The Sarcoma Immune

Subjects: Oncology

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Sarcomas are a rare disease with high rates of recurrence and poor prognosis. Available therapeutic options for advanced disease are limited and based on chemotherapeutic regimens. Immuno-oncological compounds have shown effectivity in different cancer indications, and their benefit is also expected in sarcomas. The role of the tumor microenvironment in sarcoma, prognosis, and response to novel immunotherapies are summarized here.

Keywords: sarcomas ; immunotherapy ; CAR-T cells ; PD-L1 ; chemotherapy ; radiotherapy ; targeted therapy

1. Introduction

Sarcomas are very heterogenous tumors, with more than 100 histologic subtypes characterized by the evolving recognition of distinct morphological and genetic features[1][2]. Based on a recent analysis of the Surveillance, Epidemiology and End Results cancer database, the incidence of sarcomas has been reported to increase from 6.8 cases/100.000 individuals in 2002 to 7.7 in 2014 whereas the median 5-year survival of patients with metastatic disease has remained at 30%[3]. Selected epidemiological data on the incidence and survival of sarcoma subtypes are shown in Table 1. It is thus very important to investigate novel biomarkers acting as potent therapeutic targets so to apply new treatments with improved clinical efficacy. In this frame, immunotherapies may provide new treatment options for sarcomas. However, due to the vast molecular heterogeneity of sarcomas, clinical efficacy may widely vary among patients and thus the identification of patients most likely to respond to immunotherapies is of great importance. Predictive biomarkers for immune checkpoint blockade have been proposed and their validation in clinical trials will be most useful to guide the selection of patients to benefit from immunotherapies and in this way to modify the landscape of this disease. To this end, the level of tumor-infiltrating lymphocytes (TILs) and programmed cell death-ligand 1 (PD-L1) expression have been correlated with prognosis in both soft tissue sarcomas (STS) and bone sarcomas (BS)[4]. Tumor-associated antigens (TAA) including cancer-testis (CT) antigens have been reported to induce immune responses in STS patients thus providing an important therapeutic target for this type of cancer [5][6]. In addition, the predictive and prognostic role of tumor mutational burden (TMB) in sarcoma is under intensive investigation.

Table 1. Selected clinical and molecular characteristics of sarcomas with potential targeted therapies[3][7][8][9][10][11].

Histology	% of Patients	5-y CSS (%)	Molecular Targets	Targeted Therapy
Sarcoma, NOS	14.8	55.2	TK, ALK, NTRK	sunitinib, cediranib, anlotinib, tivatinib pazopanib, crizotinib, entrectinib
Leiomyosarcoma	14.6	60.5	genomic instability	pazopanib
Liposarcoma	11.3	82.8	CDK4, MDM2, p53, TK	Palbociclib, RG7112, selinexor, sitravatinib
Gastrointestinal stroma tumors	10.8	80.4	KIT, PDGFRA	Imatinib mesylate, sunitinib, sorafenib
Malignant Fibrous Histiocytoma	7.3	77.0	ALK, SQSTM1-ALK, VCL-ALK	crizotinib
Dermatofibroma	6.5	99.2	PRKCA, PRKCB, PRKCD, KIRREL, PDPN, CD63, LAMTOR1	not available
Osteosarcoma	4.6	65.2	p53, RB, BRCAness	palbociclib
Chondrosarcoma	4.6	81.9	IDH1/2, EXT1/2, EWS-NR4A3	sunitinib
Angiosarcoma	4.4	53.8	endoglin	carotuximab

Histology	% of Patients	5-y CSS (%)	Molecular Targets	Targeted Therapy
Fibrosarcoma	3.9	82.9	COL1A1-PDGFB	imatinib, sunitinib
Stromal	3.9	75.6	JAZF1-SUZ12, YWHAE-NUTM2, ESR1	not available
Rhabdomyosarcoma	3.3	54.7	FGFR4, ALK1, PDGFRA, IGF1R, PAX3-FOXO1A	ponatinib, crizotinib, sorafenib, sunitinib, sphingosine
Other *	2.9	70.4	EWSR1-DDIT3, EWS-WT1, EWS-ATF1, MET, HGF, FOS	crizotinib, SU11274, AMG 102, FOS siRNA, ganitumab
Synovial	2.5	65.6	SS18, SS18-SSX1/2/4	tazemetostat
Malignant peripheral nerve sheath tumor	2.4	65.4	CSF1R kinase	PLX3397, sirolimus
Ewing sarcoma	2.3	64.0	IGF1R, FET-ETS	cituxumab

