Clinical Cancer Research

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. *Clin Cancer Res; 21(21); 4753–9.* *©2015 AACR.*

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 these have had disappointing results in phase II trials (2–4), and few sarcoma studies have progressed to phase III (5). Off-label use is common but has shown little evidence of efficacy (6). In this environment, there is a large unmet clinical need when local control measures are insufficient for cure.

By morphology, karyotype, and expression profile, sarcomas have been broadly categorized into two groups. Molecularly defined sarcomas often carry pathognomonic translocations, whereas pleomorphic sarcomas have highly rearranged karyotypes. Recent sequencing studies have provided more detailed insights into the biology of different subtypes and reveal an intermediate group of sarcomas carrying recurrent events in the context of complex changes (Fig. 1).

Three major genomic studies in Ewing sarcoma (defined by *EWSR1* translocations to ETS transcription factors) have confirmed its low mutation burden. Notably, all three studies reported recurrent *STAG2* inactivation in about 20% of cases (7–9). *STAG2* encodes a subunit of the cohesin complex that is also involved in imprinting, chromatin insulation, and regulation of enhancer elements (10). Rhabdomyosarcomas were also reported to harbor a low mutation burden, in line with most pediatric cancers. In general, few mutations are observed in synovial sarcoma, 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 nonsynonymous 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

Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada.

Corresponding Author: Torsten O. Nielsen, Department of Anatomical Pathology, University of British Columbia, JP1502-855W 12th Avenue, Vancouver, BC, Canada V5Z 1M9. Phone: 604-875-411166768; Fax: 1-604-8754797; E-mail: torsten@mail.ubc.ca

