Exploring the sarcoma immune landscape

Oost, S. van

1

General introduction

Sarcomas are mesenchymal tumors of uncertain cell-of-origin that can arise anywhere in the body. They are very rare and account for only 1% of all adult cancers (1). According to the World Health Organization, there are currently more than 75 malignant sarcoma subtypes, which originate in soft tissues, such as fat, muscle or blood vessels, or in bone and cartilage. Among these subtypes, some are relatively more common (e.g. chondrosarcoma), while others are considered ultra-rare (e.g. adamantinoma), with incidence rates ≤ 1 per 1.000.000 per year (2). For research purposes, sarcomas are commonly classified into two categories according to their genomic architecture: sarcomas with simple genomes or sarcomas with complex genomes. Sarcomas with simple genomes are driven by specific, recurrent genetic alterations, typically involving single gene fusions or mutations. Examples include myxoid liposarcoma with the *FUS::DDIT3* fusion (3) and alveolar soft part sarcoma (ASPS) with the *ASPSCR1::TFE3* fusion (4). Conversely, sarcomas with complex genomes are characterized by genomic instability, involving large-scale genetic alterations such as gene amplifications, deletions and chromosomal rearrangements. This genomic complexity can vary substantially, even within the same histological subtype. Such variability contributes to differences in tumor behavior and treatment response, thereby complicating the clinical management of these subtypes (5). Examples of sarcomas with complex genomes include undifferentiated pleomorphic sarcoma (UPS), myxofibrosarcoma (MFS), and osteosarcoma. The treatment of sarcomas depends on the specific subtype, but typically involves surgery, chemotherapy, or radiotherapy. Depending on the tumor's anatomical location and aggressiveness, a combination of these modalities may also be used (6, 7). Nevertheless, despite continued refinement of multidisciplinary treatment strategies, only modest improvements in clinical outcomes have been achieved over the last decade, as disease recurrence remains common and many sarcomas exhibit intrinsic or acquired resistance to conventional systemic therapies. These challenges underscore the urgent need for more effective and innovative therapeutic approaches.

The link between the immune system and cancer has long been recognized, however, only in the past two decades has the molecular basis of anti-tumor immunity been elucidated in detail. The immune system can detect and target cancer cells by identifying molecules that distinguish malignant from healthy cells. Two major classes of such molecules, collectively referred to as antigens, can elicit anti-tumor immune responses: tumor-specific antigens and tumor-associated antigens (8, 9). Tumor-specific antigens, also known as neoantigens, arise from somatic mutations that generate novel peptide sequences. As these antigens are not present in normal tissues, they are recognized by the

immune system as “non-self” and are therefore often highly immunogenic. In contrast, tumor-associated antigens are derived from normal-self proteins that are aberrantly expressed, overexpressed, or expressed in inappropriate contexts within tumor cells. Examples include oncofetal proteins such as carcinoembryonic antigen and cancer-testis antigens such as NY-ESO-1, which are normally expressed only during embryonic development or in germline tissues such as the testis. Because these tissues lack human leukocyte antigen (HLA) expression and are not surveyed by the immune system, lymphocytes recognizing these self-antigens are not eliminated during immune maturation. Consequently, when these antigens are re-expressed in tumors, they tend to induce relatively weak immune responses due to central immune tolerance.

Tumor cells present antigens on HLA class I molecules, enabling recognition and elimination by cytotoxic $CD8^+$ T cells (**Figure 1A**) (10). In addition, tumor antigens released during cell death can be taken up by dendritic cells, which process and cross-present them via HLA class I to activate cytotoxic T cells (11), or present them via HLA class II molecules to $CD4^+$ helper T cells, which in turn promote cytotoxic T cell activation through cytokine secretion (**Figure 1A**) (12). However, many tumors evade immune recognition by downregulating components of the antigen presentation machinery (**Figure 1B**) (13), recruiting immunosuppressive cell types (14), or upregulating inhibitory immune checkpoints like programmed death-ligand 1 (PD-L1) (15). These mechanisms collectively suppress T cell activation and effector function, allowing tumors to escape immune-mediated elimination and providing the rationale for immune checkpoint blockade, which aims to restore and enhance anti-tumor immunity by releasing inhibitory constraints on T cell function.

