Minireview

Innovative approaches for treatment of osteosarcoma

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Impact Statement

Osteosarcoma is not entirely curable. The main treatment of the disease is surgery combined with aggressive chemotherapy, which has not changed in almost 30 years. Thus, patients' overall survival also has not significantly improved. Currently, there are no drugs undergoing clinical trials for osteosarcoma. In the review, we have offered potential techniques to develop novel therapies for osteosarcoma patients.

Abstract

Osteosarcoma (OS) is the most common primary malignant bone tumor, which usually occurs in children and adolescents. It is generally a high-grade malignancy presenting with extreme metastases to the lungs or other bones. The etiology of the disease is multifaceted and still remains obscure. A combination of surgery and chemotherapy has played a major role in the treatment of OS over the past three decades, and consequently, the overall survival rates for the disease have remained unchanged. Therefore, there is an urgent need to employ new comprehensive analyses and technologies to develop significantly more informative classification systems, with the aim of developing more effective and less toxic therapies for OS patients. This review discusses the existing knowledge of OS therapy and potential methods to develop novel therapeutic agents for the disease.

Keywords: Osteosarcoma, sarcoma, targeted therapy

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Introduction

Sarcomas are rare malignant tumors derived from mesenchymal tissues.[1] They have been divided into two major categories; soft-tissue sarcoma (muscle, fat, blood vessels, peripheral nerves, and fibrous connective tissues) that comprises less than 10% of all adult solid tumors and bone sarcoma as the name refers a malignancy arising in bone which comprise 15% of all pediatric tumors.[2] The primary approach to treat most sarcoma malignancies is surgery. However, patients with metastatic tumors have been treated with chemotherapy. Notably, for the pediatric sarcomas, treatment has evolved to include surgery followed by chemotherapy and/or radiotherapy.[2,3] There is a clear need for a novel, less toxic, and more effective treatment for sarcoma malignancies, especially osteosarcoma (OS) which is the most common pediatric sarcoma type. In this review, we aim to summarize the current therapeutic strategies for OS and suggest several techniques, which may be used to develop potentially novel therapeutics.

Background of OS

OS, can be also called as osteogenic sarcoma, is the most common primary bone malignancy among children, adolescents, and young adults.[4] Osteogenic sarcomas are commonly locally aggressive tumor types and frequently produce early systemic metastases to the lungs.[5] They usually occur near the metaphysis of the long bones such as distal femur and proximal tibia.[4,6] The molecular pathogenesis of the OS initiation and progression remains one of the major unsolved questions of the disease pathophysiology.

According to the World Health Organization (WHO), histological classification of OS can be divided into several categories: conventional, telangiectatic, parosteal, periosteal, high-grade surface, low-grade central, and small cell.[7] The conventional OS is the most common one among other classifications, which represents approximately 80% of all cases and can be subdivided into osteoblastic, chondroblastic, and fibroblastic types depending on the predominant characteristics of the tumor cells.[5,7] The symptoms of OS usually are intermittent pain, tenderness, and swelling near the affected bone.[8] Diagnosis and tracking progression are achieved by a combination of imaging (X-ray, magnetic resonance imaging scan, positron emission tomography, and computed tomography scan) and histology assessing the characteristic appearance of tumor cells forming osteoid.[9]

Current therapeutic strategies

In cancer, therapeutic approaches and strategies are usually based on several factors including tumor stage, age of

patient, general condition, patient's quality of life, and life expectancy. Currently, there are three major therapeutic options are available for patients of OS: surgery, chemotherapy, and radiation therapy.[10]

The main aim of surgical amputation and limb-salvage in OS therapy is a complete tumor removal with a wide-ranging margin of non-cancerous normal tissue in order to avoid local reoccurrence, and improve overall survival.[10] Surgery alone creates surgical stress, which causes ischemia–reperfusion injury, activation of sympathetic nervous system, endocrine and metabolic changes, acute and chronic inflammation, and immune suppression within the body, and consequently promotes tumor metastasis.[11,12] That is preciously why current management of OS includes surgery followed by chemotherapy and radiotherapy with the aim of reducing overall tumor size as well as eliminating micrometastases.[8,13]