doi: 10.1158/1078-0432.CCR-15-0831

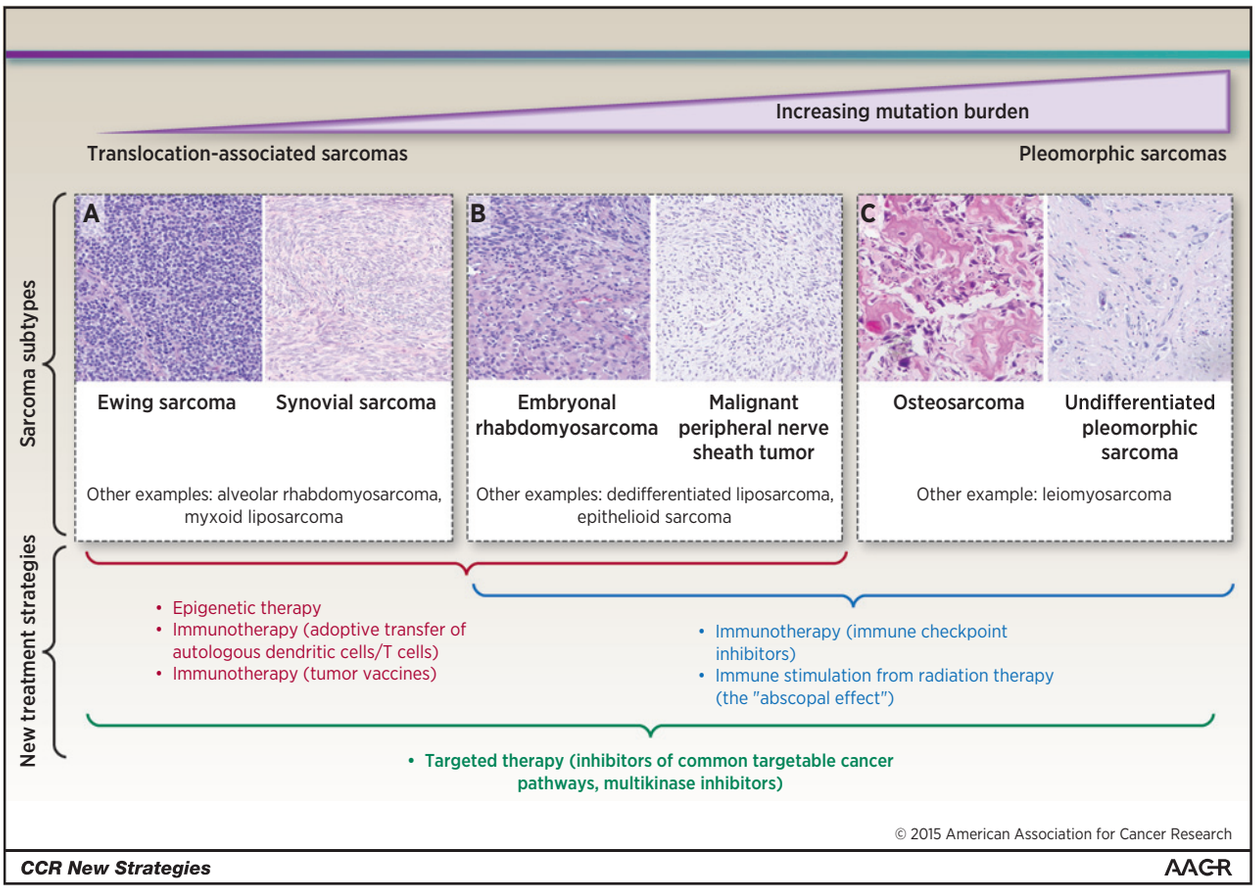

Figure 1.
Overview of major categories of sarcomas and potential treatments. Recent genomic studies confirm different mutational burdens across histologies. Targeted therapy against common cancer pathways remains one viable strategy relevant to all groups. A, translocation-associated sarcomas have low mutation burden, with underlying fusion oncoproteins that alter epigenetic regulation. In the absence of direct inhibitors against fusion oncoproteins, targeting epigenetic cofactors may be a rational strategy. Immune stimulation to help the immune system recognize tumor-associated antigens represents another strategy. B, some sarcoma subtypes have moderate-to-high mutation burdens on top of recurrent, potentially targetable mutations. C, many types of pleomorphic sarcomas in adults, as well as osteosarcoma in pediatric patients, have high mutation burdens but lack consistent targetable underlying events. In these subtypes, checkpoint inhibition and combinations with conventional and pathway-targeted therapy may be the most rational strategy.

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* fusion–positive/negative distinction in terms of single-nucleotide 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 sensitivity and specificity, 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 explain the overlap of heterogeneous mesenchymal differentiation patterns observed in MPNST and synovial sarcoma. Besides PRC2 dysregulation, recurrent *BCOR* mutations were found in some fusion-negative rhabdomyosarcoma (21). BCOR is a chromatin modifier that interacts with class I and II histone deacetylases (HDAC) as a transcriptional corepressor.

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,

Table 1. Survey of epigenetic drug trials in cancer and immunotherapy trials in sarcoma

