Immunotherapeutic Approach to Osteosarcoma

Timothy Allen[1], MD, Ph.D., Ghazaleh Shoja E Razavi[*1] MD

Global Allied Pharmaceutical, Center for Excellence in Research & Development

*Corresponding author: Dr. Ghazaleh Shoja E Razavi MD, Dir. Clinical Development- Oncology and Respiratory, Global Allied Pharmaceutical, Tel: 1- 416-520-8835; Email: ghazaleh.shoja@gapsos.com

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Abstract

Osteosarcoma is a rare form of cancer that account for less than 0.2% of all cancers diagnosed in the United States. Unfortunately, the cause is unknown, but there have been several risks factors accused of causing the disease such as genetics, Paget disease, exposure to radiation or chemicals, and bone marrow transplants. Immunotherapy is a cancer treatment alternative to chemotherapy and radiation therapy. Its goal is to use a patient's own immune system to fight the tumor. The patient's immune system is stimulated by exposing synthetic immune molecules into their system. In this paper, we discuss the potential causes of osteosarcomas, the pathophysiology of the disease, and potential ways to cure the disease using different immunotherapy techniques.

Keywords: Bone Cancer; Immunotherapy; Chondrosarcoma; Osteosarcoma

Introduction

Bone cancer is a rare cancer, which occurs due to the unwanted growth of cells in the bone tissues [1]. In 2014, the American Cancer Society estimated that there were 3,020 (1,680 men and 1,340 in women) new cases of bone cancer and 1,460 (830 men and 630 among women) deaths caused by bone cancer in the United States.

In adults, over 40% of primary bone cancers are osteosarcoma, which is less than 0.2% of all types of cancer. In children and teenagers (those younger than 20 years), osteosarcoma (56%) and Ewing Sarcoma (34%) are much more common than chondrosarcoma (6%).

The exact cause of most of the bone cancers is not known. Several risk factors have been found that increase the risk of developing bone cancer. These include genetic disorders, Paget disease, radiation, bone marrow transplant, exposure to chemicals, and hormone disorders.

Pathophysiology and molecular basis of Osteosarcoma:

The molecular pathophysiology of osteosarcoma consists of chromosomal abnormalities, tumor suppressor gene dysfunction, transcription factors, growth factors, osteosarcoma cell proliferation, apoptosis, anchorage-independent growth, tumor angiogenesis, tumor invasion and osteoclast function, which are explained below. Potential therapeutic targets are mentioned in figure 1.

A.Chromosomal abnormalities: Osteosarcoma has been reported in patients with Bloom syndrome, Rothmund-Thompson syndrome, Werner syndrome, Li-Fraumeni syndrome, and hereditary retinoblastoma [2-4]. Amplifications of chromosomes 6p21, 8q24, and 12q14, as well as loss of heterozygosity of 10q21.1 have been reported in a study of diagnostic biopsy specimens [5]. Numerical chromosomal abnormalities include loss of chromosomes 9, 10, 13, and 17, as well as gain of chromosome 1 [2].

B. Tumour suppressor Gene dysfunction: In osteosarcoma, there are mutations in both the p53 (22%) and Retinoblastoma (Rb) gene [6]. The p53 expression was higher in low Rosen grade osteosarcoma. Rosen-grade 1: <50% necrosis; grade 2: 50%–90% necrosis; grade 3: >90% necrosis; grade 4: 100% necrosis; grade 1 + 2 = low-grade; grade 3 + 4 = high grade [3]. The p53 mutation has also been shown

to be more common in high-grade conventional osteosarcoma versus low-grade central osteosarcoma [4]. The inherited mutation of the Rb gene causes retinoblastoma syndrome, a condition that predisposes a patient to multiple malignancies, including osteosarcoma [5]. Both germ-line and somatic mutations of Rb may confer an increased risk of osteosarcoma [6].

