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Exploring the sarcoma immune landscape

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General discussion & future perspectives

GENERAL DISCUSSION

Sarcomas comprise a diverse group of mesenchymal malignancies in which genomic complexity, immune contexture, and therapeutic responsiveness vary substantially across subtypes. The aim of this thesis was to gain insight into the sarcoma immune landscape to help guide patient stratification for immunotherapeutic or combination treatment strategies. A central observation emerging from this thesis is that the clinical relevance of immune infiltration differs markedly between sarcoma histologies. Across sarcoma subtypes with reported but heterogeneous sensitivity to checkpoint blockade — undifferentiated pleomorphic sarcoma (UPS), myxofibrosarcoma (MFS), and chordoma — this thesis demonstrates that similar degrees of immune infiltration can have divergent implications for prognosis and treatment responsiveness. These findings point to the need for subtype-specific and context-dependent frameworks that integrate immune features with genomic complexity, microenvironmental organization, and treatment-induced remodeling. **Chapter 2** establishes this foundation by linking patterns of immune infiltration to genomic complexity across sarcomas, providing the rationale for the subsequent subtype-specific and translational discussions.

Undifferentiated pleomorphic sarcoma and myxofibrosarcoma

A 2017 study from The Cancer Genome Atlas (TCGA) proposed that UPS and MFS may represent a biological continuum rather than distinct entities (1). In that analysis, no consistent subtype-specific differences were identified at the genomic, epigenetic, or transcriptional level, apart from the expression of myxoid matrix-associated genes in MFS with a more pronounced myxoid component. This molecular overlap, together with their shared historical classification under malignant fibrous histiocytoma (MFH), has led UPS and MFS to be frequently studied together. However, accumulating evidence indicates that they are biologically and clinically distinct (2-4), differing in histology, clinical presentation, recurrence patterns, and response to radiotherapy. **Chapter 3** adds to this evidence by demonstrating divergent associations between immune infiltration and clinical outcome, as well as subtype-specific microenvironmental responses to radiotherapy.

One key finding in Chapter 3 is that a pre-existing immune response in UPS, characterized by T cells and macrophages, is associated with improved metastasis-free survival. In contrast, despite a comparable degree of immune infiltration in MFS, this beneficial association is absent. This discrepancy suggests differences in immune infiltrate functionality (4), tumor specificity,

or spatial organization. Alternatively, the biology driving tumor aggressiveness in MFS may dominate metastatic progression, thereby offsetting any protective effect of the immune system. Consistent with this, the MFS microenvironment appears less responsive to radiotherapy than that of UPS, both histologically and immunologically. Following radiotherapy, UPS specimens exhibited extensive fibrosis/hyalinization, with clear reductions in myeloid cells, tumor and stromal cells, and vascular structures; these changes were accompanied by an increase in cytotoxic T cells, collectively indicating a coordinated, tissue-wide response. By comparison, MFS specimens post-radiotherapy remained largely viable with no significant changes in cellular composition, irrespective of tumor depth. These observations underscore differences in their immunobiology and radiotherapy response, likely reflecting distinct tumor-stroma-immune interactions.

Resistance to radiotherapy in MFS: beyond the myxoid extracellular matrix

The limited radiotherapy response of MFS raises questions about underlying microenvironmental features. One defining histological feature of MFS is its myxoid extracellular matrix — a structural component that differs substantially from UPS and may influence microenvironmental organization. TCGA analyses indicate that myxoid matrix-related gene signatures are enriched mainly in tumors with extensive myxoid areas (1). However, high-grade MFS typically displays a heterogeneous architecture, with intermixed myxoid and more cellular regions, such that matrix-rich areas do not dominate the tumor as a whole. For comparison, myxoid liposarcoma — another sarcoma subtype characterized by a prominent myxoid matrix — exhibits a strikingly different radiotherapy response (5): it is highly radiosensitive and typically demonstrates pronounced post-radiation changes, including hyalinization and, to a lesser extent, adipocytic maturation (6). Unlike the curvilinear and irregular vascular pattern characteristic of MFS, myxoid liposarcoma also features a distinctive branching capillary network. Together, these observations underscore that the presence of a myxoid matrix alone is insufficient to explain radioresistance and that vascular architecture and tumor-stroma interactions likely play an important role. Further work is needed to examine how stromal composition, vascular organization, and immune cell distribution together shape these distinct radiobiological behaviors. Recent efforts to map extracellular matrix signatures across soft tissue sarcoma (STS) subtypes provide a useful starting point for such analyses (7, 8).