NCT number	Agent	Other agent	Inclusion	Phase	Start	End	Enrollment
EZH2 inhibitor trials							
NCT02082977	GSK2816126		Diffuse large B-cell lymphoma	I	2014	2017	100
NCT01897571	E7438		Diffuse large B-cell lymphoma	I/II	2013	2015	154
NCT02395601	CPI-1205		B-cell lymphoma	I	2015	2016	41
Bromodomain inhibitor trials							
NCT02259114	OTX015		Advanced solid tumors	I	2014	2016	98
NCT02296476	OTX015		Glioblastoma multiforme	I/II	2014	2016	51
NCT02303782	OTX015	Azacitidine	Acute myeloid leukemia	I/II	2015	2016	88
NCT01949883	CPI-0610		Progressive lymphoma	I	2013	2015	33
NCT02157636	CPI-0610		Multiple myeloma	I	2014	2015	36
NCT02158858	CPI-0610		Leukemias	I	2014	2015	36
NCT02419417	BSM-986158	Paclitaxel	Advanced solid tumors	I/II	2015	2020	185
NCT01587703	GSK525762		NUT midline carcinoma	I	2012	2018	90
NCT02391480	ABBV-075		Advanced cancer	I	2015	2017	78
NCT02308761	TEN-010		AML and myelodysplastic syndrome	I	2014	2017	68
NCT01987362	TEN-010		Advanced solid tumors	I	2013	2016	66
NCT02369029	BAY1238097		Neoplasms	I	2015	2017	140
Immunotherapy trials in sarcoma							
NCT01643278	Ipilimumab	Dasatinib	GIST, advanced sarcoma	I	2012	2015	39
NCT02210104	Ipilimumab	Cyclophosphamide, CD4 T cells	NY-ESO–expressing, advanced sarcoma	I	2015	2018	12
NCT02406781	Pembrolizumab	Cyclophosphamide (metronomic)	Advanced sarcoma	II	2015	2017	163
NCT02304458	Nivolumab	(±) Ipilimumab	Recurrent childhood sarcomas	I/II	2015	2016	204
NCT02428192	Nivolumab		Advanced uterine LMS	II	2015	2016	37
NCT02301039	Pembrolizumab		Advanced sarcomas	II	2015	2018	80
NCT01241162	Decitabine	Dendritic cell (lysate pulsed)	Relapsed pediatric sarcoma, neuroblastoma	I	2010	2015	15
NCT01522820	DEC-205/NY-ESO-1 fusion	Sirolimus	Advanced, NY-ESO-1–expressing	I	2012	2016	18
NCT02387125	LV305 (viral NY-ESO-1 expression)	NY-ESO-1 vaccine	Synovial sarcoma, myxoid liposarcoma	IB	2015	2017	33
NCT01291420	Dendritic cells (transfected)		Recurrent sarcoma (limited disease)	I/II	2011	2014	10
NCT01343043	NY-ESO-1 T cells		Synovial sarcoma	I	2011	2016	10
NCT02319824	NY-ESO-1 T cells	Radiation therapy	NY-ESO-1–expressing sarcomas	I	2015	2016	12
NCT02059850	NY-ESO-1 T cells	Cyclophosphamide	Synovial sarcoma, myxoid liposarcoma	I	2014	2016	12
NCT02387125	CMB305		Metastatic, NY-ESO-1–expressing	I	2015	2017	33
NCT02457650	NY-ESO-1 T cells	Cyclophosphamide; fludarabine	NY-ESO-1–expressing cancers	I	2015	2019	36
NCT02122861	ID-LV305		Advanced, NY-ESO-1–expressing	I	2014	2016	42
NCT01883518	Dendritic cell vaccine (cancer-testis antigens)		Sarcoma	I/II	2013	2015	48

Abbreviations: AML, acute myeloid leukemia; GIST, gastrointestinal stromal tumors; LMS, leiomyosarcoma.

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 neoantigen 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 efferocytosis 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 MHCI 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 contributing to depletion of regulatory T cells and activation of effector T cells (65, 66). Thus, relations between emerging immunotherapies, epigenetic drugs, tyrosine kinase inhibitors, and cytotoxic agents will be complex, requiring further animal model studies and clinical trials to determine the best combinatorial approaches.

Sarcoma treatment: current and future prospects

The paradigm of success for sarcoma targeted therapy was the 2002 approval of imatinib (and later, sunitinib) for gastrointestinal stromal tumors. The newest FDA-approved agent added to the armamentarium for a sarcoma indication (in 2012) was pazopanib, a multikinase angiogenesis inhibitor indicated for nonadipocytic advanced soft-tissue sarcoma. In angiosarcoma, the finding of recurrent mutations in two angiogenesis genes (*PTPRB* and *PLCG1*) highlights a redundancy in angiogenesis driver mutations (67) that may explain the mixed results seen in clinical trials of antiangiogenesis agents in vascular sarcomas. The simpler genomes of translocation-associated sarcomas present fewer targetable mutations, and their driver fusion oncogenes usually encode transcription factors that are not targeted directly with available agents. A potential solution is to assess the dependence of particular sarcomas on more common targetable cancer pathways. In this regard, one recent development is the identification of aberrant Wnt/β-catenin signaling in synovial sarcoma models (68) and rhabdomyosarcoma (69). Combination targeted therapy again may have merit: a phase II trial of sorafenib and everolimus combination therapy achieved 45% 6-month progression-free survival (PFS; ref. 70). Although falling just short of its prespecified primary endpoint (50% 6-month PFS), this result is well-above the widely accepted 20% 6-month PFS benchmark for sarcoma drug activity (71) and represents the first positive study for relapsed osteosarcoma in a quarter century.

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 logistically 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.

