New Strategies in Sarcoma: Linking Genomic and Immunotherapy Approaches to Molecular Subtype
Jamie Lim, Neal M. Poulin, and Torsten O. Nielsen

Abstract
There are more than 100 sarcoma subtypes, each uncommon and diagnostically challenging. Conventional chemotherapy has little benefit for most soft-tissue sarcomas; new treatment strategies are needed. Multiple recent genomic studies have provided detailed insights into sarcoma biology, including more accurate classification by molecular subtype, identification of recurrent mutations in oncogenic pathways, and evidence of epigenetic dysregulation. Advances in immunotherapy (adoptive immune cell transfer, tumor vaccine strategies, and immune checkpoint inhibition) have also provided a better understanding of how immuno-oncology might best be applied to sarcoma treatment, including connections to oncogenic pathways that may support combination strategies with conventional and targeted therapies. In this article, we review the latest sarcoma genomic studies and immuno-oncology developments and discuss how the findings suggest potential strategies to improve diagnosis and treatment across multiple sarcoma subtypes.

Background
Sarcomas are classified into more than 100 different histologic subtypes by the World Health Organization, each rare. Most are diagnostically challenging and lack effective systemic treatments. While conventional cytotoxics do improve survival in Ewing sarcoma, rhabdomyosarcomas, and osteosarcoma, they confer little curative benefit in most soft-tissue sarcomas, as confirmed by a recent analysis of 2,665 cases across 11 clinical trials (1). Much recent effort has gone into targeting expressed proteins in activated oncogenic pathways, but metastatic and highly proliferative cases have acquired genomic instability, a finding more frequent in adult cases, which provides a biologic explanation for their worse clinical outcomes relative to pediatric patients (11).

Osteosarcoma is notable among pediatric cancers for having a high mutational burden, with median non-synonymous mutations per genome in the range of 22 to 37 (12, 13). SNP analysis reveals more severe LOH in osteosarcoma than in myxoid liposarcoma or synovial sarcoma (14). In addition, osteosarcoma has one of the highest rates of structural variation of any pediatric cancer sequenced to date (12, 15), with 50% to 85% of tumors exhibiting the hypermutation phenomenon termed kataegis (12, 13). Similar genomic complexity is present in adult sarcomas such as undifferentiated pleomorphic sarcoma and leiomyosarcoma (16).

In contrast, oncogenesis for translocation-associated sarcomas appears to be mediated less by genomic changes and more by altered epigenetic regulation of the transcriptome. A major ChIP-Seq study in Ewing sarcoma reported dual roles for EWS-FLI1 as a transcriptional activator and repressor through distinct chromatin remodeling mechanisms (17). Similar epigenetic regulatory functions have been identified in other translocation-associated sarcomas, suggesting therapeutic options (18).

These and other recent genomic studies provide new and detailed insights into sarcoma biology, with implications for strategies to improve sarcoma diagnosis and treatment.

On the Horizon
A paradigm shift from morphologic to molecular diagnosis
Sarcoma subtypes have traditionally been classified by histology, but this has proven insufficient, for example, in rhabdomyosarcoma. Alveolar rhabdomyosarcoma (ARMS) and embryonal...
rhabdomyosarcoma (ERMS) are the two main subtypes, with ARMS usually associated with translocations fusing FOXO1 to \( PAX3 \) or \( PAX7 \) (19). Cases with ARMS histology lacking \( PAX \)-\( FOXO1 \) fusions exhibit clinical behavior and karyotypic changes similar to ERMS (20) and do not require the more aggressive chemotherapy regimen typically prescribed for ARMS. Recent genomic studies now confirm the \( PAX \)-\( FOXO1 \) distinction in terms of epigenetic variation, copy number alterations, and transcriptome changes (21, 22). Fusion status is a more useful diagnostic categorization than histology, especially considering the significantly worse prognosis of \( PAX3 \) compared with \( PAX7 \) fusion cases (23, 24).

Another major recent finding is recurring MYOD1 Leu122Arg mutations in spindle and sclerosing variants of RMS as well as in a subset of clinically aggressive ERMS, albeit with spindle cell morphology (25–27). These findings all provide convincing evidence that molecular classification more accurately captures the true biology and clinical course of rhabdomyosarcoma to guide therapeutic decisions.

