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Review

Novel therapeutic strategies for spinal osteosarcomas

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ABSTRACT

At the dawn of the third millennium, cancer has become the bane of twenty-first century man, and remains a predominant public health burden, affecting welfare and life expectancy globally. Spinal osteogenic sarcoma, a primary spinal malignant tumor, is a rare and challenging neoplastic disease to treat. After the conventional therapeutic modalities of chemotherapy, radiation and surgery have been exhausted, there is currently no available alternative therapy in managing cases of spinal osteosarcoma. The defining signatures of tumor survival are characterised by cancer cell ability to stonewall immunogenic attrition and apoptosis by various means. Some of these biomarkers, namely immune-checkpoints, have recently been exploited as druggable targets in osteosarcoma and many other different cancers. These promising strides made by the use of reinvigorated immunotherapeutic approaches may lead to significant reduction in spinal osteosarcoma disease burden and corresponding reciprocity in increase of survival rates. In this review, we provide the background to spinal osteosarcoma, and proceed to elaborate on contribution of the complex ecology within tumor microenvironment giving arise to cancerous immune escape, which is currently receiving considerable attention. We follow this section on the tumor microenvironment by a brief history of cancer immunity. Also, we draw on the current knowledge of treatment gained from incidences of osteosarcoma at other locations of the skeleton (long bones of the extremities in close proximity to the metaphyseal growth plates) to make a case for application of immunity-based tools, such as immune-checkpoint inhibitors and vaccines, and draw attention to adverse upshots of immune-checkpoint blockers as well. Finally, we describe the novel biotechnique of CRISPR/Cas9 that will assist in treatment approaches for personalized medication.

1. Introduction

Cancer continues to blight the welfare of patients, and remains one of the leading causes of premature death, worldwide [1]. Moreover, in 48 countries, cancer has now become the foremost non-communicable disease with rates of mortality surpassing cardiovascular-related pathogenesis [2]. In the United States of America, bones and joints are third leading cause of cancer death in the age group of under twenties counting both females and males, annually [3]. Spinal osteosarcomas are rare, but still present a major burden on the patient, their carers, and health-connected budgets. However, the underlying pathologic mechanisms triggering the onset of cancerous growth and metastasis are complex, and incompletely comprehended. Patients with osteosarcoma receiving either monotherapy or combinatorial treatment, the prognosis for non-responders to chemotherapy, radiotherapy and surgery is usually poor. Therefore, novel therapeutic strategies are required to overcome treatment- resistance. In this regard, the immunogenicity of osteosarcoma may be low. This notion, however, can be dispelled as accumulated evidence indicates the application of immunotherapy is feasible in those types of tumors [4]. Contextually,

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during the last twenty-five years, considerable excitement has been generated with the current dawn of immune-based medication as applied to spinal osteosarcoma [5–7].

Herein, we provide an overview of spinal osteosarcoma, a contemporary synopsis of tumor microenvironment and a critical appraisal of promising immunotherapeutic options available in the management of spinal osteosarcoma. In addition, we have considered other significant biotechniques to improve immune-based drug development that are set to become fundamental players either in regressing or eliminating this neoplastic disease.

2. Osteosarcoma of the spine

Osteosarcoma is the most common primary malignant bone tumor in children (an annual incidence of 5.6 cases per million children) and adolescent with a second peak in the elderly population [8–12]. These tumors are characterized by spindle-shaped cells of mesenchymal origin depositing immature lace-like osteoid (osteoblastic) matrix [9,13]. Spinal osteosarcoma accounts for only 3%–15% of all primary spinal tumors [10,9–12]. It is frequently located in the posterior elements (pedicles and traverse, articular and spinous processes) of thoracic and sacral regions of spine, but rarely reported in cervical section of the spine processes [10,14]. In one study, 68% of the cases had sacral tumors [14,15]. Although the majority of osteosarcoma cases are initiated through sporadic mutations, but significant numbers of incidents are related to risk factors such as therapeutic radiation for other cancers, Paget's disease of bone [16], inherited cancer syndromes like Li–-Fraumeni (mutation of the *TP53* gene encodes for p53) [17], retinoblastoma (loss of pRb protein encoded by *RB1*) [18], Bloom's [19], and Werner's syndromes [20,21].

Symptoms of spinal osteosarcoma vary from radical or axial back pain, lytic lesions, cortical breakage, compression of spinal cord, or even neurologic deficits in late stages, paraparesis, and impaired bladder function. Most of these symptoms are nonspecific and arise from other diseases making spinal osteosarcoma difficult to diagnose at an early stage of tumor development.

Histologically, spinal osteosarcoma is classified into two main groups: surface and conventional. Surface osteosarcoma is usually a low-grade malignancy such as parosteal with a limited distal metastasis capacity [22–24]. Few incidences reported a dedifferentiation of low-grade parosteal to high-grade parosteal in 16% to 43% of the cases [24,25]. Conventional osteosarcoma is a high-grade tumor classified into subtypes: osteoblast, chondroblast (most common), and fibroblast (most rare) [26,27]. Small-cell and Telangiectatic are also detected as other subtypes of spinal osteosarcoma. Telangiectatic osteosarcoma is characterized by high-grade sarcomatous cells with dilated blood-filled cavities. Radiographically, Telangiectatic osteosarcoma resembles aneurysmal bone cysts, therefore, careful examination of osteoid matrix should be performed for avoiding misdiagnosis [24,28]. Small-cell osteosarcoma is a rare high-grade tumor that could be confused with Ewing's sarcoma, as they have a similar positive response to staining for CD99 marker and translocation between chromosomes 11 and 22. However, it could be differentiated from Ewing's sarcoma through the presence of osteoid matrix and spindle-shaped tumor cells. [24,29,30].