Disclosure of Potential Conflicts of Interest

T.O. Nielsen reports receiving a commercial research grant from and is a consultant/advisory board member for NanoString Technologies. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: J. Lim, T.O. Nielsen
Writing, review, and/or revision of the manuscript: J. Lim, N.M. Poulin, T.O. Nielsen
Study supervision: T.O. Nielsen

Grant Support

T.O. Nielsen was supported by grants from the Canadian Cancer Society Research Institute (Impact Grant; grant number 701582), the Terry Fox Research Institute, and the Liddy Shriver Sarcoma Initiative.

Received June 9, 2015; revised July 23, 2015; accepted August 3, 2015; published OnlineFirst September 1, 2015.

References

1. Young RJ, Natukunda A, Litière S, Woll PJ, Wardelmann E, van der Graaf WT. First-line anthracycline-based chemotherapy for angiosarcoma and other soft tissue sarcoma subtypes: pooled analysis of eleven European Organisation for Research and Treatment of Cancer Soft Tissue and Bone Sarcoma Group trials. Eur J Cancer 2014;50:3178–86.
2. Pappo AS, Vassal G, Crowley JJ, Bolejack V, Hogendoorn PC, Chugh R, et al. A phase 2 trial of R1507, a monoclonal antibody to the insulin-like growth factor-1 receptor (IGF-1R), in patients with recurrent or refractory rhabdomyosarcoma, osteosarcoma, synovial sarcoma, and other soft tissue sarcomas: results of a Sarcoma Alliance for Research Through Collaboration study. Cancer 2014;120:2448–56.
3. Wagner LM, Fouladi M, Ahmed A, Krailo MD, Weigel B, DuBois SG, et al. Phase II study of cixutumumab in combination with temsirolimus in pediatric patients and young adults with recurrent or refractory sarcoma: a report from the Children's Oncology Group. Pediatr Blood Cancer 2015;62:440–4.
4. D' Angelo SP, Mahoney MR, Van Tine BA, Adkins DR, Perdekamp MT, Condy MM, et al. Alliance A091103 a phase II study of the angiopoietin 1 and 2 peptibody trebananib for the treatment of angiosarcoma. Cancer Chemother Pharmacol 2015;75:629–38.
5. Chawla SP, Cranmer LD, Van Tine BA, Reed DR, Okuno SH, Butrynski JE, et al. Phase II study of the safety and antitumor activity of the hypoxia-activated prodrug TH-302 in combination with doxorubicin in patients with advanced soft tissue sarcoma. J Clin Oncol 2014;32:3299–306.
6. Eberst L, Cropet C, Le Cesne A, Pautier P, Penel N, Adenis A, et al. The off-label use of targeted therapies in sarcomas: the OUTC'S program. BMC Cancer 2014;14:870.
7. Brohl AS, Solomon DA, Chang W, Wang J, Song Y, Sindiri S, et al. The genomic landscape of the Ewing Sarcoma family of tumors reveals recurrent STAG2 mutation. PLoS Genet 2014;10:e1004475.
8. Crompton BD, Stewart C, Taylor-Weiner A, Alexe G, Kurek KC, Calicchio ML, et al. The genomic landscape of pediatric Ewing sarcoma. Cancer Discov 2014;4:1326–41.
9. Tirode F, Surdez D, Ma X, Parker M, Le Deley MC, Bahrami A, et al.; St. Jude Children's Research Hospital–Washington University Pediatric Cancer Genome Project and the International Cancer Genome Consortium. Genomic landscape of Ewing sarcoma defines an aggressive subtype with co-association of STAG2 and TP53 mutations. Cancer Discov 2014;4:1342–53.