Chemotherapy has been the most common treatment for OS patients since the 1970s.[14] The main chemotherapy regimens applied for the disease are high-dose methotrexate (HDMTX) with leucovorin rescue, doxorubicin, cisplatin, and ifosfamide with or without etoposide.[10]

Methotrexate (MTX) is a folate antimetabolite that has been used to treat neoplastic diseases, psoriasis, and rheumatoid arthritis for a long time.[15] It inhibits the production of pyrimidine and purine nucleotides, and thymidylic acid by binding dihydrofolate reductase to block proliferation of cancer cells.[16] Despite its efficacy, HDMTX has serious life-threatening side effects including renal failure, mucositis, hepatotoxicity, pulmonary toxicity, and neurotoxicity.[16–18] Notably, the standard chemotherapy dose of 8–12 g/m^2 HDMTX is extremely higher than the absolute lethal dose of 2–4 mg/kg.[19–21] Although, leucovorin, folinic acid, is widely used to decrease the toxic effects of HDMTX, therapy is still very harmful to the patients.[19]

Doxorubicin (DOX), also known as Adriamycin, is an anthracycline drug extracted from a bacterium species of *Streptomyces peucetius var. caesius* in the 1970s and is widely used in various cancer types, such as lung carcinoma gastric adenocarcinoma, breast cancer, ovarian cancer, thyroid carcinoma, non-Hodgkin's/Hodgkin's lymphoma, multiple myeloma, soft-tissue sarcomas, and pediatric cancers.[22–24] Its cumulative dose is from 240 to 480 mg/m^2; dose per cycle is from 60 to 90 m^2, and its mechanism of action is through intercalation into DNA double helix and disruption of topoisomerase-II-mediated DNA repair and inhibits the synthesis of DNA and RNA.[5,10,24] Despite its good effects on patients survival, like many cytotoxic drugs, DOX also has serious effects for patients such as cardiomyopathy, symptomatic cardiac toxicity, transient electrocardiographic abnormalities, alopecia, and myelosuppression.[16,25]

Cisplatin, (SP-4-2)-diamminedichloridoplatinum(II), is widely used and was the first metal-platinum-based chemotherapeutic drugs for the treatment of almost all cancers including testicular, cervical, ovarian, head and neck, blood, bladder, lung, cervical cancer, melanoma, lymphomas, sarcomas, and others.[26–28] Its cumulative dose is from 480 to 600 mg/m^2; dose per cycle is from 100 to 120 mg/m^2.[5] Cisplatin binds to the N7 reactive center on purine residues of malignant cells' DNA resulting in inhibition of DNA synthesis, DNA damage, and blocking their cell division activities, further promoting apoptotic cell death.[26,27] However, the drug also has extreme side effects such as acute and chronic renal failure, peripheral neuropathy, ototoxicity, hypomagnesemia, gastrointestinal disorders, and hemorrhage.[16,27]

Ifosfamide, (IFO), a bifunctional alkylating agent, is a member of the nitrogen mustard family and has been used to treat several tumor types including lymphoblastic leukemia, soft-tissue sarcoma, and OS.[29,30] Its common cumulative dose starts from 480 to 600 mg/m^2; dose per cycle is from 100 to 120 mg/m^2.[5] The drug's mechanism of action is through the cross-linking of DNA strands, inhibition of DNA synthesis, and protein translation.[16] IFO also has dramatic side effects such as hemorrhagic cystitis, acute kidney injury, Fanconi's syndrome, interstitial nephritis, glomerular disease, and encephalopathy.[16,29]

New therapeutic approaches

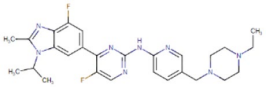
As we discussed the current therapy techniques for OS and unfortunately the results are extremely disappointing, leading to a five-year overall survival rate of 65–70%.[31] The outcomes for OS patients have not significantly improved or changed for over 30 years and this lack of new treatment strategies is reflected by the failure to improve survival rates. There is an urgent need for more effective and less toxic treatment for OS patients. Predictive biomarkers and prognostic markers are needed for use in the development of new treatments. In this section, we summarize the potential ways to develop new therapeutic approaches for the disease (Table 1).

