrosarcoma reveals critical determinants of disease progression, 2019, 4622.

[45]. Jeremy S. Whelan and Lara E. Davis, Osteosarcoma, Chondrosarcoma, and Chordoma, 2017, 36-2.

[46]. AJ Gelderblom, MD, PhDJudith VMG Bovée, MD, PhD, Chondrosarcoma, 2020, UpToDate.

[47]. Arthur G G C Kortena, Hans J W ter Berga, Geert H Spincemailleb, Ronald T van der Laana, Antoinet M Van de Welc, Intracranial chondrosarcoma: review of the literature and report of 15 cases, 2020, 163-175.

[48]. Mi-Jung Kim, Kyung-Ja Cho, Alberto G. Ayala, and Jae Y. Ro, Chondrosarcoma: With Updates on Molecular Genetics, 2011, 18

[49]. C. Michael Gibson, Rohan A. Bhimani,Chondrosarcoma pathophysiology, 2019, case study 1

[50]. Aggerholm Pedersen N, Maretty-Nielsen K, Baerentzen S, Jorgensen PH, Hansen BH, Baad-Hansen T, Keller J, Safwat A,Chondrosarcoma: the impact of comorbidity-30 years of experience from a population-based database including 199 consecutive chondrosarcoma patents,2019,109-116.

[51]. Rani P, Shyamala K, Girish H et al 'Journal of Advanced Clinical & Research Insights (2015) 2 164-168 DOI: 10.15713/ins.jcri.70

[52]. Gaspar, N., Hawkins, D. S., Dirksen, U., Lewis, I. J., Ferrari, S., Le Deley, M. C., Kovar, H., Grimer, R., Whelan, J., Claude, L., Delattre, O., Paulussen, M., Picci, P., Sundby Hall, K., van den Berg, H., Ladenstein, R., Michon, J., Hjorth, L., Judson, I., Luksch, R., … Oberlin, O. (2015). Ewing Sarcoma: Current Management and Future Approaches Through Collaboration. Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 33(27), 3036–3046. https://doi.org/10.1200/JCO.2014.59.5256

[53]. . Brown, H. K., Schiavone, K., Gouin, F., Heymann, M. F., & Heymann, D. (2018). Biology of Bone Sarcomas and New Therapeutic Developments. Calcified tissue international, 102(2), 174–195. https://doi.org/10.1007/s00223-017-0372-2

[54]. Bernstein, M., Kovar, H., Paulussen, M., Randall, R. L., Schuck, A., Teot, L. A., & Juergensg, H. (2006). Ewing's Sarcoma Family of Tumors: Current Management. The Oncologist, 11(5), 503–519. doi.org/10.1634/theoncologist.11-5-503

[55]. Ozaki, T. (2015). Diagnosis and treatment of Ewing sarcoma of the bone: a review article. Journal of Orthopaedic Science, 20(2), 250–263. doi.org/10.1007/s00776-014-0687-z

[56]. Burchill, S. A. (2003, February 1). Ewing's sarcoma: Diagnostic, prognostic, and therapeutic implications of molecular abnormalities. Journal of Clinical Pathology. https://doi.org/10.1136/jcp.56.2.96

[57]. Vohar, V. (2008). Ewing's tumour, a report on 27 cases. Indian Journal of Radiology and Imaging, 18(2), 106–112.

[58]. Dubois, S. G. (2018). Ewing sarcoma. In The 5-Minute Pediatric Consult, 8th Edition (pp. 346–347). Wolters Kluwer Health.

[59]. Lessnick, S. L., & Ladanyi, M. (2012). Molecular pathogenesis of Ewing sarcoma: New therapeutic and transcriptional targets. Annual Review of Pathology: Mechanisms of Disease. https://doi.org/10.1146/annurev-pathol-011110-130237

[60]. Juergens, C., Weston, C., Lewis, I., Whelan, J., Paulussen, M., Oberlin, O., … Craft, A. (2006). Safety assessment of intensive induction with vincristine, ifosfamide, doxorubicin, and etoposide (VIDE) in the treatment of ewing tumors in the EURO-E.W.I.N.G. 99 Clinical Trial. Pediatric Blood and Cancer, 47(1), 22–29. https://doi.org/10.1002/pbc.20820

[61]. Bilodeau, L., Prof, A., & Gaillard, F. (n.d.). Ewing sarcoma Radiographic features Related Radiopaedia articles Bone tumours Cases and figures, 22–23.

