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SARCOMAS (SR PATEL, SECTION EDITOR)

Immunotherapy as a Potential Treatment for Chordoma: a Review

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Abstract Chordoma is a locally aggressive primary malignancy of the axial skeleton. The gold standard for treatment is en bloc resection, with some centers now advocating for the use of radiation to help mitigate the risk of recurrence. Local recurrence is common, and salvaging local failures is quite difficult. Chemotherapy has been ineffective and small molecule targeted therapy has had only marginal benefits in small subsets of patients with rare tumor phenotypes or refractory disease. Recent successes utilizing immunotherapy in a variety of cancers has led to a resurgence of interest in modifying the host immune system to develop new ways to treat tumors. This review will discuss these studies and will highlight the early studies employing immune strategies for the treatment of chordoma.

Keywords Antigen processing · Bone tumors · Carbon-ion · Chordoma · Chimeric antigen receptor · CSPG4 · CTLA-4 · HLA · Immune checkpoint · Immune escape · Immune system · Immunotherapy · MHC · PD-1 · PD-L1 · PD-L2 · Proton · Radiation · Radiotherapy · Sarcoma · Tumor antigen · Vaccine

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Introduction

Chordoma is a primary malignancy of the axial skeleton originating from notochordal remnants [1]. The embryologic notochord gives rise to the nucleus pulposus of intervertebral discs; however, through a mechanism which is still poorly understood, a portion of these notochordal cells is found scattered in the vertebral bodies, and this subset, known as the notochordal remnants, can undergo malignant transformation to become chordoma [2]. These rare tumors have an annual incidence of approximately 1 per 1 million people, and they tend to be locally aggressive and have a high rate of recurrence. Chordomas can occur anywhere in the spine, but they are primarily found in the sacrococcygeal region and also often at the skull base involving the clivus [3]. Pathologically, gross specimen is gelatinous pinkish-gray masses with solid and cystic components. Low-power microscopic examination reveals lobules of epithelioid cells encompassed in clusters by surrounding fibrous septae in a background of mucinous matrix. The pathognomonic cell type for chordoma is the physaliferous cell, which has a heavily vacuolated cytoplasm. Chordoma is immunohistochemically identified by strongly positive cytokeratin and brachyury expression, as well as weak positivity for PAS and S-100. Brachyury is a transcription factor involved in notochordal development and is overexpressed in most chordomas [4].

The standard of care for chordoma is en bloc resection, with the goal of negative margins [1]. These tumors will grow if untreated, eventually involving nerve roots which leads to significant neurologic deficits [5, 6]. In sacral chordomas, this means loss of bowel, bladder, and sexual function. Marginal or subtotal resection has been shown to result in high rates of recurrence [7]. Recurrent chordoma is nearly impossible to eradicate, which has led to a paradigm shift among surgeons, who are now aiming for ablative resection with wide margins.

This often involves sacrificing nerve roots to achieve negative margins. While more aggressive surgical approaches to chordoma resection have resulted in greater local control, it often comes at the expense of function, and chordoma remains a cancer ridden with significant morbidity and mortality.

Radiation therapy for chordoma has been shown to provide both therapeutic and palliative benefit in patients [8–10, 11•, 12, 13]. In addition to standard photon beam radiation, newer modalities are beginning to be applied to chordoma treatment. Proton beam radiation allows for dose escalation with less damage to surrounding healthy tissue, thereby increasing the therapeutic ratio of radiation delivered [12]. Adjuvant proton beam radiation therapy combined with en bloc resection has been shown to provide durable local control of chordoma [10, 11•]. Carbon-ion therapy uses heavier ions to cause more DNA damage in malignant cells, which has been shown to increase the biological efficiency of radiation doses [13]. For unresectable chordomas, high-dose proton radiotherapy has been shown to be effective for definitive management in select cases, resulting in improved overall survival, chordoma-specific survival, local progression-free survival, and metastasis-free survival [9]. The major adverse effect of this radiation-only management was sacral insufficiency fractures; however, all patients remained ambulatory with minimal neurologic side effects since sacral nerve roots were spared. An early study of carbon-ion radiotherapy has found that it is safe and effective at providing local control in select primary sacral chordomas [13].