Antisense oligonucleotides

In 1978, Zamecnik and Stephenson reported that antisense oligonucleotides (ASOs) can obstruct the replication of Rous sarcoma virus *in vitro*.[32] Twenty years later, the first oligonucleotide agent, Novartis Pharmaceutical's Vitravene (Fomivirsen), was approved by the US Food and Drug Administration (FDA) to treat cytomegalovirus retinitis afflicting HIV patients.[33] Since then, ASOs have gained popularity as therapeutics for a wide range of inherited and acquired diseases. They are short, single-stranded, synthetic RNA or DNA molecules with an average length of 8–50 nucleotides that can specifically anneal to a complementary target via Watson–Crick base pairing. ASOs are able to alter RNA, reduce, and adjust protein expression through several different mechanisms.[34–36] The molecular weight of ASOs is usually between 6 and 10 kDa.[37] ASOs are able to localize in both the cytoplasm and nucleus with the aim of reaching cytoplasmic and/or nuclear targets.[38] Hence, the molecules have chemical modification for protecting them against the action of nucleases as well as allowing them to easily travel through the plasma membrane without the requirement for vectorization.[39] Phosphorodiamidate morpholino oligomer, third generation, is the most commonly used oligo, has high binding affinity and neutral charge. However, its main disadvantages are rapid renal clearance and poor uptake into the cell nucleus.[37]

Oligonucleotide therapeutics have been investigated as cancer treatments for decades with a high potential and promising *in vitro* outcomes. Currently, there are no

Table 1. Comparison of different widely used methods for drug development. (A color version of this table is available in the online journal.)

	ASO (single-stranded DNA)	siRNA (double-stranded RNA)	Synthetic mRNA	Peptides and proteins	Protacs	Small molecules
						Abemaciclib 
Molecular weight	6–10 kDa	14–18 kDa	200–400 kDa	1.5–70 kDa	Up to 1.5 kDa	200–700 Da
Physicochemical properties	High solubility Polyanionic	High solubility Polyanionic	High solubility Polyanionic	High solubility Variable charge state	Variable solubility Variable charge state	Variable solubility Variable charge state
General characteristics	Improved binding affinity Excellent nuclease resistance Does not support RNase H Rapid renal clearance Poor uptake in cell nucleus	High degree of specificity Non-infections Low toxicity Membrane impermeability Rapid renal clearance	High efficacy and rapid uptake in cell Natural degradation Non-infectious Low severity of side effects Low attainment costs	Low tissue distribution Broad range of targets High chemical and biological diversify Stability dependent on Plasma-protein binding Short half-life and rapid renal clearance	Can target proteins without an active binding site Can target mutated proteins Low toxicity Heterogeneous distribution, dependent on chemistry Rapid hepatic and renal clearance	Distribution dependent on chemistry Can target proteins on cell surface and inside a cell Heat and metabolic stability High permeability Administered orally Hepatic and renal clearance
Route of administration	Intravenous or subcutaneous	Intravenous or subcutaneous	Intravenous, subcutaneous, or intramuscular	Intravenous or subcutaneous	Intravenous, subcutaneous or oral	Oral
Examples	Name: Nusinersen Target: SMN2 exon 7 Approved indications – year: Spinal Muscular Atrophy, 2016 Corporation: Ionis Pharma Biogen	Name: Patisiran Target: Transthyretin Approved indications – year: Hereditary transthyretin-mediated amyloidosis, 2018 Corporation: Alnylam Pharmaceuticals	Name: Pegaptanib Target: VEGF Approved indications – year: Age-related macular degeneration, 2004 Corporation: NeXstar Pharmaceuticals	Name: Setmelanotide Target: MC4R Approved indications – year: Chronic weight management (Obesity), 2020 Corporation: Rhythm Pharmaceuticals	Name: Alectinib Target: TKI Approved indications – year: Non-Small Cell Lung Cancer, 2017 Corporation: Chugai Pharmaceutical	Name: Axitinib Target: VEGFR-1/2/3 Approved indications – year: Renal cell carcinoma – 2012 Corporation: Pfizer

ASO: antisense oligonucleotide; siRNA: small interfering RNA. (A color version of this table is available in the online journal.)

approved ASOs in oncology yet and most therapeutics for cancer are still in clinical Phase I or II.[40] Disappointingly, none of the current ASOs therapeutics in clinical trials for oncology are targeting OS or any sarcoma types.[41]