C. Transcription factors: The activator protein 1 complex (AP-1) is a regulator of transcription and consists of Fos and Jun proteins. Both of these proteins are found to be upregulated in high-grade osteosarcoma [7,8]. Overexpression of Myc in bone marrow stromal cells leads to osteosarcoma development [9]. Myc is amplified in U2OS osteosarcoma cell line variants with the highest resistance to doxorubicin. Gain of Myc was found in SaOS-2 methotrexate-resistant variants [10].

D. Growth factors: High-grade osteosarcoma is found to express TGF-β1 in significantly higher amounts than low-grade osteosarcoma [11].Insulin-like growth factor (IGF)-I and IGF-II are also overexpressed by osteosarcoma [12]. Connective tissue growth factor (CTGF) is related to a number of proteins in the CCN family (CTGF/Cyr61/Cef10/NOVH) and acts through integrin signaling pathways [13].

E. Osteoclast function: Osteosarcoma invasion relies on interactions between the bone matrix, osteosarcoma cells, osteoblasts, and osteoclasts.
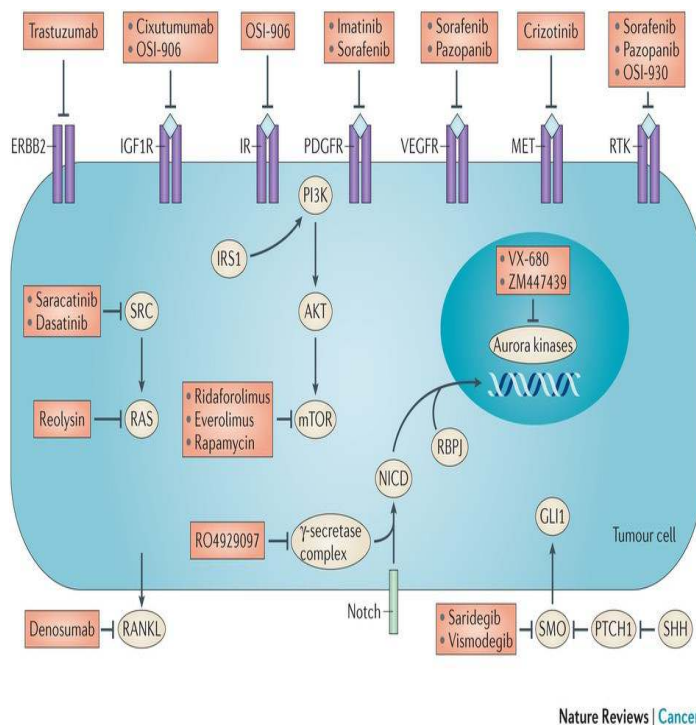

Figure 1. Potential therapeutic targets in osteosarcoma [14].

Growth factors, such as TGF-β are released from the degraded bone matrix during the initial stages of osteosarcoma invasion and act on osteosarcoma cells, stimulating the release of

PTHrP, interleukin-6 (IL-6) and interleukin-11 (IL-11) [15,16]. RANK expression is under the control of cytokines IL-1, IL-6, IL-8, tumor necrosis factor-α (TNF-α), PTHrP, and TGF-α [17]. Receptor-ligand binding initiates a cascade of events through binding of TRAF-6. This leads to the activation of both NFκB and MAPK pathways, with the resulting increase in nuclear factor of activated T-cells (NFATc1) activity. RANK/RANKL also activates the c-Fos component of AP-1, resulting in additional NFATc1 upregulation. NFATc1 is an end-point in osteoclast activity and maturation[18].Crosstalk between cells of bone lineage and immune cells is common as in figure 2.
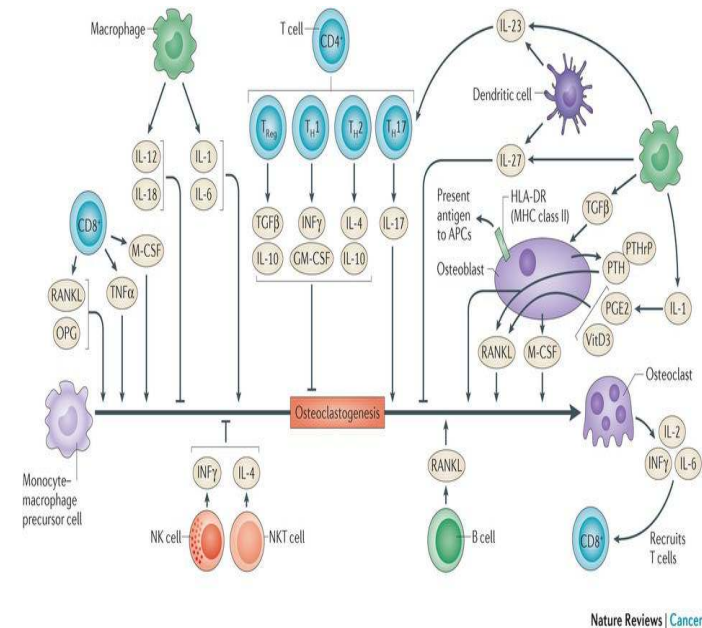