Reconsidering treatment response assessment in the context of immunotherapy

As discussed previously, the clinical efficacy of immunotherapy in MFS remains less well defined, largely because most clinical trials to date have included fewer than five MFS patients. Encouragingly, several ongoing trials now include larger cohorts of both MFS and UPS, which will help clarify subtype-specific responses to checkpoint blockade (NCT04332874; NCT04480502; NCT06128863). The findings presented in Chapter 3 may therefore inform translational analyses within such studies, particularly those seeking to link immune features with treatment response. In this context, the concept of treatment responsiveness in STS warrants careful reconsideration. Tissue responses to therapy vary substantially between subtypes and treatment modalities (9, 10), and the histopathological criteria used to define response can strongly influence prognostic interpretation. For instance, in both UPS and MFS treated with neoadjuvant radiotherapy or combined regimens, response assessment based on viable tumor percentage has shown limited prognostic value (11). In contrast, hyalinization has emerged as a more informative marker of treatment effect (12, 13). More recently, a hyalinization-based threshold of 30% has been proposed as a potential marker of response in trials combining radiotherapy with checkpoint blockade (14, 15), although this approach remains preliminary and requires validation in independent cohorts.

The need for biomarkers of immunotherapy response

A natural follow-up question concerns which immune features are relevant for response to immune checkpoint blockade. Accordingly, increasing attention has focused on tertiary lymphoid structures (TLS) as a potential biomarker of response. Accumulating evidence indicates that TLS represent a context-dependent manifestation of anti-tumor immunity rather than a standalone predictive marker for checkpoint blockade (16, 17). Consistent with this view, TLS are generally associated with a favorable prognosis across STS (14, 18-20), reflecting coordinated and chronic immune activation; however, they frequently coexist with other immune features. Notably, intratumoral CD8⁺ T cell infiltration often accompanies TLS in immunologically active subtypes such as UPS and dedifferentiated liposarcoma (DDLPS), whereas other subtypes like rhabdomyosarcoma may lack broader immune activation despite the occasional presence of TLS (21). Consequently, the predictive value of TLS varies according to histology, immune composition, and treatment context.

Importantly, TLS are dynamic structures whose presence and functionality can be altered by therapy. For example, recent data show that DDLPS may develop TLS during checkpoint blockade, whereas UPS tends to lose them over the

course of treatment (14) — likely reflecting tissue remodeling in responding tumors, as also observed in Chapter 3. These findings highlight that TLS scoring is not static and may be influenced by therapy-induced changes in the tumor microenvironment. Moreover, the relationship between anti-tumor immunity and therapeutic outcomes extends beyond TLS alone: patterns of intratumoral T cell infiltration correlate with prognosis in both UPS and DDLPS treated with checkpoint blockade, even in the absence of well-defined TLS.

Frameworks that integrate immune organization with underlying tumor biology help reconcile these observations (17). In UPS, for instance, immune infiltration inversely correlates with genomic complexity, as tumors with a higher burden of copy-number alterations exhibit reduced immune infiltration (22). Sarcoma ecotypes further contextualize this relationship by distinguishing an inflamed ecotype with fewer genomic alterations from an intermediate-inflamed ecotype with greater genomic instability. Notably, tumors in the latter ecotype show a distinct association with response to checkpoint blockade despite comparable TLS prevalence to inflamed ecotype tumors. Collectively, these insights demonstrate that ecotypes capture functionally relevant immune states not fully reflected by TLS presence alone, offering a more nuanced framework for predicting immunotherapy response in STS.

From a translational perspective, digital pathology applied to H&E-stained resections enables scalable TLS detection and phenotypic characterization (23, 24), contributing to a refined understanding of TLS biology. However, since TLS are frequently underdetected in biopsies and may be modulated by treatment, surrogate markers of functional TLS and local immune activation are needed — particularly those assessable in pre-treatment biopsies or via non-invasive approaches. For instance, intratumoral plasma cells may serve as a proxy for active TLS (16, 20), while MRI-based radiomic signatures offer a promising non-invasive method to infer TLS presence (25). Together, these strategies support integrated biomarker frameworks in which TLS are interpreted alongside histology, immune organization, and genomic context to guide patient stratification and therapeutic decision-making in STS.

Chordoma

Since it has become apparent that chordomas can respond to checkpoint blockade, interest in the immunogenomic characterization of this ultra-rare bone sarcoma has surged. Unlike genomically complex STS such as UPS and MFS, chordomas exhibit relative genomic stability, despite occasional chromosomal alterations. This genomic profile raises an intriguing question:

why do approximately 16% of chordomas respond to checkpoint blockade (26, 27)? **Chapter 4** addressed this by providing the first large-scale characterization of the chordoma immune microenvironment. This analysis revealed that, compared to other sarcomas, chordomas proved relatively immunologically active, with a subset displaying coordinated immune responses — marked by T cells co-localizing with antigen-presenting cells in the stromal compartment. These inflamed tumors also showed T cell receptor (TCR) clonal enrichment; however, levels indicative of antigen-driven responses were uncommon. This aligns with chordomas' low mutational burden and consequent scarcity of T cell-recognizable neoantigens, which may explain why most tumors retain intact antigen-presentation machinery, reflecting limited selective pressure from cytotoxic T cells. Collectively, these findings reveal a paradox: low genomic complexity alongside discernible immune responses, highlighting the need to uncover mechanisms driving chordoma immunogenicity.