Use of imaging techniques and a meticulous histopathological examination of a biopsy sample are considered as the gold standard for diagnostic purposes, which include plain radiography [31], computed tomography [32], myelography [32], magnetic resonance imaging [33–35], and positron emission tomography (PET) [36] (Fig. 1). This analysis is followed by a detailed surgical staging process for malignant tissues as to apply the most suitable and efficient treatment plan: Enneking system [37], Weinstein Boriani-Biagini system [38–40], and Tomita system [41–43]. Table 1 summarizes the different staging systems.

3. Current treatment strategies for spinal osteosarcoma

From the times of ancient Egyptians till the late 1800s, cancer care essentially comprised of surgically removing a tumor (first pillar), an invasive strategy with high incidences of relapse [44,45]. Later on, radiotherapy (1890) and chemotherapy (1940) were introduced as pillars (second and third, respectively) of cancer treatments [46]. However, the generalized effects of these treatments did not discriminate between tumor and normal cells. This led to the establishment of cancer targeted therapies that would affect only tumor cells thus rendering cancer treatments less aggressive and toxic (fourth pillar) [47]. In the 1990s, immunotherapy was presented as the fifth pillar of cancer care [46]. At the outset, immunotherapy was established in melanoma treatment and proved its efficacy especially in metastatic patients [48]. At the present time, there is much excitement in cancer research for the immunotherapy of malignant diseases, particularly osteosarcoma.

4. Tumor microenvironment: immune-mediated inflammogenesis

The immune surveillance theory was first introduced by Thomas and Burnet suggesting that malignant cells are identified and eliminated by mechanisms arising from the immune system [49]. Indeed, a healthy immune system can identify a cancerous cell and eliminate it from its vicinity, this process is termed the cancer-immunity cycle [50]. This cycle consists of seven steps: the first step, neoantigens (secreted by newly formed tumor cells) and proinflammatory cytokines (released by dying cancer cells) are detected by dendritic cells (DCs). In step 2, T cells identify the captured antigens and represent them on the major histocompatibility complex (MHCI and MHCII) molecules of DCs. Steps 3 and 4 consist of generating/priming a T cell response and activating T effector and T regulatory cells. The activated T cells infiltrate into the tumor and identify cancer cells by binding their T cell receptors (TCR) to the MHCI of cancer cells and thus eliminating the malignant cells (steps 5–7). A dysregulation taking place at any of these steps would disrupt the balance established between the patient's immune system and the generation of a cancerous cell, which ultimately leads to initiation of tumor growth.

Tumor growth and metastasis are critically dependent on the tumor microenvironment. Inflammation underlies cancer pathology. The complex milieu of immune and non-immune cells, soluble factors and releasates, as well as the metabolic reactions and hypoxic niche are all conducive to nurturing, sustaining, proliferating and metastasising the malignant cells within the tumor microenvironment (Fig. 2). In other words, this tumor-synchronised ensemble of cellular and non-cellular entities are reprogrammed to play to the tune of tumor cells, as narrated below.

4.1. Cells of adaptive immunity

In the non-tumor environment, pericytes form a tight covering envelope around the capillary network [51,52]. In contrast, in the tumor microenvironment there appear to be wide gaps due to sparse pericyte coverage along capillary trees [51]. This is a biomarker for poor prognosis for survival, and may not only facilitate increased capillary permeability, but also metastasis [52]. Due to their vascular reactivity, these pericytes regulate blood flow [52]. They are known to release vasoactive factors to promote angiogenesis, and hence progression of the tumor [51,52].

4.2. Cellular metabolism

At detriment to the host, the bioenergetic homeostatic drumbeat in the tumor microenvironment is orchestrated by the tumor (a "parasite"). This is solely for self-serving purpose of the tumor: survival. Therefore, the metabolic reprogramming entailed, in conjunction with

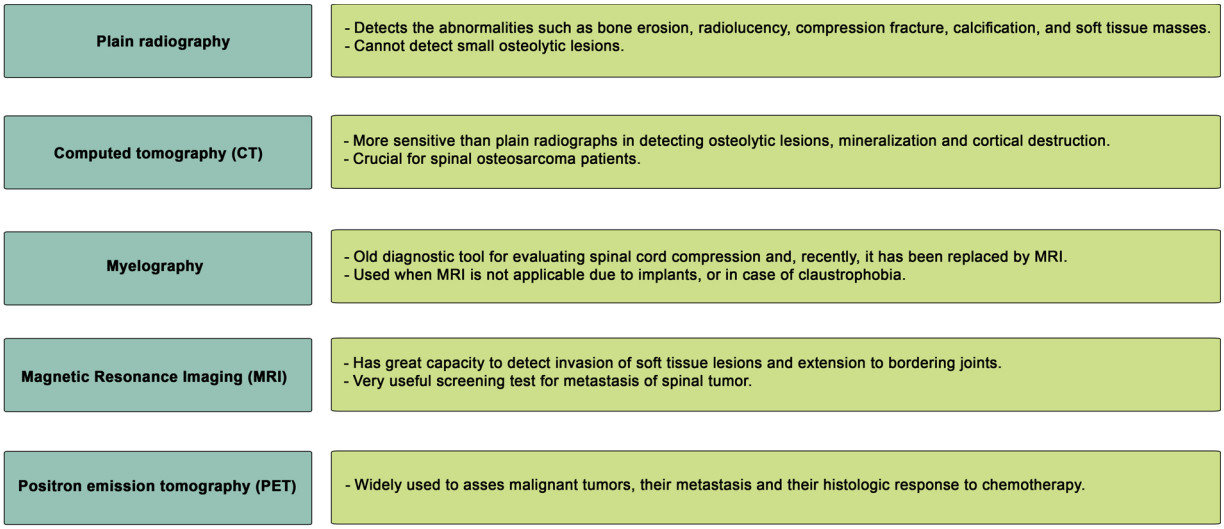

Fig. 1. Techniques used for diagnosis of spinal osteosarcoma and their properties.

immuno-avoidance, forms an integral part to promote tumor proliferation and metastasis [53].