10. Xiao T, Wallace J, Felsenfeld G. Specific sites in the C terminus of CTCF interact with the SA2 subunit of the cohesin complex and are required for cohesin-dependent insulation activity. Mol Cell Biol 2011;31:2174–83.
11. Lagarde P, Przybyl J, Brulard C, Pérot G, Pierron G, Delattre O, et al. Chromosome instability accounts for reverse metastatic outcomes of pediatric and adult synovial sarcomas. J Clin Oncol 2013;31:608–15.
12. Chen X, Bahrami A, Pappo A, Easton J, Dalton J, Hedlund E, et al.; St. Jude Children's Research Hospital–Washington University Pediatric Cancer Genome Project. Recurrent somatic structural variations contribute to tumorigenesis in pediatric osteosarcoma. Cell Rep 2014;7:104–12.
13. Perry JA, Kiezun A, Tonzi P, Van Allen EM, Carter SL, Baca SC, et al. Complementary genomic approaches highlight the PI3K/mTOR pathway as a common vulnerability in osteosarcoma. Proc Natl Acad Sci U S A 2014;111:E5564–73.
14. Joseph CG, Hwang H, Jiao Y, Wood LD, Kinde I, Wu J, et al. Exomic analysis of myxoid liposarcomas, synovial sarcomas, and osteosarcomas. Genes Chromosomes Cancer 2014;53:15–24.
15. Chen X, Stewart E, Shelat AA, Qu C, Bahrami A, Hatley M, et al; St. Jude Children's Research Hospital–Washington University Pediatric Cancer Genome Project. Targeting oxidative stress in embryonal rhabdomyosarcoma. Cancer Cell 2013;24:710–24.
16. Silveira SM, Villacis RA, Marchi FA, Barros Filho Mde C, Drigo SA, Neto CS, et al. Genomic signatures predict poor outcome in undifferentiated pleomorphic sarcomas and leiomyosarcomas. PLoS One 2013;8:e67643.
17. Riggi N, Knoechel B, Gillespie SM, Rheinbay E, Boulay G, Suvà ML, et al. EWS-FLI1 utilizes divergent chromatin remodeling mechanisms to directly activate or repress enhancer elements in Ewing sarcoma. Cancer Cell 2014;26:668–81.
18. Su L, Sampaio AV, Jones KB, Pacheco M, Goytain A, Lin S, et al. Deconstruction of the SS18-SSX fusion oncoprotein complex: insights into disease etiology and therapeutics. Cancer Cell 2012;21:333–47.
19. Parham DM, Barr FG. Classification of rhabdomyosarcoma and its molecular basis. Adv Anat Pathol 2013;20:387–97.
20. Williamson D, Missiaglia E, de Reyniès A, Pierron G, Thuille B, Palenzuela G, et al. Fusion gene-negative alveolar rhabdomyosarcoma is clinically and molecularly indistinguishable from embryonal rhabdomyosarcoma. J Clin Oncol 2010;28:2151–8.
21. Shern JF, Chen L, Chmielecki J, Wei JS, Patidar R, Rosenberg M, et al. Comprehensive genomic analysis of rhabdomyosarcoma reveals a landscape of alterations affecting a common genetic axis in fusion-positive and fusion-negative tumors. Cancer Discov 2014;4:216–31.
22. Lynn M, Shah N, Conroy J, Ennis S, Morris T, Betts D, et al. A study of alveolar rhabdomyosarcoma copy number alterations by single nucleotide polymorphism analysis. Diagn Mol Pathol 2014;22:213–21.