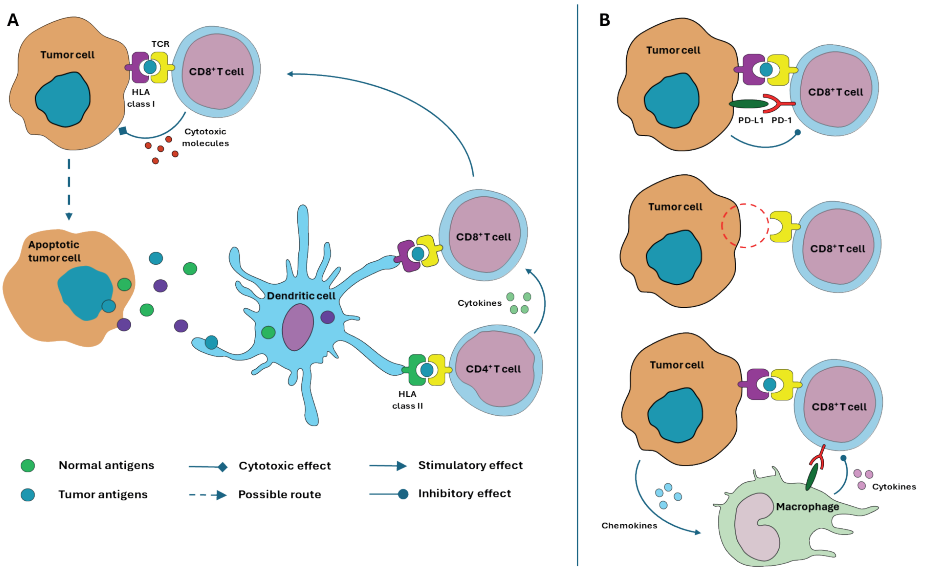

Figure 1. Schematic representation of T cell-mediated tumor recognition and mechanisms of tumor immune evasion. A) $CD8^+$ T cells recognize tumor cells through their T cell receptor (TCR) binding to neoantigens presented on HLA class I molecules. This interaction activates cytotoxic effector functions, leading to tumor cell killing. Following cell death, either via T cell-mediated killing or other mechanisms, tumor-derived antigens are released and captured by dendritic cells, which can (cross-)present them to T cells. When these antigens are tumor-associated, T cells are activated, which either triggers or enhances the anti-tumor immune response. **B**) Tumor cells have various mechanisms to evade T cell-mediated killing. They can upregulate inhibitory ligands such as PD-L1, which binds PD-1 on activated T cells and suppresses effector functions. Alternatively, tumor cells can downregulate antigen presentation machinery to escape immune recognition. In addition, tumor cells secrete chemokines that recruit immunosuppressive (myeloid) cells, which further inhibit T cell activity through immunosuppressive cytokines or inhibitory ligands.

THE CANCER IMMUNOTHERAPY REVOLUTION

Over the last decade, immune checkpoint inhibitors targeting inhibitory molecules like programmed cell death protein 1 (PD-1), PD-L1, and cytotoxic T-lymphocyte associated protein 4 (CTLA-4), have transformed the treatment landscape of several cancers, producing durable responses in melanoma (16) and non–small cell lung cancer (17). The remarkable efficacy of mono- or dual-checkpoint blockade in these tumors is largely attributed to their high mutational burden (18, 19), which enhances tumor antigenicity, and to their robust pre-existing anti-tumor immune response (20, 21). However, these features vary considerably between cancer types and even among patients

with the same tumor type, limiting the overall effectiveness of checkpoint blockade (22, 23).

In contrast, sarcomas have generally been considered poorly immunogenic, owing to their relatively low mutational burden (24) and lack of other predictive biomarkers such as PD-L1 expression or mismatch repair deficiency (25, 26). Consequently, the initial wave of immunotherapy trials yielded limited efficacy across most sarcoma subtypes. Nevertheless, objective responses have been observed in certain histologies. Notably, ASPS has emerged as the striking exception, becoming the first sarcoma subtype for which an immune checkpoint inhibitor, atezolizumab, has received FDA approval (27-29). Intriguingly, the mechanisms underlying the pronounced sensitivity of ASPS to checkpoint blockade remain poorly understood. Additional evidence of sensitivity has been observed in chordoma (30, 31), UPS (32-34), MFS (33, 35-37), and dedifferentiated liposarcoma (DDLPS) (32, 34, 38) (**Table 1**). However, the strength of evidence varies substantially among subtypes, with data for MFS, in particular, being largely limited to case reports or clinical trials enrolling fewer than 5 patients.

Table 1. Overview of clinical trials with checkpoint blockade, either as monotherapy or as a combination therapy. Only trials with ≥ 5 patients for that subtype are presented. ORR = objective response rate (as measured by RECIST); ASPS = alveolar soft part sarcoma; DDLPS = dedifferentiated liposarcoma; UPS = undifferentiated pleomorphic sarcoma; CT = chemotherapy; RT = radiotherapy; TLS = tertiary lymphoid structure.

Subtype	ORR	Drugs	Clinical trial	Reference
ASPS	37%	Atezolizumab	NCT03141684	(28)
	54%	Pembrolizumab + axitinib	NCT02636725	(27)
	82%	TQB2450 + anlotinib	CTR20190938	(29)
Chordoma	12%	Pembrolizumab	NCT03012620	(31)
	20%	Durvalumab + tremelimumab	NCT02815995	(30)
DDLPS	10%	Pembrolizumab	NCT02301039	(32) – after expansion
	42%	Pembrolizumab + CT	NCT02406781	(38) – TLS-positive
	12%	Nivolumab + RT	NCT03307616	(34) – neoadjuvant
	0%	Nivolumab + ipilimumab + RT	“”	“”
UPS	23%	Pembrolizumab	NCT02301039	(32) – after expansion
	33%	Nivolumab + ipilimumab	NCT02500797	(33)
	17%	Nivolumab + RT	NCT03307616	(34) – neoadjuvant
	25%	Nivolumab + ipilimumab + RT	“”	“”

While monotherapy shows rather limited efficacy in unselected sarcoma populations, combining checkpoint blockade with radiotherapy, chemotherapy, or antiangiogenic agents seem to enhance anti-tumor immunity and thereby improve clinical responses (**Table 1**). More recently, neoadjuvant or perioperative checkpoint blockade has produced remarkable clinical

outcomes in colorectal, gastric and gastroesophageal junction cancers (39, 40), underscoring the potential of optimizing immunotherapeutic strategies in traditionally less immunogenic cancers. Encouragingly, this concept has begun to translate into sarcoma, with the first trials combining neoadjuvant/perioperative checkpoint blockade and radiotherapy reporting promising early results in UPS (34, 41). Many clinical trials are currently underway and may soon clarify whether immunotherapy could become a mainstay in sarcoma treatment. Immunotherapeutic strategies such as tumor vaccines or chimeric antigen receptor-T cell therapy are further discussed in **Chapter 2**.