4.3. Cell death

In the tumor microenvironment, cell death occurs via different mechanisms [54]]. Necrosis is usually not controlled by the cell, but considered to be an accidental form of cell death. This type of death undergoes cell lysis, thereby prompting a tsunami of inflammatory responses. Thereby, creating an ecology for tumor progression (Fig. 3). Apoptosis is commonly referred to as programmed cell death, following a sequence of distinct steps, which are morphologically definable.

Alternative forms of cell death processes are wired into the machinery of cancerous cells, including ferroptosis [55], necroptosis [56], and pyroptosis [57]. All of these contribute to the growth and survival of tumor cells.

5. Current treatment strategies of spinal osteosarcoma

5.1. Surgical treatment

Wide en bloc resection is considered the most effective technique used for surgical intervention [39,58]. En bloc resection is defined as the removal of the tumor as a whole covered with a shell of healthy tissue called the “margin”. This healthy tissue plays a crucial role in blocking the tumor growth. The nature of the margin is more valued than its thickness, a thin fascia is a better barrier than a cancellous bone of the same thickness [59,60]. Oncologists should assess with the patients the morbidity and the functional loss against the final results. Although the variation of resection approaches, en bloc resection does not achieve a wide margin of excision due to the anatomic constrains. Furthermore, there are no large surgical series that discern the long-term results. Combination of en bloc resection and chemotherapy is

Table 1
Staging of spinal osteosarcoma.

System	Staging method	Details
Enneking stage [148,149]	In this system, staging of malignant tissues depends on three criteria: ***Grade:*** G0 = benign, G1 = low-grade malignant, G2 = high-grade malignant ***Site:*** T0 = intracapsular T1 = extracapsular, intracompartmental T2 = extracapsular, extracompartmental ***Metastasis:*** M0 = not metastatic M1 = metastatic - After detecting criteria of tumor, it could be staged to one of these stages: IA: low-grade intracompartmental IB: low-grade extracompartmental IIA: high- grade intracompartmental IIB: high- grade extracompartmental III: metastatic tumor	Staging of spinal osteosarcoma References: [148,149]
Weinstein Boriani-Biagini (WBB staging system) [38,39,40]	- In this system, the spine is divided horizontally into 12 pieces and transversely into 5 layers from paravertebral region to dural region. -WBB staging system is important in surgical en bloc resection of spinal tumor.	References: [38,39,40]
Tomita scoring system [41,42,43]	- It is the new version of Enneking system where spinal tumor is divided according to site and invasion degree into three groups: ***Intracompartmental lesion*** (type1: tumor inside vertebral body; type2: extension to the pedicel; type3: extension to whole vertebral body) ***Extracompartmental lesion*** (type 4: epidural extension; type5: paravertebral extension; type 6: tumor extend to neighboring vertebrae) ***Multiple lesions*** (type 7): extension to all spinal vertebrae. -It is the best system for spinal osteosarcoma staging.	References: [41,42,43]

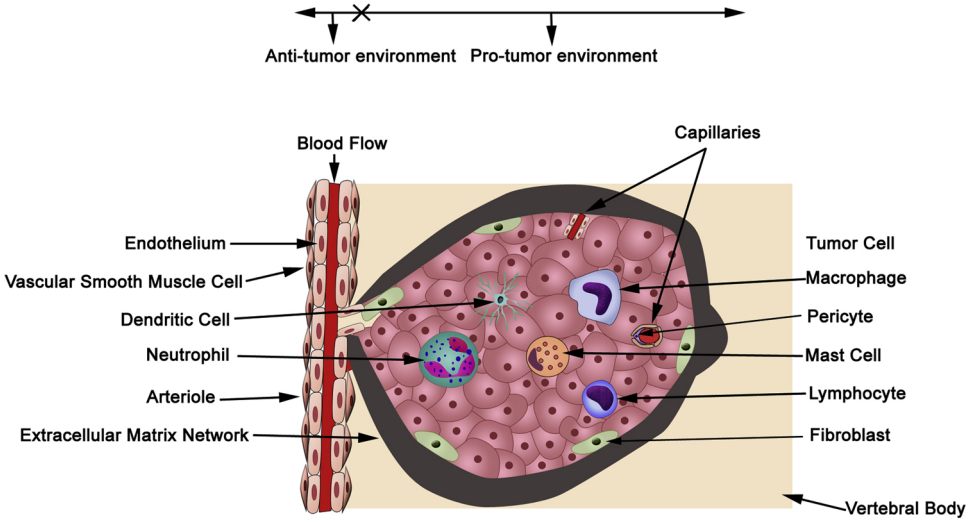

Fig. 2. Illustration of cellular heterogeneity of constituents of extradural tumor microenvironment (spinal osteosarcoma).

strongly recommended to achieve better results [27,61–63]. Also, postoperative radiotherapy is found to be beneficial.