Building on these insights, **Chapter 5** applied integrated spatial transcriptomic and metabolomic profiling to a subset of chordomas from Chapter 4, reinforcing the inflamed/non-inflamed dichotomy. As anticipated, inflamed tumors had higher densities of immune and stromal cells, with comparable tumor cell densities across groups. Notably, tumor cells in inflamed cases showed broader transcriptional repertoires — including higher expression of chordoma-associated and immune-related genes — alongside increased glycogen accumulation. These tumors also exhibited distinct metabolic and lipid adaptations consistent with oxidative stress, suggesting transcriptional and metabolic remodeling as hallmarks of chordoma immunogenicity. This prompts the question: do such adaptations reflect intrinsic tumor cell properties, microenvironmental induction, or reciprocal tumor-immune feedback? To probe this, we investigated whether interferon- γ (IFN- γ) — a key immunomodulatory cytokine — could be a driver of this transcriptional state in inflamed tumors. However, IFN- γ did not regulate *TBXT* (brachyury) expression, implicating alternative mechanisms like other cytokine signals, genomic alterations, or epigenetic regulation in the upregulation of *TBXT* and its transcriptional programs.

TBXT as a determinant of immunogenicity

In light of the association between immune responses and *TBXT*-associated transcriptional programs observed in Chapter 5, the potential contribution of *TBXT* to tumor immunogenicity warrants closer investigation. Two key questions arise: first, whether *TBXT* itself functions as an antigenic target; and second, whether its transcriptional programs actively shape the broader immune

landscape of chordomas. Addressing the first, a recent immunopeptidomic study demonstrated that *TBXT*-derived peptides are naturally presented on HLA class I molecules in most chordoma patients (28), rendering *TBXT* accessible to immune recognition. However, functional reactivity assays would be needed to confirm whether these peptides elicit robust, patient-specific T cell responses; such assays could employ *TBXT* peptides presented by autologous antigen-presenting cells to stimulate tumor-infiltrating lymphocytes.

Turning to the second question, prior work has shown that *TBXT* regulates interferon- α response networks in chordoma cells (29). Given that interferon signaling modulates antigen presentation and cytokine production, these *TBXT*-associated programs may actively shape the tumor-immune interface. Whether *TBXT* further influences immunogenicity through mechanisms such as chemokine secretion or cellular stress responses remains unclear. Elucidating these pathways will be essential to defining *TBXT*'s precise role in chordoma immunogenicity.

Associations between immunity and clinical outcome

The clinical significance of the immune microenvironment in chordoma remains unresolved. In the extended cohort analyzed in Chapter 4, T cell infiltration showed no association with disease-specific survival or recurrence-free survival. This finding aligns with other published studies (30, 31), which similarly reported no prognostic effect of immune infiltration. Interestingly, both studies observed that chromosomal instability or a higher burden of copy-number alterations correlated with worse prognosis and lower immune infiltration. These patterns suggest an indirect relationship whereby genomic complexity — rather than immune infiltration itself — may drive adverse outcomes. A potential mechanistic explanation emerged from a recent proteogenomic study (31), which showed that loss of 9p and 10q may reduce immune infiltration by decreasing expression of immune-related genes encoded on these chromosomal arms. Specifically, 9p loss was associated with reduced IL-6/JAK/STAT signaling, whereas 10q loss was linked to decreased NF- κ B signaling and diminished interferon- α responses.

However, other reports have described negative associations between immune activity and clinical outcome (32-34), highlighting substantial heterogeneity across studies and underscoring the complexity of interpreting the prognostic relevance of immunity in chordoma. This heterogeneity may stem from differences in immunobiology, the extent and nature of genomic instability, clinical management, or a combination of these factors. Anatomical location

represents another potential source of variability, as cohorts are often restricted to chordomas from a single anatomical site. Yet, results from Chapter 4 — together with a recent multimodal study that included chordomas from multiple anatomical locations (35) — suggest that anatomical site alone is unlikely to explain the inconsistent prognostic effects.