UNRAVELING THE SARCOMA IMMUNE MICROENVIRONMENT

Although the precise mechanisms underlying sarcoma sensitivity to checkpoint blockade remain elusive, the tumor immune microenvironment clearly plays a key role. In recent years, advances in molecular profiling, ranging from bulk sequencing to single-cell and spatially resolved approaches, have dramatically improved our insight into sarcoma immunity. Early studies using immunohistochemistry described the immune infiltration in various sarcoma subtypes (42-45), enabling the classification of tumors into inflamed/immune "hot", immune-excluded, or immune desert/immune "cold" phenotypes, a framework first established in carcinomas according to T cell infiltration patterns (46). In sarcoma, tumors often fall into the immune-excluded category, especially bone sarcomas due to their dense extracellular matrix, or the immune desert group with minimal T cell infiltration. Only a small subset shows an inflamed phenotype. **Chapter 2** dives further into this framework in sarcomas and how it associates with their genomic architecture.

Bulk RNA sequencing studies further refined these phenotypes by using computational deconvolution to estimate the relative composition of the immune microenvironment. A pivotal study classified soft tissue sarcomas (STS) into five immune classes (SIC A-E), representing a spectrum from poorly to highly immunogenic (47). The highly immunogenic group (SIC E) was enriched for B cells and tertiary lymphoid structures (TLS), which are clearly defined clusters of T cells, B cells, and myeloid cells, often associated with anti-tumor immunity (48). Intriguingly, SIC E was retrospectively associated with checkpoint blockade response in the SARC028 trial, and validated in the PEMBROSARC trial, where selecting TLS-positive patients increased response rates and established TLS as a predictive biomarker in sarcoma (**Table 1**) (38).

Although the presence of TLS enhances sensitivity to checkpoint blockade, they remain uncommon across sarcoma subtypes (present in 12% of the overall STS population in the Petitprez study (47); up to 75% in well-differentiated/DDLPS, and around 22% in UPS in the PEMBROSARC trial (38)). Moreover, the presence of TLS alone does not fully explain the variability in checkpoint blockade response, as roughly 40% of TLS-positive patients achieved improved 6-month progression-free survival (38). This suggests that additional immunological processes underlying effective anti-tumor immunity may complement TLS as predictive markers. In this context, the Immunologic Constant of Rejection (ICR) signature, a 20 gene signature reflecting type 1 T (Th1) cell signaling, offers an additional framework for assessing immune activation across tumor types (49). The ICR signature has been shown to stratify tumors into immune-active and immune-silent phenotypes (50, 51), including in STS cohorts, and to correlate with clinical response to checkpoint blockade (52). Although bulk transcriptomic profiling is powerful for analyzing large cohorts, it provides limited insights into immune and stromal cell composition or cellular heterogeneity. Technologies like single-cell and single-nucleus RNA sequencing overcome these limitations and have provided high-resolution profiling of the tumor microenvironment across diverse sarcoma subtypes, including chordoma (53), osteosarcoma (54), Ewing sarcoma (55), synovial sarcoma (56), UPS (57), and rhabdomyosarcoma (58). These studies have revealed subtype-specific patterns of immune infiltration, stromal composition, and mechanisms of immune evasion.

While single-cell transcriptomic analyses offer invaluable molecular insights, they lack spatial information, which limits our understanding of how cells interact and organize within tissues. Spatially resolved transcriptomic approaches address this gap by preserving tissue context and enabling detailed mapping of cellular neighborhoods, immune niches, and stromal organization. GeoMx digital spatial profiling enables compartmentalized transcriptomic profiling across regions of interest, whereas Visium provides high-resolution mapping across entire tissue sections. Using these approaches, spatially distinct patterns of inflammation have been identified in angiosarcoma (59, 60) and osteosarcoma (61). Complementary proteomic technologies such as imaging mass cytometry (IMC) and high-dimensional multiplex immunofluorescence enable subcellular-resolution quantification of approximately 40 and 60 proteins, respectively, providing detailed insights into cellular phenotypes, interactions, and spatial organization within the tumor microenvironment. For example, spatial mapping of primary osteosarcomas revealed that immunosuppressive macrophage niches correlate with metastasis-free survival (62), whereas in lung metastatic lesions, the spatial distance between effector

immune and immunosuppressive cells was associated with overall survival (63). Similarly, mass spectrometry imaging provides spatially resolved lipid and metabolite profiles and has, for instance, revealed tumor-promoting metabolic features in macrophages in malignant peripheral nerve sheath tumors (64).

Building on these insights, single-cell spatial transcriptomic platforms such as CosMx and Xenium in situ now allow gene expression to be mapped at true single-cell resolution directly onto tissue sections. By combining in-depth cellular behavior with precise spatial localization, these technologies can reveal cellular neighborhoods, immune-stromal interactions, and the spatial distribution specific cellular states, bridging the gap between single-cell transcriptional states and tissue architecture. Although still very new, the first application of this technology in sarcomas has already been reported, identifying distinct mechanisms of resistance to checkpoint blockade in Ewing sarcoma using samples from the SARC028 trial (65).

Together, these approaches have generated key insights into sarcoma immunobiology. The research presented in this thesis builds on this foundation, applying state-of-the-art bulk, single-cell, and spatial technologies to explore the immune landscape of sarcomas known to respond, albeit variably, to checkpoint blockade.

UNDIFFERENTIATED PLEOMORPHIC SARCOMA AND MYXOFIBROSARCOMA

UPS and MFS are two subtypes of STS that predominantly arise in the extremities and trunk (1). Both are defined by extensive chromosomal alterations, few recurrent mutations (66, 67), and a lack of lineage-specific differentiation markers. Despite these shared features, they present with distinct clinical and histological characteristics (**Figure 2A+B**). MFS is a spectrum of disease, ranging from low- to high-grade tumors, and can develop either superficially or deeper-seated (1). Low-grade tumors are hypocellular with abundant myxoid matrix, whereas high-grade lesions display increased cellularity, nuclear pleomorphism, and reduced myxoid stroma. In contrast, UPS presents almost exclusively as deep-seated, intermediate- to high-grade tumors. Histologically, UPS is characterized by a dense mesh of highly pleomorphic spindle and giant cells.