Small interfering RNAs

The first introduction of double-stranded RNA that can trigger gene silencing of the complementary mRNA sequences was reported by Fire and Mello and the term "RNA interference" (RNAi) was coined.[42] Recent studies have highlighted that RNAi is a fundamental pathway found in eukaryotic cells. In the cytoplasm of mammalian cells, the Dicer enzyme initiates RNA silencing by breaking down long double-stranded RNA to create small interfering RNA (siRNA) of approximately 21–23 nucleotides long.[42,43] Theoretically, siRNA can silence any disease-related transcript in a sequence-specific manner, manufacturing a promising therapeutic modality.[44] The advantages of siRNAs have certain over small molecules and monoclonal antibodies are siRNAs carry out their function through complete Watson–Crick base pairing with messenger RNA (mRNA) as opposed to the requirement of small molecules and monoclonal antibodies to recognize the complex spatial conformation of their target proteins.[44–46] Although siRNA technique has widely promising prospects in drug development, it has a few disadvantages including being negatively charged, membrane-impermeable, activation of the immune system and can be unstable in the systemic circulation.[47]

The first FDA approved siRNA drug, 20 years after RNAi was first discovered, is Patisiran used to treat hereditary transthyretin amyloidosis (hATTR).[48] The second, Givosiran, was approved to treat acute hepatic porphyria in 2019.[49] In 2020, Lumasiran was approved to treat primary hyperoxaluria type I.[50] Presently, there are seven siRNA drugs in late stages of Phase III in clinical trials some of which are very close to obtaining FDA approval. There are also 11 siRNA drugs in early stages of Phases I and II to treat different cancer types and again none of the drugs are targeted to treat OS or any sarcoma types.[51]

Synthetic mRNA

mRNAs are single-stranded RNA molecule that are encoded in genomic DNA of a gene. Furthermore, the mRNA transcripts of genes carry the genetics information to be translated into proteins.[52] The therapeutics field of mRNA vaccine had already been developing rapidly and on top of this due to COVID-19, the field has received an enormous amount of attention. The mRNA molecule is non-infectious, and non-integrating platform, and there is no possibility of risk of infection or insertional mutagenesis. Furthermore, mRNA is degraded by usual-normal mechanism of cellular processes and its half-life *in vivo* can be potentially regulated with several different modifications and delivery methods. The mRNA vaccines generally have high efficiency through the use of several modifications which make mRNA more stable as well as extensively translatable.[53] In addition, the method has a remarkably low severity of reactions and side effects as well as low attainment costs; therefore, it is easier to be part of preclinical and clinical trials against wide range of diseases including cancer.[54]

Currently, three core pharmaceutical and biotechnology companies that focus on developing mRNA therapeutic agents: Moderna, Inc. (founded: 2010, Boston, MA, USA), CureVac (founded: 2000, Tubingen, Germany), and BioNTech (founded: 2008, Mainz, Germany).[51,53,54] These companies all have an incredibly diversified portfolio of gene therapy products in the pipeline that cover metabolic disorders, cardiovascular diseases, and immune modulators for applications in immunotherapy on cancer.[51,54] Not surprisingly, there are many mRNA therapeutics in cancer that are undergoing clinical trials; sadly, none of the drugs were designed to target OS or any sarcoma types.

Peptides and proteins

Peptides and proteins both have limitless potential as therapeutic agents. Contemporary, the market for peptide and protein drugs is estimated to be higher than US$40 billion per annum.[55] This market is still continuously growing and is expected to comprise an even greater proportion of the market in the future. Currently, there are already more than 100 approved peptide-based therapeutic agents on the market, and the most of the drugs are shorter than 20 amino acids and molecular weight of 1.5–70 kDa.[55–57] Peptides and proteins could be particularly selective due to the various points of contact they have with their potential target; consequently, higher selectivity results in reduction of toxicity as well as after affects.[55] Peptides can be easily designed to target a wide range of molecules, and as such they hold endless possibilities in a range of fields including immunology, infectious disease, endocrinology, and oncology.[55]

Mifamurtide (L-MTP-PE), a macrophage activator, was designed to treat children and adolescents with non-metastatic OS.[58,59] In a Phase III clinical trial in approximately 800 newly diagnosed OS patients, mifamurtide was combined with other OS antineoplastic agents (doxorubicin and methotrexate), with or without cisplatin and ifosfamide. Six years after the treatment, 78% of patients were still alive with no evidence of cancer. However, after Phase III trial, the drug was rejected by the FDA panel in 2007 (US National Library of Medicine, ClinicalTrials.gov, access date 08 October 2021). Interestingly, mifamurtide has been licensed by the European Medicines Agency (EMA) since 2009 and it was approved in the 27 European member states (European Medicines Agency, ema.europa.eu, access date 8 October 2021).