23. Skapek SX, Anderson J, Barr FG, Bridge JA, Gastier-Foster JM, Parham DM, et al. PAX-FOXO1 fusion status drives unfavorable outcome for children with rhabdomyosarcoma: a children's oncology group report. Pediatr Blood Cancer 2013;60:1411–7.
24. Missiaglia E, Williamson D, Chisholm J, Wirapati P, Pierron G, Petel F, et al. PAX3/FOXO1 fusion gene status is the key prognostic molecular marker in rhabdomyosarcoma and significantly improves current risk stratification. J Clin Oncol 2012;30:1670–7.
25. Agaram NP, Chen CL, Zhang L, LaQuaglia MP, Wexler L, Antonescu CR. Recurrent MYOD1 mutations in pediatric and adult sclerosing and spindle cell rhabdomyosarcomas: evidence for a common pathogenesis. Genes Chromosomes Cancer 2014;53:779–87.
26. Szuhai K, de Jong D, Leung WY, Fletcher CD, Hogendoorn PC. Transactivating mutation of the MYOD1 gene is a frequent event in adult spindle cell rhabdomyosarcoma. J Pathol 2014;232:300–7.
27. Kohsaka S, Shukla N, Ameur N, Ito T, Ng CK, Wang L, et al. A recurrent neomorphic mutation in MYOD1 defines a clinically aggressive subset of embryonal rhabdomyosarcoma associated with PI3K-AKT pathway mutations. Nat Genet 2014;46:595–600.
28. Puls F, Niblett A, Marland G, Gaston CL, Douis H, Mangham DC, et al. BCOR-CCNB3 (Ewing-like) sarcoma: a clinicopathologic analysis of 10 cases, in comparison with conventional Ewing sarcoma. Am J Surg Pathol 2014;38:1307–18.
29. Cohen-Gogo S, Cellier C, Coindre JM, Mosseri V, Pierron G, Guillemet C, et al. Ewing-like sarcomas with BCOR-CCNB3 fusion transcript: a clinical, radiological and pathological retrospective study from the Societe Francaise des Cancers de L'Enfant. Pediatr Blood Cancer 2014; 61:2191–8.
30. Peters TL, Kumar V, Polikepahad S, Lin FY, Sarabia SF, Liang Y, et al. BCOR-CCNB3 fusions are frequent in undifferentiated sarcomas of male children. Mod Pathol 2015;28:575–86.
31. Specht K, Sung YS, Zhang L, Richter GH, Fletcher CD, Antonescu CR. Distinct transcriptional signature and immunoprofile of CIC-DUX4 fusion-positive round cell tumors compared to EWSR1-rearranged Ewing sarcomas: further evidence toward distinct pathologic entities. Genes Chromosomes Cancer 2014;53:622–33.
32. Smith SC, Buehler D, Choi EY, McHugh JB, Rubin BP, Billings SD, et al. CIC-DUX sarcomas demonstrate frequent MYC amplification and ETS-family transcription factor expression. Mod Pathol 2015;28:57–68.
33. Sugita S, Arai Y, Tonooka A, Hama N, Totoki Y, Fujii T, et al. A novel CIC-FOXO4 gene fusion in undifferentiated small round cell sarcoma: a genetically distinct variant of Ewing-like sarcoma. Am J Surg Pathol 2014; 38:1571–6.
34. Solomon DA, Brohl AS, Khan J, Miettinen M. Clinicopathologic features of a second patient with Ewing-like sarcoma harboring CIC-FOXO4 gene fusion. Am J Surg Pathol 2014;38:1724–5.
35. Lira ME, Kim TM, Huang D, Deng S, Koh Y, Jang B, et al. Multiplexed gene expression and fusion transcript analysis to detect ALK fusions in lung cancer. J Mol Diagn 2013;15:51–61.
36. Zheng Z, Liebers M, Zhelyazkova B, Cao Y, Panditi D, Lynch KD, et al. Anchored multiplex PCR for targeted next-generation sequencing. Nat Med 2014;20:1479–84.
37. Kadoch C, Crabtree GR. Reversible disruption of mSWI/SNF (BAF) complexes by the SS18-SSX oncogenic fusion in synovial sarcoma. Cell 2013;153:71–85.
38. Ciarapica R, De Salvo M, Carcarino E, Bracaglia G, Adesso L, Leoncini PP, et al. The Polycomb group (PcG) protein EZH2 supports the survival of PAX3-FOXO1 alveolar rhabdomyosarcoma by repressing FBXO32 (Atrogin1/MAFbx). Oncogene 2014;33:4173–84.
39. Zhang M, Wang Y, Jones S, Sausen M, McMahon K, Sharma R, et al. Somatic mutations of SUZ12 in malignant peripheral nerve sheath tumors. Nat Genet 2014;46:1170–2.
40. Lee W, Teckie S, Wiesner T, Ran L, Prieto Granada CN, Lin M, et al. PRC2 is recurrently inactivated through EED or SUZ12 loss in malignant peripheral nerve sheath tumors. Nat Genet 2014;46:1227–32.
41. Cassier PA, Lefranc A, Amela EY, Chevreau C, Bui BN, Lecesne A, et al. A phase II trial of panobinostat in patients with advanced pretreated soft tissue sarcoma. A study from the French Sarcoma Group. Br J Cancer 2013;109:909–14.
42. Chu QS, Nielsen TO, Alcindor T, Gupta A, Endo M, Goytain A, et al. A phase II study of SB939, a novel pan-histone deacetylase inhibitor, in patients with translocation-associated recurrent/metastatic sarcomas-NCIC-CTG IND 200dagger. Ann Oncol 2015;26:973–81.