The current standard-of-care for UPS and high-grade MFS consists of surgery accompanied with neoadjuvant radiotherapy (7). And although this is effective for local disease control, a substantial proportion of patients still develop

metastases (68), underscoring the necessity for more effective systemic therapies. From an immunological perspective, UPS has emerged as one of the more responsive sarcoma subtypes to T cell checkpoint blockade, while responses in MFS have also been observed, though evidence remains more limited. Because of their shared genomic complexity and lack of differentiation, UPS and MFS are often grouped together in translational research and clinical trials. However, whether they truly share the same immunogenic landscape, or instead harbor meaningful differences that shape therapeutic response, remains unresolved. Exploring these similarities and divergences is therefore a critical step toward optimizing immunotherapeutic strategies in these aggressive sarcomas.

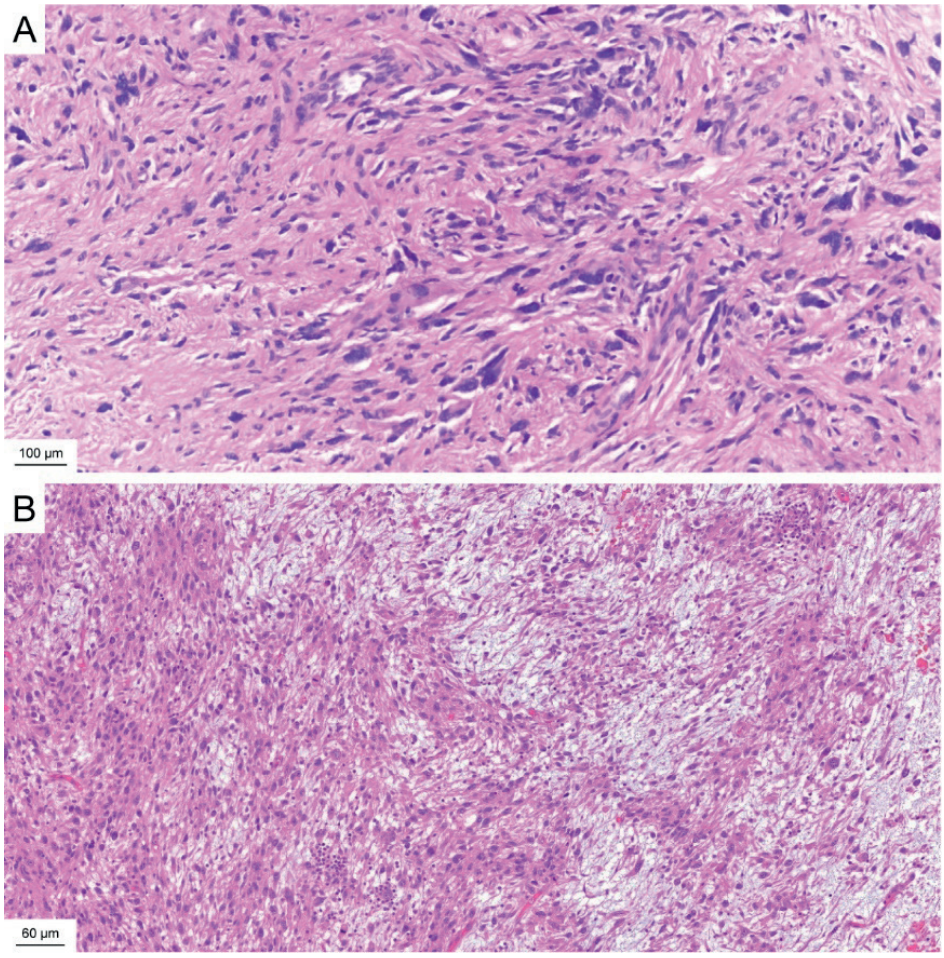

Figure 2. Histological images of UPS and MFS. A) UPS showing pleomorphism. **B**) High-grade MFS showing areas with myxoid matrix.

CHORDOMA

Chordomas are ultra-rare bone sarcomas showing notochordal differentiation that predominantly arise in the axial skeleton, including the skull base, mobile spine, and sacrococcygeal region (1). They express the notochordal transcription factor brachyury, encoded by the *TBXT* gene, which drives their distinctive biology and confers both epithelial and mesenchymal characteristics (69). Genetically, chordomas are relatively stable and exhibit a low mutational burden, though chromosomal alterations can occur, with *CDKN2A/B* loss being the most frequent (70, 71). Histologically, chordomas are typically organized in lobules separated by fibrous septa (1), often containing a myxoid or chondroid matrix (**Figure 3A**), and the tumor cells stain positive for brachyury (**Figure 3B**). The hallmark physaliphorous cells have large vacuolated cytoplasm giving them a "bubbly" appearance (**Figure 3C**). Nuclear pleomorphism is generally uncommon, observed only in a subset of tumors. Conventional chordomas may also present with a chondroid pattern, characterized by a hyaline-cartilage like matrix resembling that of chondrosarcomas, a feature seen more frequently at the skull base. Rare, more aggressive subtypes include poorly differentiated chordoma, marked by *SMARCB1* (INI1) loss with retention of brachyury expression, and dedifferentiated chordoma, which contains high-grade sarcomatous areas lacking brachyury expression, typically adjacent to conventional chordoma areas.

Standard-of-care treatment aims for *en bloc* surgical resection whenever feasible, often complemented by (proton beam) radiotherapy (72). However, complete resection is often difficult due to the tumor's proximity to critical neurovascular structures, contributing to high rates of local recurrence (73). In addition, conventional chemotherapy has minimal efficacy because of the indolent nature of chordomas (74), highlighting the urgent need for improved therapeutic strategies. Intriguingly, chordomas have demonstrated responsiveness to T cell checkpoint blockade despite their low mutational burden and abundant extracellular matrix. This underscores the importance of characterizing their immune microenvironment to uncover mechanisms that may explain their sensitivity to immunotherapy.