5.2. Non-surgical treatment

5.2.1. Metastasis of spinal osteosarcoma

Metastasis is an expected consequence for many malignant tumors including spinal osteosarcoma. Both surgical inefficiency and delayed or misdiagnosis of spinal osteosarcoma are the main causes of recurrence and metastasis. In clinical study included 26 patients of spinal osteosarcoma, local recurrence occurs in 27% of patients and metastasis is initiated in 62% of patients (most of patients had metastases at one site and three patients had multi localized metastasis) [16]. The lung is the most common location of metastasis [64–66], and the eventuality of which is death.

5.3. Chemotherapy

Developing effective chemotherapeutic protocols for spinal osteosarcoma has been based mainly on the trials for osteosarcoma of the extremities. Four agents have been found to be effective: Adriamycin (doxorubicin), cisplatin, high-dose methotrexate, and ifosfamide [10]. Recent studies recommend a combination of these agents, for instance, the addition of biologic immunostimulant agent muramyl tripeptide-phosphatidilehtanolamine (MTP-PE) and ifosfamide to standard high-dose of methotrexate, doxorubicin, and cisplatin improved the three-

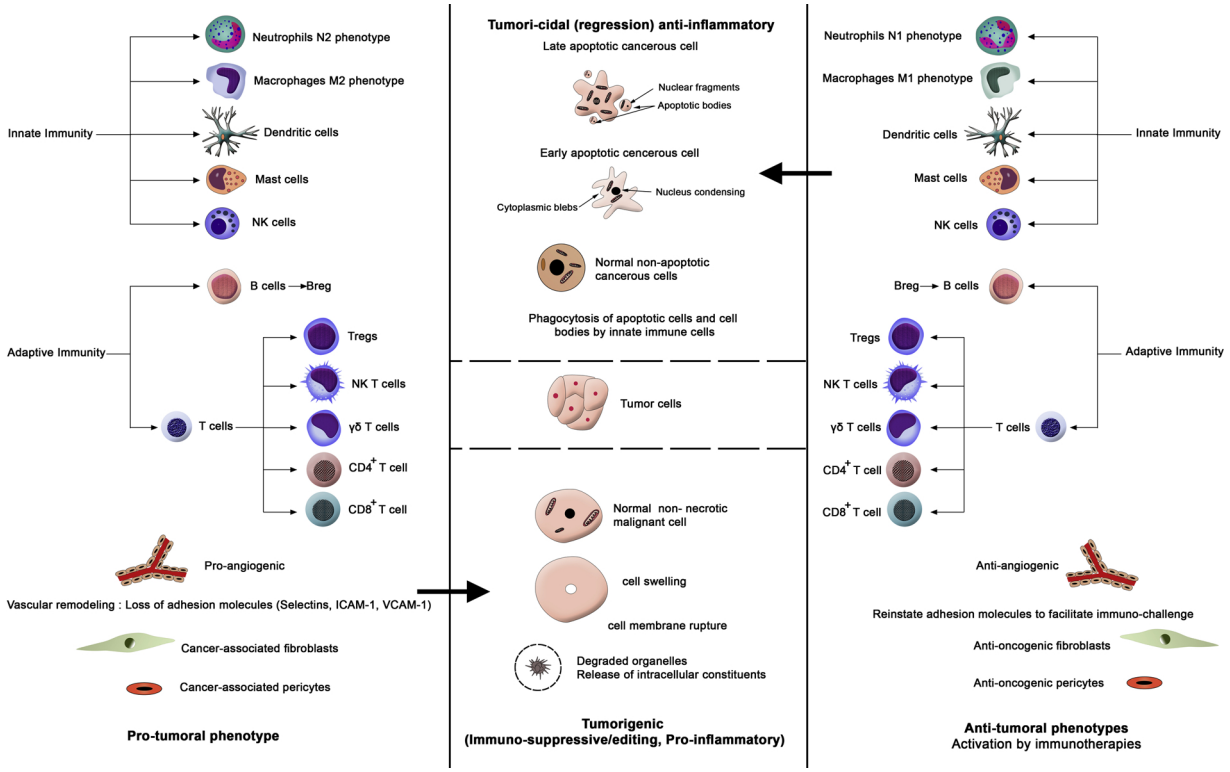

Fig. 3. Pro-tumoral, anti-tumoral, and immuno-therapeutic strategies: monoclonal antibodies, adjuvants, adoptive T-cell therapy, vaccines, immune-checkpoint inhibitors, cytokines, selectively targeted therapies to switch immune cell phenotype.

year event-free survival rate from 71% to 78% [67].

5.4. Radiation therapy

Radiation is significantly constrained due to the presence of spinal cord and thoracic abdominal organs. However, lower than radiation tolerance of the spinal cord (at 45 Gy) is required to control microscopically positive margins or gross residual disease [68,69]. A recent technique called Carbon-Ion Radiotherapy is proved to achieve precise localization and sufficient dose of the target tumor in a shorter treatment time while avoiding damage to nearby organs and surrounding healthy tissues [70]. Carbon ion beams emit a low dose of radiation after penetrating the body, and they deliver their maximum dose at the end of their range, beyond which the dose drops sharply (the Bragg peak). Furthermore, carbon ion beams also can feasibly reduce pulmonary metastasis of tumor cells [70].