43. Fritzsching B, Fellenberg J, Moskovszky L, Sápi Z, Krenacs T, Machado I, et al. CD8/FOXP3-ratio in osteosarcoma microenvironment separates survivors from non-survivors: a multicenter validated retrospective study. Oncoimmunology 2015;4:e990800.
44. Fujii H, Arakawa A, Utsumi D, Sumiyoshi S, Yamamoto Y, Kitoh A, et al. CD8(+) tumor-infiltrating lymphocytes at primary sites as a possible prognostic factor of cutaneous angiosarcoma. Int J Cancer 2014;134: 2393–402.
45. Hemminger JA, Toland AE, Scharschmidt TJ, Mayerson JL, Guttridge DC, Iwenofu OH. Expression of cancer-testis antigens MAGEA1, MAGEA3, ACRBP, PRAME, SSX2, and CTAG2 in myxoid and round cell liposarcoma. Mod Pathol 2014;27:1238–45.
46. Endo M, de Graaff MA, Ingram DR, Lim S, Lev DC, Briaire-de Bruijn IH, et al. NY-ESO-1 (CTAG1B) expression in mesenchymal tumors. Mod Pathol 2015;28:587–95.
47. Lu L, Yan H, Shyam-Sundar V, Janowitz T. Cross-sectional and longitudinal analysis of cancer vaccination trials registered on the US Clinical Trials Database demonstrates paucity of immunological trial endpoints and decline in registration since 2008. Drug Des Devel Ther 2014;8:1539–53.
48. Robbins PF, Kassim SH, Tran TL, Crystal JS, Morgan RA, Feldman SA, et al. A pilot trial using lymphocytes genetically engineered with an NY-ESO-1-reactive T-cell receptor: long-term follow-up and correlates with response. Clin Cancer Res 2015;21:1019–27.
49. Jing W, Gershan JA, Weber J, Tlomak D, McOlash L, Sabatos-Peyton C, et al. Combined immune checkpoint protein blockade and low dose whole body irradiation as immunotherapy for myeloma. J Immunother Cancer 2015;3:2.
50. Shin DS, Ribas A. The evolution of checkpoint blockade as a cancer therapy: what's here, what's next? Curr Opin Immunol 2015;33:23–35.
51. Lussier DM, Johnson JL, Hingorani P, Blattman JN. Combination immunotherapy with alpha-CTLA-4 and alpha-PD-L1 antibody blockade prevents immune escape and leads to complete control of metastatic osteosarcoma. J Immunother Cancer 2015;3:21.
52. Maki RG, Jungbluth AA, Gnjatic S, Schwartz GK, D'Adamo DR, Keohan ML, et al. A pilot study of anti-CTLA4 antibody ipilimumab in patients with synovial sarcoma. Sarcoma 2013;2013:168145.
53. Komohara Y, Morita T, Annan DA, Horlad H, Ohnishi K, Yamada S, et al. The coordinated actions of TIM-3 on cancer and myeloid cells in the regulation of tumorigenicity and clinical prognosis in clear cell renal cell carcinomas. Cancer Immunol Res 2015;3:999–1007.
54. Taube JM, Young GD, McMiller TL, Chen S, Salas JT, Pritchard TS, et al. Differential expression of immune-regulatory genes associated with PD-L1 display in melanoma: implications for PD-1 pathway blockade. Clin Cancer Res 2015;21:3969–76.
55. Tran Janco JM, Lamichhane P, Karyampudi L, Knutson KL. Tumor-infiltrating dendritic cells in cancer pathogenesis. J Immunol 2015;194:2985–91.
56. Caronni N, Savino B, Bonecchi R. Myeloid cells in cancer-related inflammation. Immunobiology 2015;220:249–53.
57. Lee CH, Espinosa I, Vrijaldenhoven S, Subramanian S, Montgomery KD, Zhu S, et al. Prognostic significance of macrophage infiltration in leiomyosarcomas. Clin Cancer Res 2008;14:1423–30.
58. Nabeshima A, Matsumoto Y, Fukushi J, Iura K, Matsunobu T, Endo M, et al. Tumour-associated macrophages correlate with poor prognosis in myxoid liposarcoma and promote cell motility and invasion via the HB-EGF-EGFR-PI3K/Akt pathways. Br J Cancer 2015;112:547–55.
59. Kepp O, Senovilla L, Vitale I, Vacchelli E, Adjemian S, Agostinis P. Consensus guidelines for the detection of immunogenic cell death. Oncoimmunology 2014;3:e955691.
60. Kitagawa D, Yokota K, Gouda M, Narumi Y, Ohmoto H, Nishiwaki E, et al. Activity-based kinase profiling of approved tyrosine kinase inhibitors. Genes Cells 2013;18:110–22.
61. Dehlin M, Andersson S, Erlandsson M, Brisslert M, Bokarewa M. Inhibition of fms-like tyrosine kinase 3 alleviates experimental arthritis by reducing formation of dendritic cells and antigen presentation. J Leukoc Biol 2011;90:811–7.
62. Rosborough BR, Mathews LR, Matta BM, Liu Q, Raïch-Regué D, Thomson AW, et al. Cutting edge: Flt3 ligand mediates STAT3-independent expansion but STAT3-dependent activation of myeloid-derived suppressor cells. J Immunol 2014;192:3470–3.