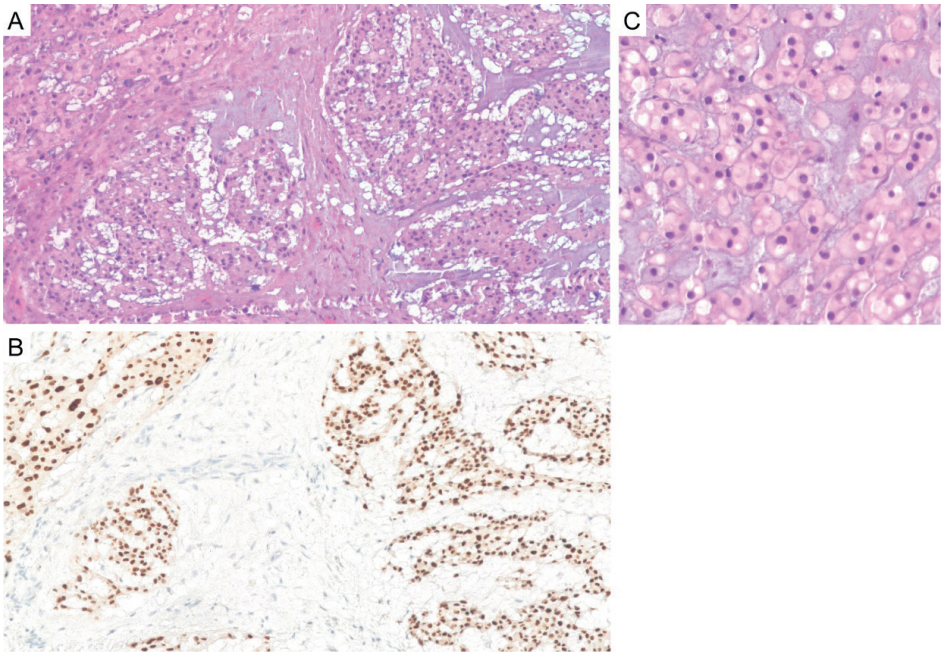

Figure 3. Histological images of chordoma. A) H&E image of a chordoma, rich in myxoid matrix, with some fibrous septations. **B**) Immunohistochemistry for brachyury, the diagnostic marker for chordoma (*TBXT*). **C**) H&E image of physaliphorous cells in a myxoid matrix.

OUTLINE OF THIS THESIS

The work presented in this thesis aims to gain insight into the sarcoma immune landscape across subtypes with reported, yet heterogeneous, sensitivity to checkpoint blockade, with the goal of informing patient stratification for rational immunotherapeutic combination strategies.

Building on recent advances in cancer immunotherapy, **Chapter 2** provides a conceptual overview of genomic complexity and its association with sarcoma immunogenicity. It explores how features of the immune microenvironment relate to genomic architecture and responsiveness to checkpoint blockade, thereby establishing a framework for the marked heterogeneity of immunotherapy responses across sarcoma subtypes.

Chapter 3 investigates two genomically complex STS subtypes — UPS and MFS — characterizing the immune microenvironment of pre-treatment samples using a combination of bulk RNA sequencing and IMC. T cells and macrophages are further analyzed in an extended cohort and associated with clinical outcome. Additionally, the impact of neoadjuvant radiotherapy on the

tumor microenvironment is assessed by comparing pre-treatment biopsies with post-treatment resections through IMC.

In contrast, chordoma — an intriguing sarcoma subtype — exhibits responses to checkpoint blockade despite its relatively low genomic complexity. **Chapter 4** provides the first large-scale characterization of the chordoma immune microenvironment, integrating bulk RNA sequencing, bulk T cell receptor sequencing, IMC and multiplex immunofluorescence to define distinct immune contextures. **Chapter 5** builds directly on these findings by applying spatial transcriptomics alongside lipidomic and metabolomic profiling to the same tumors; analyses of sequential tissue sections enable integration of tumor- and stroma-specific transcriptional profiles with spatially resolved lipid and metabolite data.

Finally, **Chapter 6** synthesizes the overall findings, discusses their biological and clinical implications, and outlines perspectives for future research and potential therapeutic strategies.