Figure 2. Crosstalk between cells of bone lineage and immune cells [14].

Immunotherapy for osteosarcoma:

A. Monoclonal antibodies:

a. Non FDA approved monoclonal antibodies:

1. **Bevacizumab:** Bevacizumab is a recombinant humanized monoclonal antibody. Its target is vascular endothelial growth factor (VEGF), a pro-angiogenic cytokine. Bevacizumab binds to VEGF and inhibits VEGF receptor binding, thereby preventing the growth and maintenance of tumor blood vessels [19]. Bevacizumab has shown to be clinically beneficial in both preclinical studies and animal models. However, its safety in pediatric groups of patients has been a cause for concern [20].

2. **Cixutumumab:** A fully human IgG-1 monoclonal, antineoplastic antibody directed against the human insulin-like growth factor-1 receptor (IGF-1R). Cixutumumab selectively binds to membrane-bound IGF-1R, thereby preventing the binding of the natural ligand IGF-1 and the subsequent activation of PI3K/AKT signaling pathway [21].Preclinical

studies with cixutumumab in osteosarcoma have shown promising results, however, phase II clinical study with the combination of cixutumumab and temsirolimus did not result in objective responses in pediatric and young adults with recurrent or refractory sarcoma [22].

3. Trastuzumab: A recombinant humanized monoclonal antibody directed against the human epidermal growth factor receptor 2 (HER2) [23]. A phase II clinical trial on Trastuzumab, plus chemotherapy in cases with metastatic osteosarcoma, failed to demonstrate clinical benefit in those cases with tumors overexpressing Her-2-neu. Besides, both tumors with and without Her-2/neu over expression have shown similar poor results [24].

4. Dinutuximab: Dinutuximab is a chimeric monoclonal antibody that binds to ganglioside GD2, which is commonly overexpressed in malignant melanoma, neuroblastoma, osteosarcoma, and small cell lung cancer [25]. Dinutuximab, in combination with Sargramostim in treating patients with recurrent osteosarcoma, is in a phase II clinical trial.

5.Glembatumumab Vedotin: Targeting glycoprotein-non-metastatic b (GPNMB) with the antibody-drug conjugate, Glembatumumab vedotin, has been suggested based on the preclinical studies evaluating the presence of GPNMB protein expression by immunohistochemistry in human osteosarcoma tumor samples and by enzyme-linked immunosorbent assay (ELISA) in osteosarcoma cell lines. In a single study, GPNMB was expressed in 92.5% (62/67) of osteosarcoma samples. All primary osteosarcoma samples expressed high levels of GPNMB mRNA. Glembatumumab induced cytotoxic effects in 74% (14/19) of osteosarcoma cell lines, and GPNMB protein levels correlated with glembatumumab in vitro cytotoxicity (r = -0.46, P = 0.04) [26].A phase II clinical study is currently active in treating patients with recurrent or refractory osteosarcomas.