Classifying “undifferentiated round cell sarcomas” that have Ewing-like morphology and presentation but lack \( EWSR1 \) translocations has also been problematic. Recent studies have identified that some of these cases carry translocations involving \( BCOR-CCNB3 \) (28–30), \( CIC-DUX4 \) (31, 32), or \( CIC-FOXO4 \) (33, 34). These variants are genetically distinct from Ewing sarcoma and portend different prognoses, for example, with \( BCOR-CCNB3 \) tumors (28, 29) showing good chemosensitivity, which must now be considered as distinct entities for treatment selection.

The identification of these and many other translocations in sarcomas highlights the need for one generic diagnostic test that can capture all clinically relevant fusions with high specificity and sensitivity, something not possible with standard clinical FISH and RT-PCR techniques. Strategies under development for sarcoma diagnosis include FFPE-compatible high-throughput screens using NanoString’s nCounter technology or anchored multiplex PCR for targeted sequencing, methods recently brought into use for lung cancer (35, 36).
Accurate diagnosis is the cornerstone of therapy. Lumping "soft-tissue sarcomas" together as a group for logistical reasons makes neither biologic nor clinical sense and has held back the field. Where few effective therapies exist, clinical trials have the greatest chance of success when applied to well-defined patient groups, which for many sarcomas can only be achieved using molecular diagnostics.

Epigenetic therapy

Evidence implicating alterations in chromatin remodeling in, for example, synovial sarcoma (18, 37), Ewing sarcoma (17), and fusion-positive ARMS (38) suggests that many translocation-associated sarcomas might be targetable with new epigenetic agents. Indeed, these strategies may also apply to other sarcomas, as recent genomic screens of malignant peripheral nerve sheath tumors (MPNST) identified inactivating mutations in two core members of the polycomb-repressive complex 2 (PRC2), most commonly SUZ12 (39, 40). PRC2 effects could be targeted in different ways using EZH2 or bromodomain inhibitors, to reactivate or block transcription. It is worth noting that PRC2 dysregulation activates developmentally suppressed pathways and may have neither biologic nor clinical sense and has held back the "soft-tissue sarcomas" together as a group for logistical reasons.

Epigenetic therapy for sarcomas

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HDAC inhibitors represent the first generation of approved epigenetic therapy agents, although their use as monotherapy in advanced sarcomas has had disappointing results beyond some prolongation of stable disease (41, 42). Like other drugs tested in advanced tumors, HDAC inhibitors may be more effective in combination with other agents and/or when tested against time-to-event rather than objective response endpoints. Beyond the HDAC inhibitors, phase I and II trials are ongoing for at least three EZH2 and seven bromodomain inhibitors in a wide range of cancer types (Table 1), with sarcomas eligible for some advanced solid tumor trials.

Advances in immunotherapy

Stimulation of host immunity for the eradication of tumor cells has been a long-sought goal of cancer therapy. Cytotoxic T lymphocytes (CTL) are critical effectors of tumor cell killing in animal models, and intense CTL infiltration correlates with good prognosis in sarcomas (43, 44). To stimulate CTL responses, MHCI-directed peptide vaccines have been pursued extensively,
usually targeting self-antigens highly expressed on tumor cells but showing restricted expression in other tissues. These include cancer-testes antigens such as NY-ESO-1 and others with known relevance to sarcoma (45, 46). Trials of such vaccines have historically shown disappointing clinical effects (47), likely due in part to insufficiency of vaccine adjuvants but also to the mutability of advanced cancers and to mechanisms of immunoevasion. This strategy may be most relevant to cancers with low mutation rates that consistently express such antigens. In synovial sarcoma, NY-ESO-1 has been targeted by autologous T cells transduced with high-affinity T-cell receptors, with partial responses in 10 of 18 cases and one durable complete response in recently reported phase II results (48). Trials using other NY-ESO-1–specific T cell and dendritic cell vaccines are open in several types of soft-tissue sarcomas (Table 1).

The role of targetable immune checkpoint proteins such as CTLA-4 and PD-1 in sarcomas is not yet well characterized but is a subject of active investigation. Preclinical investigations of other inhibitory receptors expressed on dysfunctional and regulatory T-cell infiltrates have shown great promise. High expression of PD-1 and LAG-3 or TIM-3 is regarded as a “fingerprint” of T-cell exhaustion; genetic and preclinical studies show dramatic synergies with combinatorial loss of signaling (49). While further removed from detailed characterization and therapeutic application, the list of agents targeting inhibitory immune receptors and ligands continues to grow (50). Because these agents modify inhibitory signals in previously activated, antigen-specific T cells, as single agents they may be most effective in highly mutated tumor subtypes with the most neantigen production, as shown, for example, in animal models of osteosarcoma (51). By way of comparison, 0 of 6 patients with synovial sarcoma responded to ipilimumab (52).