REFERENCES

1. WHO Classification of Tumours Editorial Board. Soft Tissue and Bone Tumours. WHO Classification of Tumours. 5 ed. Lyon, France: IARC Publications; 2020.
2. Stacchiotti S, Frezza AM, Blay JY, Baldini EH, Bonvalot S, Bovee J, et al. Ultra-rare sarcomas: A consensus paper from the Connective Tissue Oncology Society community of experts on the incidence threshold and the list of entities. Cancer. 2021;127(16):2934-42.
3. Crozat A, Aman P, Mandahl N, Ron D. Fusion of CHOP to a novel RNA-binding protein in human myxoid liposarcoma. Nature. 1993;363(6430):640-4.
4. Ladanyi M, Lui MY, Antonescu CR, Krause-Boehm A, Meindl A, Argani P, et al. The der(17)t(X;17)(p11;q25) of human alveolar soft part sarcoma fuses the TFE3 transcription factor gene to ASPL, a novel gene at 17q25. Oncogene. 2001;20(1):48-57.
5. Soupir A, Ospina OE, Hampton O, Churchman M, Radmacher M, Hedges D, et al. Genomic, transcriptomic, and immunogenomic landscape of over 1300 sarcomas of diverse histology subtypes. Nat Commun. 2025;16(1):4206.
6. Strauss SJ, Frezza AM, Abecassis N, Bajpai J, Bauer S, Biagini R, et al. Bone sarcomas: ESMO-EURACAN-GENTURIS-ERN PaedCan Clinical Practice Guideline for diagnosis, treatment and follow-up. Ann Oncol. 2021;32(12):1520-36.
7. Gronchi A, Miah AB, Dei Tos AP, Abecassis N, Bajpai J, Bauer S, et al. Soft tissue and visceral sarcomas: ESMO-EURACAN-GENTURIS Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2021;32(11):1348-65.
8. Peri A, Salomon N, Wolf Y, Kreiter S, Diken M, Samuels Y. The landscape of T cell antigens for cancer immunotherapy. Nat Cancer. 2023;4(7):937-54.
9. Shah S, Al-Omari A, Cook KW, Paston SJ, Durrant LG, Brentville VA. What do cancer-specific T cells 'see'? Discov Immunol. 2023;2(1):kyac011.
10. Yewdell JW, Reits E, Neefjes J. Making sense of mass destruction: quantitating MHC class I antigen presentation. Nat Rev Immunol. 2003;3(12):952-61.
11. Joffre OP, Segura E, Savina A, Amigorena S. Cross-presentation by dendritic cells. Nat Rev Immunol. 2012;12(8):557-69.
12. den Haan JM, Arens R, van Zelm MC. The activation of the adaptive immune system: cross-talk between antigen-presenting cells, T cells and B cells. Immunol Lett. 2014;162(2 Pt B):103-12.
13. Jhunjhunwala S, Hammer C, Delamarre L. Antigen presentation in cancer: insights into tumour immunogenicity and immune evasion. Nat Rev Cancer. 2021;21(5):298-312.
14. Lasser SA, Ozbay Kurt FG, Arkhypov I, Utikal J, Umansky V. Myeloid-derived suppressor cells in cancer and cancer therapy. Nat Rev Clin Oncol. 2024;21(2):147-64.
15. Lin X, Kang K, Chen P, Zeng Z, Li G, Xiong W, et al. Regulatory mechanisms of PD-1/PD-L1 in cancers. Mol Cancer. 2024;23(1):108.
16. Wolchok JD, Chiarion-Sileni V, Rutkowski P, Cowey CL, Schadendorf D, Wagstaff J, et al. Final, 10-Year Outcomes with Nivolumab plus Ipilimumab in Advanced Melanoma. N Engl J Med. 2025;392(1):11-22.

17. Borghaei H, Gettinger S, Vokes EE, Chow LQM, Burgio MA, de Castro Carpeno J, et al. Five-Year Outcomes From the Randomized, Phase III Trials CheckMate 017 and 057: Nivolumab Versus Docetaxel in Previously Treated Non-Small-Cell Lung Cancer. J Clin Oncol. 2021;39(7):723-33.

18. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science. 2015;348(6230):124-8.

19. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med. 2014;371(23):2189-99.

20. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014;515(7528):568-71.

21. Shien K, Papadimitrakopoulou VA, Wistuba, II. Predictive biomarkers of response to PD-1/PD-L1 immune checkpoint inhibitors in non-small cell lung cancer. Lung Cancer. 2016;99:79-87.

22. Lee M, Samstein RM, Valero C, Chan TA, Morris LGT. Tumor mutational burden as a predictive biomarker for checkpoint inhibitor immunotherapy. Hum Vaccin Immunother. 2020;16(1):112-5.

23. Roelands J, Kuppen PJK, Ahmed EI, Mall R, Masoodi T, Singh P, et al. An integrated tumor, immune and microbiome atlas of colon cancer. Nat Med. 2023;29(5):1273-86.

24. Chalmers ZR, Connelly CF, Fabrizio D, Gay L, Ali SM, Ennis R, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Med. 2017;9(1):34.

25. Lam SW, Kostine M, de Miranda N, Schoffski P, Lee CJ, Morreau H, et al. Mismatch repair deficiency is rare in bone and soft tissue tumors. Histopathology. 2021.

26. D'Angelo SP, Shoushtari AN, Agaram NP, Kuk D, Qin LX, Carvajal RD, et al. Prevalence of tumor-infiltrating lymphocytes and PD-L1 expression in the soft tissue sarcoma microenvironment. Hum Pathol. 2015;46(3):357-65.

27. Wilky BA, Trucco MM, Subhawong TK, Florou V, Park W, Kwon D, et al. Axitinib plus pembrolizumab in patients with advanced sarcomas including alveolar soft-part sarcoma: a single-centre, single-arm, phase 2 trial. Lancet Oncol. 2019;20(6):837-48.

28. Chen AP, Sharon E, O'Sullivan-Coyne G, Moore N, Foster JC, Hu JS, et al. Atezolizumab for Advanced Alveolar Soft Part Sarcoma. N Engl J Med. 2023;389(10):911-21.

29. Tan Z, Wu Y, Fan Z, Gao T, Guo W, Bai C, et al. Anlotinib plus TQB2450, a PD-L1 Antibody, in Patients with Advanced Alveolar Soft Part Sarcoma: A Single-Arm, Phase II Trial. Clin Cancer Res. 2024;30(24):5577-83.

30. Somaiah N, Conley AP, Parra ER, Lin H, Amini B, Solis Soto L, et al. Durvalumab plus tremelimumab in advanced or metastatic soft tissue and bone sarcomas: a single-centre phase 2 trial. Lancet Oncol. 2022;23(9):1156-66.

31. Blay JY, Chevret S, Le Cesne A, Brahmi M, Penel N, Cousin S, et al. Pembrolizumab in patients with rare and ultra-rare sarcomas (AcSe Pembrolizumab): analysis of a subgroup from a non-randomised, open-label, phase 2, basket trial. Lancet Oncol. 2023.

32. Tawbi HA, Burgess M, Bolejack V, Van Tine BA, Schuetze SM, Hu J, et al. Pembrolizumab in advanced soft-tissue sarcoma and bone sarcoma (SARC028): a multicentre, two-cohort, single-arm, open-label, phase 2 trial. Lancet Oncol. 2017;18(11):1493-501.