6. Immunotherapy

Previous research has established that cancer cells can escape immune checkpoints and down-regulate defense mechanisms by activating immunosuppressing mediators [71]. According to this theory, a dynamic sequence of steps rules tumor formation: first, the immune system is capable of eliminating cancer cells [72]. Then an equilibrium ensues between adaptive immunity and tumor growth [73]. Finally, cancer cells escape the immune system by down-regulating tumor-associated antigens and up-regulating proliferative genes that ultimately lead to metastasis [74,75].

Immunotherapeutic strategies are directed towards modifying a patient's immune system directly to the elimination of cancerous cells rather than escaping or even settling in an equilibrated status quo with the immune system. Treatment approaches are, therefore, classified as either active or passive immunotherapies [76] (Fig. 3**)**. Stimulating endogenous immune responses, the active immunotherapy can be made applicable through cancer vaccines, adoptive cell therapy (ACT), checkpoint inhibitors, and cytokines depending on the type and stage of cancer. Alternatively, passive immunotherapies consist of monoclonal antibodies and adjuvants administrated to stimulate specific and non-specific defense responses that are mostly transient thus requiring continuous administration. However, combining passive immunotherapies with vaccines or checkpoint inhibitors improves efficacy of anti-tumor treatment protocols [77–79].

6.1. Synopsis of historical aspects of immunotherapy

Past and present research endeavours have set the foundations for future discoveries and innovations, potentially leading to cancer immunotherapy. During the past 110 years, ten researchers in the field of immunotherapy have been honoured with the accolade of Nobel laureate (Fig. 4). Briefly, most areas of immunotherapy have been recognized, and for the interested reader, the major hallmarks are mentioned in Fig. 4. Notably, the molecular procedure using clustered regularly interspaced short palindromic repeat (CRISPR)-associated Cas9 nuclease (CRISPR/Cas9; [108–110]) is set to revolutionise the treatment of cancer.

6.2. Checkpoint inhibitors

Treatment resistance gained through dysregulation of the immune checkpoints is a characteristic of neoplastic cells. Programmed cell death protein 1 (PD-1) was identified as an activated T-cell marker that promotes T-cell arrest and allows tolerance of foreign bodies [80,81]. In cancer patients, PD-1 is highly expressed on infiltrating T cells rendering them dysfunctional thus allowing tumor growth. Therefore, inhibiting PD-1 or its ligands, PD-L1 and PD-L2 (markers of antigen-presenting cells), can restore a correct immune system response [82]. In 2018, Allison and Honjo received the Nobel Prize in Physiology or Medicine for combining PD-1 and another T-cell stopper, the cell marker cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) [83], and suggesting it as a targeted patient-specific immunotherapy [84].

In 2011, the USA Food and Drug Administration (FDA) approved the first CTLA-4 inhibitor, Ipilimumab for metastatic melanoma. In under a decade, PD-1 inhibitors (Nivolumab, Pembrolizumab) and PD-L1 inhibitors (Atezolizumab, Avelumab, Durvalumab) were FDA approved to treat up to 12 different types of advanced solid tumors: Hodgkin and non-Hodgkin lymphomas, melanoma, Merkel cell carcinoma, and liver, kidney, cervical, head and neck, lung, gastric, colorectal, and bladder cancers [46]. High levels of PD-L1 and PD-1 are expressed by different sarcoma types and sarcoma infiltrating T-cells [85,86], respectively, making this cancer type more than eligible to immunotherapy as it will be covered in this review.

6.2.1. Programmed cell death ligand 1 (PD-L1, B7-H1, CD274)

This transmembrane immune checkpoint protein, expressed on animal and patient tumor cells, suppresses the T cell-driven immune response [87,88]. Raised levels of PD-L1 expression correlate with shorter survival rates and with increased risk of metastasis in osteosarcoma patients and other various cancers. However, the reverse is true for low-levels of cellular expression [85,89,90]. Therapies targeting the PD-L1 pathway promote anti-tumor immunity, and appear to have beneficial effects in different cancer typing [91].

6.2.2. Immune-checkpoint therapy and osteosarcoma

Immunologic checkpoint inhibitors are being evaluated in osteosarcoma because of the success witnessed with this class of agents in other types of cancers. Osteosarcoma cells express PD-L1 *in vitro* [92]. PD-L1 and PD-1 are biomarkers associated with poor prognosis in osteosarcoma patients [85]. Cytotoxic T cells (CD8/Tia 1) were linked to improved prognostic value, whereas PD-L1 expression was noted for poor survival prediction [93]. A number of factors have impeded the application of immunotherapy in osteosarcoma. There are no valid biomarkers as prognostic tools, osteosarcoma tumors are rare, and there is evidence for tumor heterogeneity [94]. Palmerini et al. measured $CD8^+/FOX3^+$ ratios above the median of 3.08 equated to improved survival [93].

Most of malignant cells including osteosarcoma have the ability to escape immune surveillance via checkpoint ligands such as: CTLA-4 and PD-L1. CTLA-4 is a transmembrane glycoprotein receptor expressed on Tregs or memory T cells, where it inhibits immune response through binding to CD80/86 on DCs [95]. PD-1 is another transmembrane immunoglobulin family member expressed on activated T cells and it mainly acts as a brake of the immune system by suppressing CTLs and activating Treg cells. Increased expression of CTLA-4 in osteosarcoma patients and presence of PD-L1 ligand on a subset of osteosarcoma tumor cells support the proposed use of checkpoint inhibitors (including those targeting CTLA-4, PD-1, and PD- L1) for patients with osteosarcoma [96]. However, these inhibitors reinvigorate the T-cell-mediated antitumor responses against tumor antigens especially that are distinct from those on host tissues [97].