Proteolysis targeting chimeras

Proteolysis targeting chimeras (PROTACs), also called a bivalent chemical protein degrader, is a method which able to ubiquitinate the unwanted proteins by the ubiquitin–proteasome system (UPS).[60] The UPS is responsible for controlling nearly all basic cellular processes including cell cycle progression, cell signaling, apoptosis, immune responses, cell metabolism, protein quality control, and eliminating denatured, mutated, or structurally abnormal proteins in cells.[61,62] Fundamentally, PROTAC uses the cell's protein destruction system to remove unwanted "big garbage" proteins from cells by proteolysis. Consequently, it is possible to target various unwanted or abnormal proteins using the technology such as transcription factors, biological catalysts,

cytoskeletal, and regularity proteins. Many studies highlighted that degrading protein is more efficient than translational blocking or inhibiting the function of a protein for anticancer activities.[63,64]

The first oral PROTAC drugs, ARV-110 and ARV-471, have indicated promising results in clinical trials for the treatments of prostate and breast cancer, respectively.[65] The outcome encourages scientists and creates a greater enthusiasm for PROTACs drug development.

Small molecules

Virtually almost all traditional drugs, as well as >90% of therapeutics already marketed are small molecule drugs.[66,67] They are mainly referring to chemically synthesized or organic compounds with a low molecular weight, between 200 and 700 Da.[68] Small molecules are able to affect the function of many proteins and their interactions with other proteins by forming complexes with their targets.[67]

The first small molecule cancer drug, tyrosine kinase-inhibitor imatinib, was approved by FDA panel in 2001. Since then, numerous small molecule targeted drugs have been developed for the treatment of different cancer types. The histone deacetylase inhibitors (HDACi) are, rapidly developing, new class of cytostatic agents that inhibit the proliferation of cancer cells by inducing tumor cell cycle arrest, cell differentiation, and cell death, reduction of angiogenesis and modulation of immune response.[69] Growing evidence from both *in vitro* and *in vivo* studies has highlighted that HDACi exert their antitumor activity in OS, sensitize tumor cells to radiation, promote cell death, and increase natural killer cell–mediated (innate immunity) cytotoxicity.[70,71]

By December 2020, 89 small molecule targeted antitumor drugs have been approved by the FDA. One of the 89 small molecule drugs, Tazverik (Tazemetostat-EPZ-6438), was designed to treat metastatic or locally advanced epithelioid sarcoma.[72] The small molecule is also in current clinical trials testing for other sarcoma and cancer types including soft-tissue sarcoma (clinical trial ID: NCT04705818), synovial sarcoma (clinical trial ID: NCT02875548), and follicular lymphoma (clinical trial ID: NCT04762160).[73] Notably, the HDACi agent Abexinostat (PCI24781) is in clinical trials (Phase II) for sarcoma and lymphoma (clinical trial ID: NCT00724984).[69] Currently, no small molecule drug has been approved by FDA or undergoing any clinical trials for OS.

Conclusions

This review has discussed the current treatment for OS and possible ways to develop novel therapeutic agents for the disease with the aim of less toxic and more effective treatment. Due to the complex and unknown mechanism of OS, researchers could not establish effective therapeutic treatments based on underlying disease mechanisms. In fact, the disease still does not have an accurate prognostic biomarker. Therefore, there is, certainly, a need to employ new comprehensive investigation of technologies and methods to develop significantly more informative classification systems and to identify and develop novel therapeutic agents. Genomic analysis of OS samples can help to identify the molecular profiles predicting the outcomes and drug response. This profile can help to develop a classification for a genomics-driven management of the OS and eventually change the outcome of this malignancy. A simple question: what are we waiting for?

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