Short Communication
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Profiling of Targets for Immunotherapy in Soft Tissue Sarcoma

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Short Communication

The use of anti-human signal molecules monoclonal antibodies (mAbs) for cancer or malignant tumor therapy has achieved considerable success in recent years. Antibody drug conjugates are powerful new treatment options for lymphomas and solid tumors, and immunomodulatory antibodies have also recently achieved remarkable clinical success. The development of therapeutic antibodies requires a deep understanding of cancer serology, protein-engineering techniques, mechanisms of action and resistance, and the interplay between the immune system and cancer cells. This review outlines the fundamental strategies that are required to develop antibody therapies for patients with soft tissue tumors through iterative approaches to target and antibody selection, extending from preclinical studies to human trials.

A type of drug that blocks certain proteins made by some types of immune system cells, such as T cells, and some malignant tumor cells. These proteins help keep immune responses in check and can keep T cells from killing cancer cells. When these proteins are blocked, the "brakes" on the immune system are released and T cells are able to kill cancer cells better. Examples of checkpoint proteins found on T cells or cancer cells include programed death 1 (PD-1)/programed death ligand 1 (PD-L1) and cytotoxic T-lymphocyte associated protein 4 (CTLA-4)/B7-homolog-1(B7-1)/ B7-homolog-2(B7-2) (Figure 1). Some immune checkpoint inhibitors are used to treat cancer or malignant tumors. The probability of response to a checkpoint inhibitor is closely related to the nature of a tumor's immune microenvironment. Non-small cell lung cancer has emerged as the prototypical tumor type that is responsive to checkpoint inhibition and has a PD-L1 immunohistohemical (IHC) companion diagnostic biomarker which is used to determine whether pembrolizumab, which is anti-human PD-1 humanized monoclonal immunoglobulin (Ig) G4 antibody, monotherapy will be given [1,2]. In contrast, the soft tissue sarcoma immune microenvironment is still poorly understood and biomarkers predictive of immunotherapy response are greatly needed.

Soft tissue sarcoma diagnoses are rare and published case series typically lump heterogeneous sarcomas together. Due to this and because of the use of various different PD-L1 antibodies in different studies, the reported incidence of PD-L1 positivity in soft tissue sarcomas has varied greatly in the literature, ranging from 0% to 60% [3,4,5-12]. In soft tissue sarcomas, high expression of PD-L1 by mRNA is associated with shorter metastasis-free survival [13]. In two meta-analyses the expression of PD-L1 was found to be a poor prognosticator in soft tissue sarcomas [14].

Immune-checkpoint inhibitors have yielded mixed results in sarcoma. Ipilimumab, which binds to a substance called CTLA-4, had no activity in 6 patients with synovial sarcoma [15].

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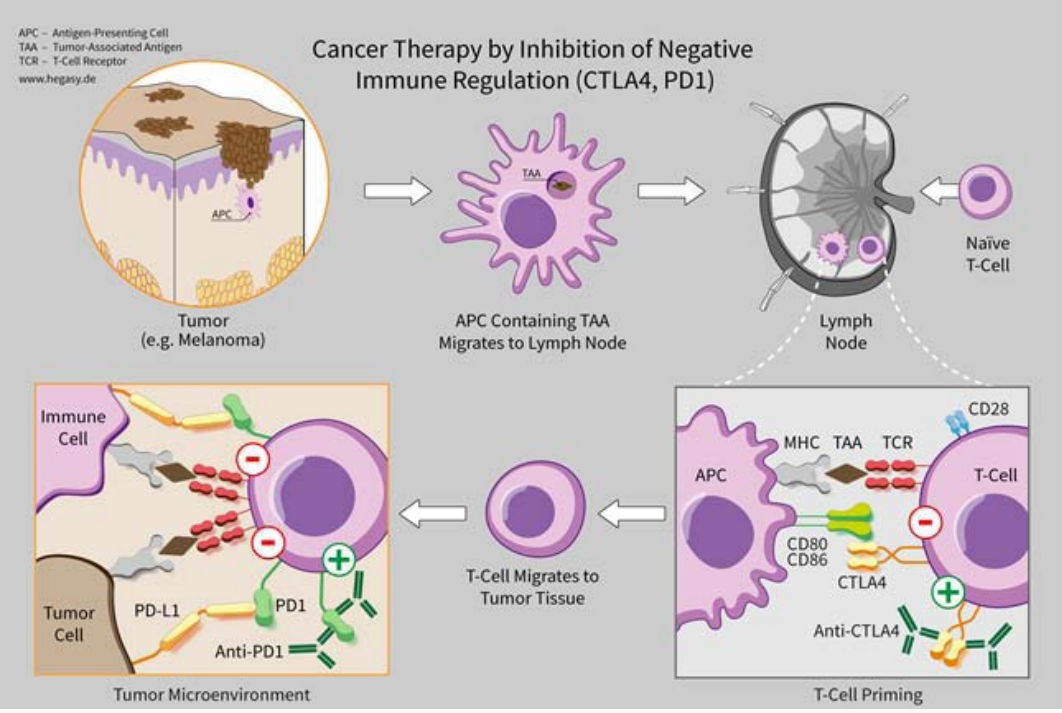