63. Rivera LB, Meyronet D, Hervieu V, Frederick MJ, Bergsland E, Bergers G. Intratumoral myeloid cells regulate responsiveness and resistance to anti-angiogenic therapy. Cell Rep 2015;11:577–91.
64. Kroesen M, Gielen P, Brok IC, Armandari I, Hoogerbrugge PM, Adema GJ. HDAC inhibitors and immunotherapy; a double edged sword? Oncotarget 2014;5:6558–72.
65. DuPage M, Chopra G, Quiros J, Rosenthal WL, Morar MM, Holohan D, et al. The chromatin-modifying enzyme Ezh2 is critical for the maintenance of regulatory T cell identity after activation. Immunity 2015;42:227–38.
66. Tumes DJ, Onodera A, Suzuki A, Shinoda K, Endo Y, Iwamura C, et al. The polycomb protein Ezh2 regulates differentiation and plasticity of CD4(+) T helper type 1 and type 2 cells. Immunity 2013;39:819–32.
67. Behjati S, Tarpey PS, Sheldon H, Martincorena I, Van Loo P, Gundem G, et al. Recurrent PTPRB and PLCG1 mutations in angiosarcoma. Nat Genet 2014;46:376–9.
68. Barham W, Frump AL, Sherrill TP, Garcia CB, Saito-Diaz K, VanSaun MN, et al. Targeting the Wnt pathway in synovial sarcoma models. Cancer Discov 2013;3:1286–301.
69. Chen EY, DeRan MT, Ignatius MS, Grandinetti KB, Clagg R, McCarthy KM, et al. Glycogen synthase kinase 3 inhibitors induce the canonical WNT/beta-catenin pathway to suppress growth and self-renewal in embryonal rhabdomyosarcoma. Proc Natl Acad Sci U S A 2014;111:5349–54.
70. Grignani G, Palmerini E, Ferraresi V, D'Ambrosio L, Bertulli R, Asaftei SD, et al. Sorafenib and everolimus for patients with unresectable high-grade osteosarcoma progressing after standard treatment: a non-randomised phase 2 clinical trial. Lancet Oncol 2015;16:98–107.
71. Van Glabbeke M, Verweij J, Judson I, Nielsen OS EORTC Soft Tissue and Bone Sarcoma Group. Progression-free rate as the principal end-point for phase II trials in soft-tissue sarcomas. Eur J Cancer 2002;38:543–9.
72. Choy E, Butrynski JE, Harmon DC, Morgan JA, George S, Wagner AJ, et al. Phase II study of olaparib in patients with refractory Ewing sarcoma following failure of standard chemotherapy. BMC Cancer 2014;14:813.
73. Willyard C. 'Basket studies' will hold intricate data for cancer drug approvals. Nat Med 2013;19:655.