6.2.3. Second and third generation immune-checkpoints

A significant number of cancer patients are refractory to PD-1 and CTLA-4 blockade [98]. Therefore, attention is diverting to other immune checkpoint receptors. Several candidates in this research endeavour have been investigated and recognised for anti-tumor immunity, including T-cell immunoglobulin and mucin-domain containing-3 (Tim-3), V-domian Ig suppressor of T cell activation (VISTA), lymphocyte-activation gene 3 (LAG-3), indoleamine 2,3-dioxygenase (IDO), and killer cell immunoglobulin-like receptors (KIRs) [99]. In the framework of osteosarcoma, there is dearth of data on most these checkpoints, but two have been examined to some extent, Tim-3 and IDO.

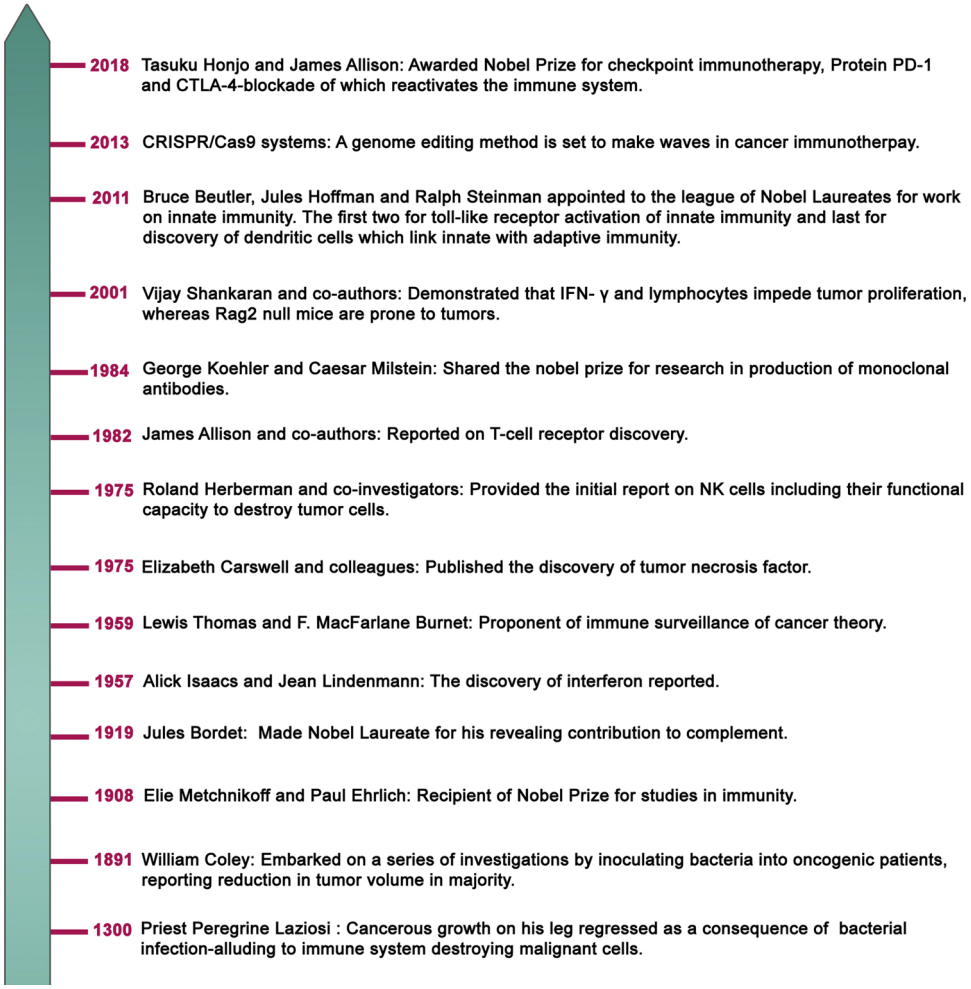

Fig. 4. Timeline of advances in immunotherapy against cancer.

6.2.4. T-cell immunoglobulin and mucin-domain containing-3

The immune checkpoint (inhibitory) receptor Tim-3 regulates both innate and adaptive immune cells. Tim-3 is expressed on the adaptive branch of the immune system: IFN-γ-releasing $CD4^+$ T helper 1 (Th1) and $CD8^+$ T cytotoxic 1 (Tc1) T cells, as well as by the innate immunity: NK cells, DCs, monocytes, and macrophages; and its endogenous ligand is galectin-9 (C-type lectin) [100,101]. Tim-3 is co-expressed with PD-1 on $CD4^+$ and $CD8^+$ T cells in various cancer types with reduced proliferation and subnormal cytokine release (IL-2, TNF-α, and IFN-γ), [101]. Also, Tim-3 is expressed on tumor associated macrophages of osteogenic tumors [101].

Serum concentrations of soluble Tim-3 were increased in osteosarcoma patients, and this corresponded to lower survival. Similarly, patients with large tumor size and metastases had raised quantity of Tim-3. Moreover, Tim-3 positive $CD8^+$ and $CD4^+$ T cells were elevated in the peripheral circulation of patients [102,103]. In addition, a negative correlation was observed between serum concentrations of cytokines (Il-2, IFN-γ, and TNF-α) and Tim-3^+ $CD4^+$ T and Tim-3^+ $CD8^+$ T cells [102].