6. Denosumab: Bisphosphonates, which inhibit osteoclast-mediated bone resorption, have been shown to suppress pulmonary metastasis and improve overall survival in an osteosarcoma model, *in vivo* [27].Several different types of bisphosphonates have been studied in vitro and in osteosarcoma models *in vivo*. Denosumab, which inhibits bone resorption by osteoclasts, is a humanized monoclonal antibody to RANKL for the treatment of osteoporosis and bone metastasis. A recent study showed that denosumab was able to reduce tumor size by more than 90% in all patients with RANK-positive giant cell tumors [28].A phase II clinical trial of Denosumab in relapsed refractory cases of osteosarcoma is currently ongoing.

Drug	Clinical trial identifier no.	Phase	Study Design	Target
Bevacizumab	NCT00667342	Phase II	Non-Randomized, Safety/Efficacy Study, Open Label	VEGF
Cixutumumab	NCT00831844	Phase II	Safety/Efficacy Study, Open Label	EGFR
Trastuzumab	NCT00005033	Phase II	Treatment	HER2
Dinutuximab	NCT02484443	Phase II	Biomarker/Laboratory analysis, Treatment	Ganglioside GD2
Glembatumumab	NCT02487979	Phase II	Biomarker/Laboratory analysis, Treatment	GPNMB
Denosumab	NCT02470091	Phase II	Biomarker/Laboratory analysis, Treatment	RANKL

Table 1. Monoclonal antibody drugs in phase I-III [19-28].

B. Vaccines

1. DEC-205-NY-ESO-1 fusion protein vaccine: A phase I clinical trial is ongoing of mTOR inhibition with Rapamycin for enhancing intranodal, dendritic cell vaccine induced, anti-tumor immunity in patients with NY-ESO-1 expressing solid tumors.

Drug	Clinical trial identifier no.	Phase	Study Design	Target
DEC-205-NY-ESO-1 fusion protein vaccine	NCT01522820	Phase I	Non-Randomized, Safety Study, Open Label	Tumour cells, which express NY-ESO-1

Table 2. Vaccine in phase I trial [29].

C. Adoptive cell therapy

1. GD2 bispecific antibody [30]: Ganglioside GD2 is highly expressed on neuroectoderm derived tumors and sarcomas such as osteosarcoma, rhabdomyosarcoma, Ewing's sarcoma in children and adolescents, liposarcoma, fibrosarcoma, leiomyosarcoma and other soft tissue sarcomas in adults. GD2 expression in normal tissues is restricted to the brain and is inaccessible to circulating antibodies. GD2 expression in peripheral

nerves and melanocytes is considered as a suitable target for systemic tumor immunotherapy. Anti-GD2 antibodies tested in clinical trials for neuroblastoma for over the past two decades has proven the safety and efficacy [31]. T cells coated with GD2 bispecific antibody helps the T-cells to recognize neuroblastoma and osteosarcoma cells and kill these cancer cells.

Drug	Clinical trial identifier no.	Phase	Study Design	Target
GD2 bispecific antibody	NCT02173093	Phase II	Safety/Efficacy Study, Open Label	GD2 positive cells

Table 3. Adoptive cell therapy in clinical trials [30-31].

D. mTOR inhibitors:

1. Sirolimus [32]: A natural macrocyclic lactone produced by the bacterium, Streptomyces hygroscopicus, with immunosuppressant properties. In cells, sirolimus binds to the immunophilin FK Binding Protein-12 (FKBP-12) to generate an immunosuppressive complex that binds to and inhibits the activation of the mammalian target of rapamycin (mTOR), a key regulatory kinase. This results in the inhibition of T lymphocyte activation and proliferation in response to antigenic and cytokine (IL-2, IL-4, and IL-15) stimulation and inhibition of antibody production. It has been investigated in a phase II clinical trial in combination with cyclophosphamide in advanced sarcoma patients. It has been well tolerated with a partial response rate of about 20% as a stable disease lasting for 6 months [33].