The most critical aspect of these modulations relates to interruption of signals involving the myeloid component of tumor infiltrates (53, 54). In the tumor environment, antigen presentation is inhibited (55), and myeloid differentiation is polarized toward regulatory, proangiogenic, and prometastatic phenotypes (56). Monocytes and tumor-associated macrophages comprise the predominant cell type in the infiltrate of many sarcomas, where their high frequency is commonly correlated with poor prognosis (57, 58).

Rational combinations of immune checkpoint inhibitors with available avenues of immune stimulation may translate to substantially improved outcomes for patients with sarcoma in the near future. In the setting of genetically unstable, refractory lesions, many have proposed mutational loss of epitope-specific antigen presentation as an inherent flaw in the design of approaches targeting a single antigen. However, the mutational landscape of metastases in different subtypes, response-associated reversals in the polarization of myeloid cells, and the effects of combinations with checkpoint inhibitors or with conventional radiation or cytotoxic chemotherapy all remain to be addressed in this context.

The cutting edge: interface between immuno-oncology and precision medicine

The modulation of host immunity by conventional and targeted therapies is becoming a central theme in modern cancer therapy. Immunostimulatory activity of radiation (the “abscopal effect”) and a number of cytotoxic chemotherapies is recognized and has been related to the stimulation of immunogenic tumor cell death, associated with release of damage-associated molecular patterns (59). Immune stimulation by cytotoxics has also been attributed to depletion of regulatory T and myeloid cells, as well as to direct stimulation of antigen-presenting cells.

Multitargeted tyrosine kinase inhibitors are among the newer agents being used in sarcoma therapy, many of which have immunomodulating off-target effects at pharmacologic doses (60). Sunitinib and pazopanib have comparable IC50 values toward their target VEGF and PDGF receptors, but sunitinib potently inhibits FLT3, a receptor inducing myeloid and dendritic cell maturation (61), and three main effecrocytosis receptors (TYRO3, AXL, and MERTK) with IC50 values in the single-digit nanomolar range. However, when scheduled after vaccination, sunitinib stimulates immunity by depleting suppressive myeloid populations, an effect which is again attributable to FLT3 inhibition (62). No such pronounced effect on myeloid cells has been attributed to pazopanib. These observations are especially relevant in light of the “rebound” of myeloid infiltrates that constitutes a major avenue for resistance to angiogenesis inhibitors (63).

Drugs targeting chromatin modifiers, mentioned above as of particular interest in translocation-associated sarcomas, may also have immune effects. HDAC inhibitors upregulate MHC-I antigen presentation of natural killer (NK) ligands on tumor cells but may have repressive effects on other immune subsets (64). Polycomb repression of chromatin, linked to neoplastic processes in Ewing, synovial, and rhabdomyosarcomas is also critical for stable lineage commitments and T-cell tolerance, with loss of function commitments and T-cell tolerance, with loss of function

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Challenges remain in developing targeted therapies for sarcomas. Anecdotal case reports of complete responses in patients and of cures in mouse models have not translated into successful clinical trials (2, 3, 72). The relative rarity of each individual subtype has led to their grouping together in clinical trials, but in this setting, the underlying genetic differences among sarcomas will complicate assessment of any drug’s efficacy. With insights gained into sarcoma biology from recent genomic studies, a more appropriate way forward may be the use of basket trials (73), in which treatment is determined by genotype instead of subtype. Rare sarcomas can then be combined with rare variants of more common cancers to make a trial simultaneously logarithmically feasible and biologically rational.

There are other reasons for the dearth of successful targeted therapies in sarcoma: drugs are targeting secondary instead of driver mutations, genomic instability promotes drug resistance, and de novo resistance via alternative pathways is often present. To address these hurdles, multiagent approaches will be needed that ideally include direct targeting of driver mutations and immune-oncology treatments that can evolve as rapidly as the tumor. In this regard, while many sarcomas will be similar to other types of cancer, at least for some types, the driver mutations are more clear and the genetics less complex.

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