[62]. Mackintosh, C., Madoz-Gúrpide, J., Ordóñez, J. L., Osuna, D., & Herrero-Martín, D. (2010, May 1). The molecular pathogenesis of Ewing's sarcoma. Cancer Biology and Therapy.

https://doi.org/10.4161/cbt.9.9.11511

[63]. Thaler, M., & Khosravi, I. (2020, June 1). Therapy management in bone sarcoma. Memo - Magazine of European Medical Oncology. Springer Medizin. https://doi.org/10.1007/s12254-020-00595-z

[64]. Hosseini, A., Mirzaei, A., Salimi, V., Jamshidi, K., Babaheidarian, P., Fallah, S., … Tavakoli-Yaraki, M. (2020). The local and circulating SOX9 as a potential biomarker for the diagnosis of primary bone cancer. Journal of Bone Oncology, 23. https://doi.org/10.1016/j.jbo.2020.100300

[65]. Khanna, N., Pandey, A., & Bajpai, J. (2017). Metastatic Ewing's Sarcoma: Revisiting the "Evidence on the Fence". Indian journal of medical and paediatric oncology : official journal of Indian Society of Medical & Paediatric Oncology, 38(2), 173–181. https://doi.org/10.4103/ijmpo.ijmpo_24_17

[66]. Toomey, E. C., Schiffman, J. D., & Lessnick, S. L. (2010). Recent advances in the molecular pathogenesis of Ewing's sarcoma. Oncogene, 29(32), 4504–4516. https://doi.org/10.1038/onc.2010.205

[67]. Lessnick, S. L., Kowalewski, A. A., & Randall, R. L. (2011). Cell cycle deregulation in Ewing's sarcoma pathogenesis. Sarcoma. https://doi.org/10.1155/2011/598704

[68]. Kelleher, F. C., & Thomas, D. M. (2012). Molecular pathogenesis and targeted therapeutics in Ewing sarcoma/primitive neuroectodermal tumours. Clinical Sarcoma Research, 2(1). https://doi.org/10.1186/2045-3329-2-6

[69]. Requilé, A., Clement, P. M., Bechter, O. E., Dumez, H., Verbiest, A., Sciot, R., … Schöffski, P. (2017). Single-Centre Experience of Systemic Treatment with Vincristine, Ifosfamide, and Doxorubicin Alternating with Etoposide, Ifosfamide, and Cisplatin in Adult Patients with Ewing Sarcoma. Sarcoma, 2017. https://doi.org/10.1155/2017/1781087

[70]. Randall, L., Calvert, G., Spraker, H., & Teddy, T. N. (2010). Ewing ' s Sarcoma Family of Tumors (ESFT) What is Ewing ' s Sarcoma Family of Tumors ? What is a Translocation ? The EWS / FLI Fusion Protein Who Gets ESFT ? Differential Diagnosis in Young Children How Does Someone with ESFT Feel ? How is Ewing ' s Sar, 1–3.

[71]. (Al-Naggar et al., 2013) Al-Naggar, R. A., Bobryshev, Y. V., Abdulghani, M., Rammohan, S., Osman, M. T., & Abdul Kadir, S. Y. (2013). Complementary/alternative medicine use among cancer patients in Malaysia. World Journal of Medical Sciences, 8(2), 157–164. https://doi.org/10.5829/idosi.wjms.2013.8.2.7358

[72]. Ganasegeran, K., Rajendran, A. K., & Al-Dubai, S. A. R. (2014). Psycho-Socioeconomic Factors Affecting Complementary and Alternative Medicine Use among Selected Rural Communities in Malaysia: A Cross-Sectional Study. PLoS ONE, 9(11), e112124. doi:10.1371/journal.pone.0112124

[73]. Al-Naggar, R. A., Bobryshev, Y. V., Abdulghani, M. A.-M. M., Rammohan, S., & Al-Jashamy, K. (2012). Knowledge and Perceptions of Cancer and Cancer Prevention among Malaysian Traditional Healers: a Qualitative Study. Asian Pacific Journal of Cancer Prevention, 13(8), 3841–3850. https://doi.org/10.7314/apjcp.2012.13.8.3841

[74]. Rajendran, P., Alzahrani, A. M., Hanieh, H. N., Kumar, S. A., Ben Ammar, R., Rengarajan, T., & Alhoot, M. A. (2019). Autophagy and senescence: A new insight in selected human diseases. Journal of Cellular Physiology. doi:10.1002/jcp.28895