2. Everolimus [34]: A derivative of the natural macrocyclic lactone sirolimus with immunosuppressant and anti-angiogenic properties. In cells, everolimus binds to the immunophilin FK Binding Protein-12 (FKBP-12) to generate an immunosuppressive complex that binds to and inhibits the activation of mTOR. Inhibition of mTOR activation results in the inhibition of T lymphocyte activation and proliferation associated with antigen and cytokine (IL-2, IL-4, and IL-15) stimulation and the inhibition of antibody production. A non-randomized phase II clinical trial using Everolimus in combination with sorafenib has been completed in patients with unresectable high-grade osteosarcoma progressing after standard treatment. Thirty-eight patients have been recruited between June 16, 2011, and June 4, 2013. Seventeen (45%; 95% CI 28–61) of 38 patients were progression free at 6 months. Toxic effects led to dose reductions, or short interruptions, or both in 25 (66%) of 38 patients and permanent discontinuation for 2 (5%) patients. The most common grade 3–4 adverse events were lymphopenia and hypophosphataemia each in 6 (16%) patients, hand and foot syndrome in 5 (13%), thrombocytopenia in 4 (11%), and fatigue, oral mucositis, diarrhoea, and anaemia each in 2 (5%). One patient (3%) had a grade 3 pneumothorax that required trans-thoracic drainage. That had recurred at the time of disease progression. This was reported as a serious adverse event related to the study drugs in both instances. No other serious adverse events were reported during the trial. There were no treatment-related deaths [35].

3. Ridaforolimus [36]: A small molecule and non-prodrug analogue of the lipophilic macrolide antibiotic rapamycin with potential antitumor activity. Ridaforolimus binds to and inhibits the mTOR, which may result in cell cycle arrest and, consequently, the inhibition of tumor cell growth and proliferation. Upregulated in some tumors, mTOR is a serine/threonine kinase involved in regulating cellular proliferation, motility, and survival that is located downstream of the PI3K/Akt signaling pathway.A phase II clinical trial with ridaforolimus in patients with advanced bone and soft tissue sarcoma has led to PFS results that compare favorably with historical metrics. Adverse events were mostly mild to moderate and mainly consisted of stomatitis, mucosal inflammation, mouth ulceration, rash, and fatigue [37].

4. Temsirolimus [38]: An ester analog of rapamycin. Temsirolimus binds to and inhibits the mammalian target of rapamycin (mTOR), resulting in decreased expression of mRNAs necessary for cell cycle progression and arresting cells in the G1 phase of the cell cycle. mTOR is a serine/threonine kinase which plays a role in the PI3K/AKT pathway that is upregulated in some tumors. Efficacy of temsirolimus in osteosarcoma models has been evaluated, combined with cisplatin or bevacizumab on the growth of human osteosarcoma xenografts (OS-33 and OS-1) *in vivo*. This incorporates functional imaging techniques and microscopic analyses to unravel mechanisms of response. In both OS-33 and OS-1 models, the activity of temsirolimus was significantly enhanced by the addition of cisplatin (TC) or bevacizumab (TB) [39].

Drug	Clinical trial identifier no.	Phase	Study Design	Target
Sirolimus	NCT00743509	Phase II	Non-Randomized, Open Label	mTOR kinase
Everolimus	NCT01830153	Phase II	Non-Randomized, Safety/Efficacy Study, Open Label	mTOR kinase
Ridaforolimus	NCT00538239	Phase III	Randomized, Double Blind	mTOR kinase
Temsirolimus	NCT01016015	Phase II	Efficacy Study, Open Label	mTOR kinase
Everolimus	NCT01216826	Phase II	Non-Randomized, Safety/Efficacy Study, Open Label	mTOR kinase

Table 4. mTOR inhibitors under clinical trials phase I-III [32-40].