Interestingly, Tim-3 is expressed on $CD31^+$ endothelial cells (ECs), as well as on CK-18^+ epithelial cells, including Bcl-2^+ and $PCNA^+$ osteogenic tumor cells. This implies that the tumor cells are proliferating while the cellular apoptotic rate is low. In addition, $CD68^+$ macrophages surface expressed Tim-3. Further, epithelial-mesenchymal transition (EMT) biomarkers (Slug, Snail and Smad) co-expressed with Tim-3, hence enabling tumor growth [104]. Evidently, Tim-3 is linked to immune escape, and a plausible target to eradicate spinal osteosarcoma.

6.2.5. Indoleamine 2,3-dioxygenase

Indoleamine 2,3-dioxygenase (IDO), a tryptophan degrading enzyme, is induced by IFN-γ. Increased IDO activity depletes tryptophan in the intracellular compartment. This step initiates restraining of T lymphocyte proliferation, amplifies immunosuppression, leading to tumor escape [105]. Also, cytokines IL-12 and IL-18 augment IDO activity in HOS and MG-63 cells (human osteosarcoma cell lines) in the presence of stimulated lymphocytes. However, the observable IDO expression and activity was independent of IFN-γ [106]. Osteosarcoma patients with elevated levels of IDO expression have a low rate of overall survival [107]. The investigators suggested that IDO-mediated immune tolerance played a significant role in osteosarcoma tumorigenesis. Yet again, another checkpoint protein as therapeutic target for elimination of osteosarcoma. Interestingly, IDO inhibitors (Epacadostat, NLG-8189 and Roxyl-WL) are available to quench enzyme activity, and several are currently undergoing clinical trials [105].

6.2.6. Adverse effects and limitations

Immune checkpoint inhibitors are slowly, but irrefutably, being entrenched into the armamentarium of cancer immunotherapy. These checkpoints are associated with a kaleidoscope of side-effects, or more aptly named as immune-related adverse events. Indeed, immune hyperactivation is a recognised feature of anti-tumor immune system stimulation following therapeutic application of anti-PD-1 inhibitors [108]. A characteristic of over-active immune stimulation is the

cytokine release syndrome or a cytokine storm (proinflammatory cytokines, such as IL-6, TNF-α) eventually evolving into a systems failure of various organs. These life-threatening toxicities include: cardiovascular-associated and cerebrovascular-related events, coagulation disorders, encephalopathy, endocrinopathies, fever, gastro-hepatic-intestinal derangements, and hypoxia [109–112]. Recently, these antagonistic actions were counteracted by corticosteroids and tocilizumab (IL-6 blocker) infusion [108]. Consequently, careful management of immune-related adverse events displayed by the cancer patient following administration of immune checkpoint inhibitors is indispensable [4].

The hype created by the media is a far shout from the reality that exists in oncological practice. Reports of resistance to immune checkpoint blockade are slowly beginning to emerge. In patients with melanoma, resistance to anti-PD-1 immunotherapy builds-up over the duration of the course of treatment. This outcome arises as a consequence of JAK 1/2 (Janus kinase 1/Janus kinase 2, coupled to interferon receptor transduction networks) mutations, leading to loss of effect to IFN-γ. Hence, the recurrence of immune evasion [113].

In general, almost 30% of cancer patients which constitutes a major limitation, benefit from therapies targeting checkpoint proteins [99]. Therefore, a change in therapeutic strategy from monotherapy to combinatory treatment is required to benefit more patients. Another elemental limitation is financial distress/toxicity. The interventions mentioned in this review are high-priced (prohibitly expensive) immuno-antioncogenic medication, which will create financial hardship not only for survivors, but also for their families [114,115]. Globally, immune checkpoint inhibitors will not benefit the economically challenged population, that includes almost 99.5% of all people, due to substantial heterogeneity in economic wealth.

7. Adoptive T-cell therapy

Adoptive T-cell transfer (ATCT) is another active immunotherapeutic strategy by which T cells with chimeric antigen receptors (CAR) or tumor infiltrating lymphocytes (TILs) extracted from patient tumors are isolated then re-injected along with IL-2 in the same patient to overcome the tumor immunosuppressing effects [116]. ATCT has proven its efficacy in a wide panel of solid malignancies like sarcoma, glioblastoma, melanoma, multiple myeloma, and colorectal cancer [117–120].

In this type of immunotherapy, T-cells are isolated from the patient, manipulated and expanded *ex-vivo* then reinfused into patients where they migrate to the tumor site and mediates an antitumor effect. According to the type of T-cells transferred there are different kinds of adoptive T-cell therapy such as TILs, $CD8^+$ Cytotoxic T Lymphocytes (CTL), and γδ T Cells [121,122].

As a result of unreliable isolation and expansion of TILs from osteosarcoma tissues, there are no clinical reports of use of ATCT with TILs for osteosarcoma as yet. However, recent evidence has shown that combining checkpoint inhibitors with adoptively transferred neoantigen-specific T cells from TILs may represent an effective treatment option for osteosarcoma patients [123].

Adoptive transfer of tumor-reactive CD8+ CTLs is another promising immunotherapy that recognize tumor cells through specific tumor spefic antigens called cancer/testis antigen family (CTAs). Several CTAs, such as the MAGE-A family proteins and LAGE-1/NY-ESO-1, are known to be expressed in osteosarcoma. However, in some types of osteosarcoma, CTA genes are silenced and this can be overcome via elevated CTA expression in the tumors using synergistic effects from combining demethylating treatment. CTLs against osteosarcoma is MHC-dependent. Hence, down regulation of HLA prevent recognition of tumor cell by CTLs [124].