33. D'Angelo SP, Mahoney MR, Van Tine BA, Atkins J, Milhem MM, Jahagirdar BN, et al. Nivolumab with or without ipilimumab treatment for metastatic sarcoma (Alliance A091401): two open-label, non-comparative, randomised, phase 2 trials. Lancet Oncol. 2018;19(3):416-26.

34. Roland CL, Nassif Haddad EF, Keung EZ, Wang WL, Lazar AJ, Lin H, et al. A randomized, non-comparative phase 2 study of neoadjuvant immune-checkpoint blockade in retroperitoneal dedifferentiated liposarcoma and extremity/truncal undifferentiated pleomorphic sarcoma. Nat Cancer. 2024.

35. Kelly CM, Antonescu CR, Bowler T, Munhoz R, Chi P, Dickson MA, et al. Objective Response Rate Among Patients With Locally Advanced or Metastatic Sarcoma Treated With Talimogene Laherparepvec in Combination With Pembrolizumab: A Phase 2 Clinical Trial. JAMA Oncol. 2020;6(3):402-8.

36. Zhou M, Bui N, Lohman M, van de Rjin M, Hwang G, Ganjoo K. Long-Term Remission with Ipilimumab/Nivolumab in Two Patients with Different Soft Tissue Sarcoma Subtypes and No PD-L1 Expression. Case Rep Oncol. 2021;14(1):459-65.

37. Kalofonou F, Napolitano A, Benson C, Miah A, Zaidi S, Lindsay D, et al. The efficacy of combination immunotherapy with ipilimumab plus nivolumab in metastatic myxofibrosarcoma. Rare Tumors. 2024;16:20363613241290788.

38. Italiano A, Bessede A, Pulido M, Bompas E, Piperno-Neumann S, Chevreau C, et al. Pembrolizumab in soft-tissue sarcomas with tertiary lymphoid structures: a phase 2 PEMBROSARC trial cohort. Nat Med. 2022;28(6):1199-206.

39. Chalabi M, Fanchi LF, Dijkstra KK, Van den Berg JG, Aalbers AG, Sikorska K, et al. Neoadjuvant immunotherapy leads to pathological responses in MMR-proficient and MMR-deficient early-stage colon cancers. Nat Med. 2020;26(4):566-76.

40. Verschoor YL, van de Haar J, van den Berg JG, van Sandick JW, Kodach LL, van Dieren JM, et al. Neoadjuvant atezolizumab plus chemotherapy in gastric and gastroesophageal junction adenocarcinoma: the phase 2 PANDA trial. Nat Med. 2024;30(2):519-30.

41. Mowery YM, Ballman KV, Hong AM, Schuetze SM, Wagner AJ, Monga V, et al. Safety and efficacy of pembrolizumab, radiation therapy, and surgery versus radiation therapy and surgery for stage III soft tissue sarcoma of the extremity (SU2C-SARC032): an open-label, randomised clinical trial. Lancet. 2024;404(10467):2053-64.

42. Ogose A, Kawashima H, Hotta T, Ariizumi T, Yamagishi T, Oike N, et al. Frequent expression of human leukocyte antigen class I and the status of intratumoral immune cells in alveolar soft part sarcoma. Oncol Lett. 2017;13(4):2169-76.

43. Pollack SM, He Q, Yearley JH, Emerson R, Vignali M, Zhang Y, et al. T-cell infiltration and clonality correlate with programmed cell death protein 1 and programmed death-ligand 1 expression in patients with soft tissue sarcomas. Cancer. 2017;123(17):3291-304.

44. Dancsok AR, Gao D, Lee AF, Steigen SE, Blay JY, Thomas DM, et al. Tumor-associated macrophages and macrophage-related immune checkpoint expression in sarcomas. Oncoimmunology. 2020;9(1):1747340.

45. Zou MX, Pan Y, Huang W, Zhang TL, Escobar D, Wang XB, et al. A four-factor immune risk score signature predicts the clinical outcome of patients with spinal chordoma. Clin Transl Med. 2020;10(1):224-37.

46. Galon J, Bruni D. Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. Nat Rev Drug Discov. 2019;18(3):197-218.

47. Petitprez F, de Reynies A, Keung EZ, Chen TW, Sun CM, Calderaro J, et al. B cells are associated with survival and immunotherapy response in sarcoma. Nature. 2020;577(7791):556-60.

48. Fridman WH, Meylan M, Petitprez F, Sun CM, Italiano A, Sautes-Fridman C. B cells and tertiary lymphoid structures as determinants of tumour immune contexture and clinical outcome. Nat Rev Clin Oncol. 2022;19(7):441-57.

49. Bertucci F, Finetti P, Simeone I, Hendrickx W, Wang E, Marincola FM, et al. The immunologic constant of rejection classification refines the prognostic value of conventional prognostic signatures in breast cancer. Br J Cancer. 2018;119(11):1383-91.

50. Roelands J, Hendrickx W, Zoppoli G, Mall R, Saad M, Halliwill K, et al. Oncogenic states dictate the prognostic and predictive connotations of intratumoral immune response. J Immunother Cancer. 2020;8(1).

51. Bertucci F, Niziers V, de Nonneville A, Finetti P, Mescam L, Mir O, et al. Immunologic constant of rejection signature is prognostic in soft-tissue sarcoma and refines the CINSARC signature. J Immunother Cancer. 2022;10(1).

52. Mogenet A, Finetti P, Denicolai E, Greillier L, Boudou-Rouquette P, Goldwasser F, et al. Immunologic constant of rejection as a predictive biomarker of immune checkpoint inhibitors efficacy in non-small cell lung cancer. J Transl Med. 2023;21(1):637.