CSS: cause-specific survival; NOS: not otherwise specified; IGF1R: insulin-like growth factor 1 receptor; TK: tyrosine kinases; TKI: tyrosine kinase inhibitors; ALK: anaplastic lymphoma kinase; NTRK: neurotrophic receptor kinase; CDK4: Cyclin-dependent kinase 4; MDM2: mouse double minute 2 homolog; PDGFRA: platelet-derived growth factor receptor A; SQSTM1: sequestosome 1; VCL: vinculin; PRKCA/B/D: protein kinase C alpha/beta/delta; KIRREL: Kin Of IRRE Like; PDPN: podoplanin; LAMTOR1: late endosomal/lysosomal adaptor, MAPK and MTOR activator 1; RB: retinoblastoma; IDH1/2: isocitrate dehydrogenase 1/2; EXT1/2: exostosin-1/2; COL1A1: collagen type I alpha 1 chain; ESR1: estrogen receptor 1; HGF: hepatocyte growth factor; CSF1R: colony-stimulating factor 1 receptor; * including malignant mesenchymoma, odontogenic tumor, clear cell sarcoma, myxosarcoma, malignant hemangiopericytoma, malignant giant cell tumor, malignant granular cell tumor, alveolar soft part sarcoma, and desmoplastic small round cell tumor.

2. The Immune Tumor Microenvironment in Sarcomas

The immune system has the potential to identify and destroy nascent tumor cells in a process named cancer immunosurveillance[12]. The process is initiated by innate immunity involving cells of the immune system such as macrophages, dendritic cells, myeloid-derived suppressor cells, and natural killers[13]. T-cell priming and activation of effector T-cells against tumor cells constitute an important part of the adaptive immunity[5]. However, the interplay between innate and adaptive immune system can also promote tumor progression[5]. Together, the dual host-protective and tumor-promoting actions of immunity are referred to as cancer immunoediting. According to the immunoediting theory, the tumor microenvironment (TME) represents the prominent site where tumor evolution is taking place based on continuous and dynamic interactions mainly between tumor cells and elements of the immune system[6]. The relative balance of effector and memory immune cells, on one hand, and immunosuppressive populations in the TME, on the other, determines the fate of the tumor[14]. In sarcomas, the TME is highly immunosuppressive with high densities of hypoxia-inducible factor 1 α (HIF1α), macrophages, neutrophils, and decreased T-cell levels[15]. A previous study has also implicated tumor-associated macrophages (TAMs) in the establishment of an immune-hostile TME promoting the growth, angiogenesis, and metastasis of sarcomas posing an obstacle for the development of an effective antitumor adaptive immunity[16]. The activation of the STAT3 pathway, besides promoting the immunosuppressive effect of myeloid-derived suppressor cells, has an antiapoptotic effect and confers insensitivity to chemotherapy mediated by the secretion of IL-22 by T-cells[17][18]. An immunosuppressive TME can also be characterized by programmed cell death protein-1 (PD-1) and PD-L1 expression which dampen adaptive antitumor immune responses. In a recent systematic meta-analysis of 14 studies including 868 patients with STS and BS, the expression of PD-L1 was positively correlated with the infiltration of PD-1 positive T-lymphocytes and significantly correlated with metastasis, mortality risk, and poorer overall survival in patients with BS[19].

The relatively non-immunogenic phenotype attributed to sarcomas could be due to the low-intermediate TMB displayed by some STS subtypes as compared to the other cancer types[20]. On the other hand, non-synonymous somatic mutations are coding for neoantigens, exclusively expressed by cancer cells, which could induce potent immune responses, because the quality of the T-cell repertoire recognizing these antigens in the context of particular major histocompatibility complex (MHC) alleles is not affected by central T-cell tolerance[21]. Some subtypes such as undifferentiated pleomorphic sarcoma (UPS) have a higher number of non-synonymous mutations and greater TMB opening a window of opportunity for immunotherapeutic approaches such as vaccines and anti-PD-1/PD-L1 therapies[21].

3. Biomarkers for Immunotherapy in Sarcoma

3.1. Intratumoral Immune Infiltrates and PD-L1

Tumor immune cell infiltrates, such as effector CD8+ T-cells, are essential in hindering cancer progression and may complement the classification systems of cancer, as in the case of breast cancer and melanoma[22][23]. Immunological biomarkers are preferentially explored for immunotherapies as a result of the immune contexture of the TME and our comprehensive knowledge about the pathways which are targeted by immunotherapy and in particular by immune checkpoint inhibitors (ICI). IFNγ produced by activated cells of the innate and adaptive immunity induces the expression of PD-L1 on the surface of tumor cells and myeloid cells such as macrophages and myeloid-derived suppressor cells[16]. PD-L1 binds on its natural ligand PD-1 which is expressed by the activated CD8 + cytotoxic T-lymphocytes (CTLs) and de-phosphorylates substrates downstream the nucleus, resulting in reduced proliferation and cytokine production[24]. Administration of anti-PD-1 and/or anti-PD-L1 monoclonal antibodies (MAbs) unblocks immune inhibition via PD-1/PD-L1 bridging and enhances tumor cell killing by the CD8 + CTLs[24]. As thoroughly addressed in this review, multiple clinical trials have addressed the therapeutic efficacy of ICI, including ipilimumab, nivolumab, pembrolizumab, and atezolizumab, in sarcoma patients[25][26][27][28][29][30]. Although producing remarkable clinical responses, ICI are effective in a rather low percentage of cancer patients and therefore the identification of biomarkers for selecting patients most likely to respond to ICI is of paramount importance.