Figure 1: One ligand-receptor interaction under investigation is the interaction between the transmembrane programmed cell death 1 protein (PDCD1, PD-1; also known as CD279) and its ligand, PD-1 ligand 1 (PD-L1, CD274). PD-L1 on the cell surface binds to PD1 on an immune cell surface, which inhibits immune cell activity. Among PD-L1 functions is a key regulatory role on T cell activities. It appears that (cancer-mediated) upregulation of PD-L1 on the cell surface may inhibit T cells that might otherwise attack. PD-L1 on cancer cells also inhibits FAS- and interferon-dependent apoptosis, protecting cells from cytotoxic molecules produced by T cells. Antibodies that bind to either PD-1 or PD-L1 and therefore block the interaction may allow the T-cells to attack the tumor. The first checkpoint antibody approved by the Food and Drug Administration (FDA) was ipilimumab, approved in 2011 for treatment of melanoma. It blocks the immune-checkpoint molecule CTLA-4. Clinical trials have also shown some benefits of anti-CTLA-4 therapy on lung cancer or pancreatic cancer, specifically in combination with other drugs. In on-going trials the combination of CTLA-4 blockade with PD-1 or PD-L1 inhibitors is tested on different types of cancer. Figure is modified from Figure presented at the URL (https://en.wikipedia.org/wiki/Cancer_immunotherapy).

Ben-Ami reported that none of 12 uterine leiomyosarcoma patients responded to nivolumab, which is anti-human PD-1 fully human immunoglobulin (Ig) G4 monoclonal antibody [16]. Two of four patients with alveolar soft part sarcoma were reported to have partial response to anti-PD-L1 therapy [17]. A microsatellite stable, low tumor mutation burden (TMB) chondrosarcoma with 1% PD-L1 positivity was reported to respond to nivolumab [18]. Finally, the SARC028 clinical trial reported that 18% of patients with soft tissue sarcomas had an objective response to pembrolizumab. Twenty percent of patients with liposarcoma, 10% with synovial sarcoma, and 5% with bone sarcoma had a response. No response was seen in patients with leiomyosarcoma or Ewing sarcoma. Importantly, 4 of 10 patients with undifferentiated soft tissue sarcoma had a response and 2 of these 4 patients had at least 1% neoplastic cell PD-L1 positivity. Tumors from all 6 pembrolizumab unresponsive patients with soft tissue sarcoma were PD-L1 negative [8]. High RNA expression of *B7-homolog 3 (B7-H3)*, *Transforming growth factor beta-1 (TGFB1)*, and *T-cell immunoglobulin and mucin domains-containing prptein 3 (TIM3)*, which are putative immunotherapy targets, has been described in sarcoma, specifically in dedifferentiated liposarcoma, undifferentiated pleomorphic sarcoma, and myxofibrosarcoma [19].

Most of the immune-therapeutic focus in soft tissue sarcoma has centered on the PD-1 axis [3,4,9-14]. The expression of a few alternate immunotherapeutic targets, such as B7-H3, TGFB1, and TIM3, has been previously described in some soft tissue sarcoma types [19,20]. Pathological studies with anti-PD-L1 antibody have yielded inconsistent findings in soft tissue sarcoma partially because of the inclusion of heterogeneous sarcoma entities in many of the relevant studies [3-11]. Using various criteria and antibodies most studies show that 40-82% of soft tissue sarcoma have expression of PD-L1 [3,10,11]. The population of soft tissue sarcoma had reportedly PD-L1 positivity in 38% of cases, substantially more than the non-soft tissue sarcoma population which was PD-L1 positive in 19% of cases. The mRNA expression of *PD-L1* gene in soft tissue sarcoma was not significantly different from non-sarcoma cases and was significantly under expressed in non-soft tissue sarcoma. However, PD-L2 emerged as significantly overexpressed in soft tissue sarcoma. PD-L2 expresses in both neoplastic and non-neoplastic cells in multiple neoplasms and its expression correlates with pembrolizumab response in head and neck squamous cell carcinoma independent of PD-L1 expression [21]. Along with the high positivity PD-L1 in soft tissue sarcoma, the overexpression of PD-L2 in soft tissue sarcoma suggests that the communication of PD-1/PD-L1/PD-L2 axis may be a key role of protective mechanism against host immune response in soft tissue sarcoma. In turn, this raises the possibility that agents targeting PD-L1 may not be as therapeutically efficacious as agents targeting the PD-1 receptor in soft tissue sarcoma, and provides a possible reason for the high response rate of soft tissue sarcoma to pembrolizumab [8]. As additional putative immunotherapy targets, B7-H3 and TGFB1 were remarkable in that they wereboth overexpressed in soft tissue sarcoma and non-soft tissue sarcoma.