53. Duan W, Zhang B, Li X, Chen W, Jia S, Xin Z, et al. Single-cell transcriptome profiling reveals intra-tumoral heterogeneity in human chordomas. Cancer Immunol Immunother. 2022;71(9):2185-95.

54. Zhou Y, Yang D, Yang Q, Lv X, Huang W, Zhou Z, et al. Single-cell RNA landscape of intratumoral heterogeneity and immunosuppressive microenvironment in advanced osteosarcoma. Nat Commun. 2020;11(1):6322.

55. Visser LL, Bleijs M, Margaritis T, van de Wetering M, Holstege FCP, Clevers H. Ewing Sarcoma Single-cell Transcriptome Analysis Reveals Functionally Impaired Antigen-presenting Cells. Cancer Res Commun. 2023;3(10):2158-69.

56. Jerby-Arnon L, Neftel C, Shore ME, Weisman HR, Mathewson ND, McBride MJ, et al. Opposing immune and genetic mechanisms shape oncogenic programs in synovial sarcoma. Nat Med. 2021;27(2):289-300.

57. Luthria K, Shah P, Caldwell B, Melms JC, Abuzaid S, Jakubikova V, et al. Single-Cell Profiling of Sarcomas from Archival Tissue Reveals Programs Associated with Resistance to Immune Checkpoint Blockade. Clin Cancer Res. 2024;30(19):4530-41.

58. DeMartino J, Meister MT, Visser LL, Brok M, Groot Koerkamp MJA, Wezenaar AKL, et al. Single-cell transcriptomics reveals immune suppression and cell states predictive of patient outcomes in rhabdomyosarcoma. Nat Commun. 2023;14(1):3074.

59. Loh JW, Lee JY, Lim AH, Guan P, Lim BY, Kannan B, et al. Spatial transcriptomics reveal topological immune landscapes of Asian head and neck angiosarcoma. Commun Biol. 2023;6(1):461.

60. Ko TK, Guo Z, Kannan B, Lim BY, Lee JY, Li Z, et al. Multiomics characterization of breast angiosarcoma from an Asian cohort reveals enrichment for angiogenesis signaling pathway and tumor-infiltrating macrophages. Front Immunol. 2024;15:1515935.

61. Zheng X, Liu X, Zhang X, Zhao Z, Wu W, Yu S. A single-cell and spatially resolved atlas of human osteosarcomas. J Hematol Oncol. 2024;17(1):71.

62. Gyau BB, Wang J, Wu W, Scull B, Major AM, Jin W, et al. Multiplex Imaging Mass Cytometry Reveals Prognostic Immunosuppressive Subpopulations and Macrophage-Driven Metastasis in Osteosarcoma. Cancers (Basel). 2025;17(17).

63. Lacinski RA, Dziadowicz SA, Melemai VK, Fitzpatrick B, Pisquiy JJ, Heim T, et al. Spatial multiplexed immunofluorescence analysis reveals coordinated cellular networks associated with overall survival in metastatic osteosarcoma. Bone Res. 2024;12(1):55.

64. Ursem R, Groen JL, Malessy MJA, Briaire-de Bruijn I, McDonnell LA, Heijs B, et al. Spatial Lipidomics Reveals Myelin Defects and Protumor Macrophage Infiltration in Malignant Peripheral Nerve Sheath Tumor Adjacent Nerves. Lab Invest. 2025;105(1):102186.

65. Daley JD, Mukherjee E, Ferraro D, Bhaskar S, Green A, Meyer EM, et al. SARC028 samples reveal an interplay between TGFbeta, interferon signaling and low HLA class I expression as contributors to Ewing sarcoma checkpoint blockade resistance. Clin Cancer Res. 2025.

66. Cancer Genome Atlas Research Network. Comprehensive and Integrated Genomic Characterization of Adult Soft Tissue Sarcomas. Cell. 2017;171(4):950-65 e28.

67. Mitra S, Farswan A, Piccinelli P, Sydow S, Hesla A, Tsagkozis P, et al. Transcriptomic profiles of myxofibrosarcoma and undifferentiated pleomorphic sarcoma correlate with clinical and genomic features. J Pathol. 2024;264(3):293-304.

68. Yoshimoto M, Yamada Y, Ishihara S, Kohashi K, Toda Y, Ito Y, et al. Comparative Study of Myxofibrosarcoma With Undifferentiated Pleomorphic Sarcoma: Histopathologic and Clinicopathologic Review. Am J Surg Pathol. 2020;44(1):87-97.

69. Nelson AC, Pillay N, Henderson S, Presneau N, Tirabosco R, Halai D, et al. An integrated functional genomics approach identifies the regulatory network directed by brachyury (T) in chordoma. J Pathol. 2012;228(3):274-85.

70. Tarpey PS, Behjati S, Young MD, Martincorena I, Alexandrov LB, Farndon SJ, et al. The driver landscape of sporadic chordoma. Nature Communications. 2017;8.

71. Bai J, Shi J, Li C, Wang S, Zhang T, Hua X, et al. Whole genome sequencing of skull-base chordoma reveals genomic alterations associated with recurrence and chordoma-specific survival. Nat Commun. 2021;12(1):757.

72. Stacchiotti S, Gronchi A, Fossati P, Akiyama T, Alapetite C, Baumann M, et al. Best practices for the management of local-regional recurrent chordoma: a position paper by the Chordoma Global Consensus Group. Ann Oncol. 2017;28(6):1230-42.

73. Walcott BP, Nahed BV, Mohyeldin A, Coumans JV, Kahle KT, Ferreira MJ. Chordoma: current concepts, management, and future directions. Lancet Oncol. 2012;13(2):e69-76.

74. Colia V, Stacchiotti S. Medical treatment of advanced chordomas. Eur J Cancer. 2017;83:220-8.