Although the role of intratumoral CD8 + T-cell densities in sarcomas as prognosticators has not yet been firmly established, levels of PD-1 + CD8 + (TILs and intratumoral expression of PD-L1 in sarcoma subtypes have been correlated with prognosis[31][32], suggesting that PD-1/PD-L1 interaction could regulate T-cell-mediated control of tumor growth and pointing to the use of ICI for sarcomas. To this point, it should be mentioned that PD-L1 expression might not always be a reliable biomarker for predicting responses to ICI. This was better shown in a large phase III study with advanced-stage melanoma patients but also in a meta-analysis of anti-PD1/PD-L1 clinical trials across different malignancies indicating that clinical responses to immunotherapies could be detected also among patients with PD-L1 negative tumors[33][34]. PD-L1 can be expressed in tumor cells as a result of genetic modifications including PTEN loss, EGFR mutations, MYC overexpression, mutations in the PI3K/AKT signaling pathway, and PDJ amplification[35]. Interestingly, in a recent report, it was shown that not PD-L1 per se, but rather the composition of the TME in which PD-L1 is induced, determines tumor recurrence[36]. In the same study, it was found that PD-L1 + tumor cells generated in the context of activated TAMs were resistant to chemotherapeutic regimens and therapy with ICI, whereas PD-L1 + tumor cells in the context of activated T-cells producing IFNγ, were sensitive to ICI. The role of tertiary lymphoid structures and a proposed immune classification system in patients with sarcoma was underscored in a recent study[37]. Based on the immune cell composition of the TME and the expression of gene signatures related to the functional orientation of the immune TME and to immune checkpoints, sarcomas were classified as A “immune desert”, B “immune-low profile”, C “vascularized”, D “immune-high profile” and E “immune and CTLS high”. Patients in class E, characterized by the presence of tertiary lymphoid structures containing T cells, follicular dendritic cells, and B cells, demonstrated improved survival and a high response rate to pembrolizumab[37].

3.2. Tumor Mutational Burden (TMB)

High TMB is largely being considered as a response biomarker to modern immunotherapeutics across different malignancies[38]. In recent years, high-throughput sequencing technologies have made it possible to detect somatic mutations in tumors and to identify TMB profiles. The numbers are still being defined, but, in general, 20 mutations per Mb (m/Mb) are considered high[39]. Accumulated evidence indicates large heterogeneity of TMB among sarcoma subtypes ranging from very low (0.15 m/MB) to high (29 m/MB)[39][40][41][42]. Most of the large cohort studies indicate that undifferentiated and high-grade sarcoma subtypes are most likely to exhibit TMB high values[39][42]. A recent analysis of 133 sarcoma samples, mostly low grade, identified only two cases with high TMB; one UPS and one high-grade LMS[43]. In another study, characterization of TMB in a large sarcoma cohort including different subtypes, identified a median TMB of 1.7 in most patients and TMB high in only 2% of patients[44]. In a homogeneous study including tissue samples from 26 cardiac sarcomas, whole-exome sequencing and NGS analysis identified high TMB in 92.3% of patients[41]. In another study investigating the TMB values in 16 patients with UPS who underwent complete local resection twice due to relapse identified a statistical increase of the TMB after recurrence (4.6 vs. 7.5 m/MB)[45]. A recent study identified a subgroup of synovial sarcomas with high TMB and suggested that this might explain the 10% response rate to ICI observed in clinical trials including patients with synovial sarcoma[46].

The predictive and prognostic role of TMB in sarcoma has not been fully elucidated. A case series reported results on heavily pretreated with chemotherapy angiosarcoma patients with one patient demonstrating CR on anti-CTLA-4 therapy with low TMB (0.09 mutations/mb) and 2 patients achieving partial responses on pembrolizumab and

nivolumab/ipilimumab with high TMB (12 and 15 mutations/mb)[47]. A retrospective analysis of TMB as predictive marker of response to anti-PD1 therapy identified 1 patient (out of 3) with UPS with intermediate TMB who responded to therapy[38]. Another recent report indicated that patients with TMB high and elevated effector immune cells infiltrate exhibited the highest survival[48].

Further proof-of-concept studies are required to determine which specific biomarkers including TMB are clinically meaningful to predict the efficacy of anti-PD1 blockade in sarcomas.

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