E. Kinase inhibitors:

1. Pazopanib [41] : A small molecule inhibitor of multiple protein tyrosine kinases with potential antineoplastic activity. Pazopanib selectively inhibits vascular endothelial growth factor receptors (VEGFR) -1,-2 and -3, c-kit and platelet derived growth factor receptor (PDGF-R), which may result in the inhibition of angiogenesis in tumors, in which these receptors are upregulated. It has been approved by the FDA for the treatment of specific soft tissue sarcomas. Moreover, case studies in relapsed refractory cases of osteosarcoma showed that pazopanib might be effective in matastatic cases of osteosarcoma. The efficacy and safety of this targeted therapy needs to be validated in large randomized clinical trials [42].

2. Regorafenib [43]: Regorafenib binds to and inhibits vascular endothelial growth factor receptors (VEGFRs) 2 and 3, and Ret, Kit, PDGFR and Raf kinases, which may result in the inhibition of tumor angiogenesis and tumor cell proliferation. VEGFRs are receptor tyrosine kinase that plays an important role in tumor angiogenesis; the receptor tyrosine kinases RET, KIT, and PDGFR, and the serine/threonine-specific Raf kinase are involved in tumor cell signaling. A phase I clinical study in patients with advanced solid tumors showed its benefit [44]. Phase II clinical trial in patients with osteosarcoma is currently recruiting patients.

3. Cabozantinib [45]: Cabozantinib strongly binds to and inhibits several RTKs, which are often overexpressed in a variety of cancer cell types, including hepatocyte growth factor receptor (MET), RET (rearranged during transfection), vascular endothelial growth factor receptor types 1 (VEGFR-1), 2 (VEGFR-2), and 3 (VEGFR-3), mast/stem cell growth factor (KIT), FMS-like tyrosine kinase 3 (FLT-3), TIE-2 (TEK tyrosine kinase, endothelial), tropomyosin-related kinase B (TRKB) and AXL. This may result in an inhibition of both tumor growth and angiogenesis, and eventually, lead to tumor regression.

Drug	Clinical trial identifier no.	Phase	Study design	Target
Pazopanib	NCT01759303	Phase II	Efficacy Study, Open Label	VEGFR-1, -2 and -3, c-kit and platelet derived growth factor receptor (PDGF-R)
Regorafenib	NCT02048371	Phase II	Randomized, Efficacy Study, Double blind	VEGFRs 2 and 3, and Ret, Kit, PDGFR and Raf kinases
Cabozantinib-s-malate	NCT02243605	Phase II	Efficacy Study, Open Label	RTK

Table 5. Kinase inhibitors in clinical trials phase I-III [41-45].

Conclusion

Bone cancer is not a common cancer. Conventional treatment for bone cancer consists of chemotherapy, surgical resection and radiation therapy. Although early tumor detection, novel surgical technics, neoadjuvant chemotherapy with effective high dose protocols and a multidisciplinary approach to osteosarcoma has led to better tumor control and limb salvage tumor removal and even cure in selected patients, the prognosis for locally advanced, metastatic and recurrent cases is still poor and the clinical outcomes provided by these therapeutic modalities have not significantly improved in recent decades. Advanced knowledge of the tumorigenesis and tumor promoting molecular pathways and interaction between immune system and tumor cells in different types of cancer as well as osteosarcoma has led to the concept of targeting these known molecular pathways and augmenting anti-tumor immune response as a potential therapeutic modality either alone or in combination with conventional therapeutic approach for an optimal response and potential less toxic therapeutic modality. Multiple drugs of these categories are under clinical trials for the treatment of osteosarcomas, such as mAbs, adoptive therapy and vaccines as well as targeted therapies addressing mediators in the carcinogenesis pathways such as mTOR inhibitors and kinase inhibitors. The available preclinical data on animal models has shown the feasibility of these therapeutic options, however, many of these treatment modalities are still under clinical investigation in early phase clinical trials and none of them has been approved for the treatment of osteosarcoma yet. Further clinical studies and enrolling patients in clinical trial may turn the light into these novel treatment modalities and their place in the therapeutic approach to osteosarcoma.

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