B7-H3 is immunohistochemically present in most osteosarcomas,

is associated with a poor prognosis and with poor infiltration by $CD8^+$ T-cells [22]. Our knowledge of its functional significance in other sarcomas is limited, but its known immune-suppressive function and relatively high expression in both soft tissue sarcoma and non-soft tissue sarcoma suggests that it may be an important immune-suppressor in soft tissue sarcoma. Overexpression of TGFB1 as the immune-suppressive cytokine has been described in soft tissue sarcomas [19,20]. Importantly, TGFB1 physiologically induces epithelial to mesenchymal transition (EMT) [23]. The elevated expression of multiple EMT-related factors also induce the process of protection from host immune-system, these factors are exploitable as immunotherapy targets.

Colony stimulating factor 1 receptor (CSF1R) is well-known to associate with macrophages and there is preclinical evidence that its blockade increases $CD8^+$ T-cell motility and tumor infiltration [24]. The significantly differential expression of TIM3 is observed in soft tissue sarcoma or non-soft tissue sarcoma. This is in conflict with the previously described overexpression of TIM3 in the Cancer Genome Atlas (TCGA) sarcoma cohort [19]. The reasons for this discrepancy are unclear, although the differences in the specific subtypes of soft tissue sarcomas included in ours and TCGA cohort may be an explanation. Notably, other potential immunotherapy targets such as CTLA4, Glucocorticoid induced TNF-related Protein (GITR), Inducible T-cell co-Stimulator (ICOS), Indoleamine 2,3-dioxygenase 1 (IDO1), and PD-1 were either under expressed or had no expression difference in soft tissue sarcoma and non-soft tissue sarcoma. Functional analysis in a responding patient demonstrated rapid *in vivo* expansion of neoantigen-specific Tcell clones that were reactive to mutant neopeptides found in the tumor. These data support the hypothesis that the large proportion of mutant neoantigens in mismatch repair-deficient (dMMR) make them sensitive to immune checkpoint blockade, regardless of the cancers' tissue of origin. Recent report demonstrated that uterine sarcomas were mismatch repair-deficient [8,25-27]. Sarcoma, 10% of stage I to stage III malignant tumors and 5% of stage IV malignant tumors were mismatch repair-deficient [8,25-27].

The causal relationship between metastatic status and presence of CD8 is currently unclear, although there is preclinical evidence indicating that depletion of cytotoxic $CD8^+$ T-cells plays an important role in the proliferation of metastatic neoplastic cells [25]. The recent research findings suggest that inflammatory status can be used to categorize sarcomas and, possibly, their clinical behavior. Further clinical studies will elucidate whether this type of profiling is also predictive of immunotherapy response. The sample size is small and we were only able to include a smattering of various sarcomas in the non-soft tissue sarcoma category. Further studies will need large numbers of each specific sarcoma histology. Finally, histopathological studies and functional or clinical studies are needed to confirm our findings.

In conclusion, both soft tissue sarcoma and non-soft tissue sarcoma possible have a high incidence of expression of PD-L1. Soft tissue sarcoma in particular has a very high rate of positive mRNA expression of *PD-L1* gene and high mRNA expression of *PD-L2* gene, which suggests a mechanism of immune-suppression and therefore soft tissue sarcoma has a high response rate to pembrolizumab. The expression profiling of proteins demonstrated the overexpression of PD-L1, and dMMR in sarcoma [8,25-27]. The clinical evidences of lymphocytes infiltration and negative signal-ligands such like PD-L1 in soft tissue sarcoma suggest that the tumor microenvironment plays an important role of anti-tumor by immune-checkpoint inhibitors.

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