As mentioned above there are different mechanisms by which tumors evade the immune system. To overcome this mechanism and to be more specific to tumor surface antigens rather than antigens of normal cells, T-cells can be engineered to effectively and specifically respond to tumor surface antigens. These engineered T cells are called chimeric antigen receptor T cells (CAR-Ts) [96], and consist of an extracellular domain derived from a monoclonal antibody specific for a tumor surface antigen, a spacer domain, a transmembrane domain, and an intracellular signal-transducing chain of the T cell receptor.

8. Vaccines

Cancer vaccines, offered as preventive or therapeutic means, activate specific immunity [125]. They can delay development or reduce the size of a tumor. The latest addition to the cancer vaccines family is the FDA-approved Sipuleucel-T against metastatic prostate cancer [126].

Tumor vaccines is another immune-based therapeutic approach that induces anti-tumor effect through the exposure of tumor antigens of whole cells, lysates, proteins, DNA, RNA, or peptides. Dendritic cell vaccines are a common type of tumor vaccine in which DCs (antigen presenting cells have ability to activate T-cells) can be loaded with the particles treated with immunoadjuvants *ex vivo*, and then re-injected into the patient. The safety and feasibility of the DC vaccination strategy were observed in relapsed osteosarcoma patients. However, only 2 out of 12 vaccinated patients showed a significant anti-tumor response [96].

8.1. Genetic regulation of osteosarcoma

As a result of genetic complexity of osteosarcoma, the main etiology is not completely clear. Accumulated data from a number of studies have demonstrated that the loss of tumor suppressor function is a critical step in pathogenesis of spinal osteosarcoma, hence, future novel therapeutics could be discovered based on a comprehensive understanding of the molecular neoplasticity [127,128].

8.2. Epigenetic regulation of osteosarcoma

Epigenetics is a term referring to chemical modifications of DNA that cause changes in gene expression, without any resultant alteration in the DNA sequence [129]. The non-genetic factors that modify gene activity are sensitive to the milieu of the tumor microenvironment, which is favorable to epigenetic alterations [130,131]. Various mechanisms are known to induce epigenetic modifications, including DNA methylation, histone-mediated changes, and non-coding RNAs [132]. Recently, a combinatory treatment of epigenetic approaches with immune checkpoint inhibition immunotherapy has been advocated, and moreover clinical trials are in progress to address the diverse mechanisms resisting anti-tumor immunity [133].

MicroRNAs (miRNAs) regulate hundreds of target genes, and deregulation of expression is associated with many human diseases including several types of cancer [134]. Hence, examining the potential of miRNA is clinically helpful [135–137]. Table 2 shows the effect of miRNAs on spinal osteosarcoma tissues.

8.3. CRISPR/Cas9 system

In 2014, the advent of CRISPR/Cas9 technology to enable genomic modification has heralded a transformational shift in cancer treatment, as reflected by the initial publications [138–140]. Applications of CRISPR/Cas9 tools for osteosarcoma therapeutics embrace inactivation [141], silencing [142], editing [143], repair [144], and knock-in [145] of target genes. Moreover, CRISPR/Cas9 system is being used to disrupt PD-1, and produce efficaciously enhanced CAR T cell-based adoptive immunotherapy [146]. In addition, deletion of genomic PD-L1 in osteosarcoma cell lines by CRISPR/Cas9, vastly improved the sensitivity to pharmaceutical agents doxorubicin and paclitaxel [147]. Clearly, CRISPR/Cas9 biotechnology is making headway, and will lead to

Table 2
Effect of some miRNAs on spinal osteosarcoma tissues.

Types of miRNA	Effect on spinal osteosarcoma	Reference
miR-520b	Significantly downregulates expression in spinal osteosarcoma tissues and in three osteosarcoma cell lines. The authors found that miR-520b overexpression inhibited cell proliferation, migration, and invasion, while the effect of miR-520b knockdown was just the opposite.	[135]
miR-373	Promotes growth and cellular invasion in osteosarcoma cells by activating the PI3K/AKT– Rac1–JNK pathway. Therefore, miR-373 might be a candidate for molecular targeted therapy of spinal osteosarcoma.	[136]
miR-103a	miR-103a acts as an oncogene in osteosarcoma, probably through activating the JNK/STAT and mTOR pathways by inhibiting p57 expression.	[137]

personalised drugs in the near future.

9. Perspectives and conclusion

The present goal of this review is to dissect and understand the complexity of spinal osteosarcoma multifactorial oncogenic disease. In particular, the intricacies in concept of tumor evasion, including reduced apoptotic capacity, immune avoidance, augmented proliferation, proangiogenesis milieu, proinflammatory state, and dysregulated bioenergetics within the tumor microenvironment. The more knowledge that is acquired will aid in finding novel approaches to target the various types of neoantigens that have become embedded in the ensemble of signalling mechanisms driving the proliferative action of the osteosarcoma tumor. Moreover, the quicker this momentum is energised in identifying novel targets for combinatorial immunotherapeutic strategies rather than a monotherapic agent or the "magic bullet", the easier it will become not only to give relief from this debilitating disease, but also to increase the life expectancy of patients with spinal osteosarcoma. Moreover, one may argue that for teenagers blighted by osteosarcoma, immunotherapy treatments should become the primary choice rather than the last resort.

Conflict of interest

The authors declare that they have no potential conflicts of interest to disclose.

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