In this context, the complexity and heterogeneity of chordoma treatment, together with patient comorbidities and anatomical constraints that complicate surgery and radiotherapy, may obscure any contribution of natural anti-tumor immunity. Moreover, immune responses in chordoma may lack the robustness needed for effective tumor control, owing to limited antigenicity, immunosuppressive mechanisms within the tumor microenvironment, restricted intratumoral infiltration, or infiltration that reflects local inflammation rather than coordinated anti-tumor activity. Despite these challenges, the identification of shared and expanded TCR clonotypes between tumor tissue and peripheral blood supports systemic, antigen-driven T cell responses in a subset of patients (35), indicating that clinically meaningful immunity may occur even without broad prognostic value. Overall, the limited prognostic value of immune infiltration in chordoma likely reflects biologically modest immune responses combined with clinical factors that mask their effects, rather than a true absence of immunogenic potential.

Several strategies could help disentangle these clinical and biological factors. For instance, analyzing pre-treatment biopsies from patients receiving exclusively proton beam radiotherapy — an increasingly common definitive treatment for clival chordomas and a standard local-control option for sacrococcygeal tumors in older patients (36-38) — would minimize treatment-related confounders and enable a cleaner assessment of baseline immunity and its association with clinical outcome. Pairing such analyses with prospective FDG-PET imaging could further clarify whether metabolic features correlate with immune activity, building on the metabolic adaptations observed in Chapter 5.

FUTURE PERSPECTIVES

Building on the findings presented in this thesis, future research in sarcoma immunobiology should focus on identifying immune features that are functionally relevant and therapeutically actionable. In particular, the divergent prognostic implications of immune infiltration across UPS, MFS, and

chordoma underscore the need to define how immune functionality and spatial organization interact with genomic complexity to influence clinical relevance.

Over the past decade, bulk, single-cell, and spatial profiling have mapped the immunogenomic landscape of sarcomas with increasing resolution. These efforts have defined baseline immune features and identified immunologically active subgroups. However, without well-annotated treatment cohorts, clinical validation of these immune states remains limited. Integrative analyses of samples from patients in immunotherapy trials — directly linking immune features to treatment response and clinical outcomes — will therefore be crucial. Embedding high-resolution multi-omic profiling into ongoing and future clinical studies will help determine which immune features or integrative frameworks, such as Sarcoma Immune Classes (18), TLS, intratumoral CD8⁺ T cell infiltration, the Immunologic Constant of Rejection signature (39), and sarcoma ecotypes (17), can serve as robust, clinically actionable biomarkers for patient stratification across sarcoma subtypes.

A related opportunity involves characterizing antigenicity in both genomically simple and complex sarcomas. Although recurrent, mutation-driven neoantigens are rare, alternative sources — including fusion-derived peptides (40) and aberrant RNA processing (41) — may drive immune recognition. Combining immunopeptidomics with emerging technologies like long-read sequencing and single-cell genomics will define sarcoma antigen landscapes and reveal how T cells recognize noncanonical tumor-associated antigens. These methods will also deepen insights into innate immune recognition, which may be particularly relevant in sarcomas with limited classical antigenicity. NK cells, gd-T cells, and innate lymphoid cells respond to stress signals, cytokines, and nonclassical ligands rather than MHC-restricted peptides (42, 43). Elucidating how genomic instability and stress responses shape these innate pathways could unlock therapies beyond conventional checkpoint blockade.

The development of single-cell spatial profiling enables subtype-specific interrogation of the sarcoma immune landscape. In UPS and MFS, this tool can dissect their divergent immunobiology by resolving differences in tumor-intrinsic programs, stromal organization, immune functionality, and spatial arrangement. In chordoma, where immune infiltration shows limited, context-dependent prognostic associations, this approach may uncover cellular neighborhoods or functional states that better predict immunogenicity, prognosis, or treatment sensitivity than bulk measures. Hypotheses from these descriptive studies can then be tested in selected functional model systems to probe specific tumor-immune interactions under controlled conditions.

Collectively, these approaches provide a framework for connecting tumor biology to immune organization and function across sarcoma subtypes, refining interpretations of patient-derived immune phenotypes.

For chordoma specifically, this thesis highlights key areas for investigation. Although *TBXT* drives chordoma biology, its role in immunogenicity remains unclear. Beyond confirming the consistent presentation and immunogenicity of *TBXT*-derived peptides, studies must explore whether *TBXT*'s transcriptional program influences broader immunogenicity. Clarifying these mechanisms will help inform *TBXT*-targeted therapies, including peptide vaccines, adoptive TCR approaches, and checkpoint blockade combinations.

Taken together, the future of sarcoma immunobiology lies in leveraging high-resolution multi-omic profiling, with particular emphasis on embedding these efforts within translational work in clinical trials, and validating those findings with mechanistic studies. This will elucidate how genomic aberrations generate antigenicity; how tumor, stromal, and immune cells orchestrate anti-tumor responses; and how to refine patient selection and therapeutic strategies. As molecular profiling advances and sarcoma trials expand, these efforts promise to translate immunogenomic insights into meaningful improvements in patient care.

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