Effective first-line small molecule-based targeted therapy does not exist for chordoma. Rare cases of advanced PDGFR-β-positive chordomas treated with imatinib have shown a modest survival benefit and halting of disease progression, suggesting that imatinib may have some antitumor activity in chordoma [14]. Imatinib has also been used as off-label salvage therapy in patients with disease refractory to conventional treatment. Chemotherapy has proven to be largely ineffective [15, 16]. Systemic therapy for chordoma is limited and is reserved for patients with metastasis and local recurrence where surgical morbidity outweighs the benefits [16].

The lack of curative options for chordoma has led to the investigation of other strategies to devise new ways to treat this disease. Recent work studying both classic and newer immunotherapeutic strategies, including tumor antigen vaccines and immune checkpoint blockade, for chordoma has created the possibility for utilizing these methods in the treatment of this cancer. This review article will touch on these studies, and will also explore how lessons from other studies in other tumors may further develop immunotherapy as a new treatment strategy for chordoma. The hope of these studies and for those generating research programs focused on immunotherapy for chordoma is to create new treatment paradigms that may improve patient outcomes in a classically difficult-to-treat tumor with significant morbidity and mortality.

The Role of the Immune System in Cancer

The interaction between the immune system and malignancy has long been studied. The immune system plays a critical role in eliminating abnormal cells [17]. These abnormal cells harbor molecular signatures that are recognized as non-self antigens by immune cells that are constantly surveying the cells in our body. When an immune cell, such as a cytotoxic T lymphocyte (CTL), encounters a non-self antigen, it engages in a process that leads to cell death for the abnormal or intruder cell. As will be described below, tumor antigens (TA) are processed by malignant cells and are displayed on the tumor cell surface via the human leukocyte antigen (HLA) class I antigen complex. Studies have shown that these TA are viewed as non-self antigens by circulating immune cells, leading to tumor cell destruction as a result of interactions with the immune system. These interactions, and mechanisms that allow tumors to specifically evade such interactions, will be further described in the following sections.

The HLA Class I Antigen Complex and Tumor Antigen Processing

The HLA system is the human analog of the major histocompatibility complex (MHC), a group of genes on chromosome 6 that encode cell surface markers and antigen processing machinery (APM) involved in the innate and adaptive immune responses, as well as in infection, autoimmunity, and malignancies [18]. The HLA class I region contains genes that encode both classical and non-classical class I antigens. For the purposes of this review, only the classical antigens will be discussed, as they are the molecules involved in antigen presentation and the immune interactions that will be detailed below.

The classical HLA class I antigens, HLA-A, HLA-B, and HLA-C, are normally expressed on most human cell types, with some exceptions including erythrocytes and trophoblasts [17]. The complete HLA class I antigen is a dimer composed of a transmembrane heavy (alpha) chain and an associated light (beta) chain, beta-2-microglobulin (β2m). The β2m component is non-polymorphic and is encoded on chromosome 15, which is not part of the MHC.

One of the major roles of the HLA in human cells is to process endogenous proteins intracellularly and present short amino acid sequences on the cell surface via the HLA class I antigen complex [17]. In malignant cells, these unfamiliar tumor antigens are exhibited on the cell surface, allowing for recognition by cognate T lymphocytes [19]. Primed T cells

recognize TA presented by the HLA class I antigen via interaction with the T cell receptor (TCR). This interaction leads to a cytotoxic effect that leads to tumor cell destruction.

Antigen processing begins with the synthesis of the heavy chain and β2m components in the endoplasmic reticulum (ER) [17]. These components associate with the chaperone proteins calnexin, calreticulin, and Erp57, which stabilize the heavy and beta chains and prevent loading of self-peptides from the ER itself. The heavy and beta chains then associate with the amino acid transporter TAP, which is stabilized by the chaperone protein tapasin. In the cytosol, proteins to be degraded are ubiquitinated and thereby directed toward the proteasome. In the context of immune activation or inflammation, IFN-γ induced expression of LMP-2 and LMP-7 leads to proteasome augmentation with these new subunits. This augmented proteasome creates peptides of optimal length for HLA class I presentation, approximately 8–10 amino acids long. TAP then transports these short peptides from the cytosol into the ER, where they associate with the heavy and beta chains, stabilizing the complex. This peptide-HLA antigen complex is then transported to the cell surface for antigen presentation. An intact HLA class I antigen complex is needed in order to present TA on the tumor cell surface for possible interaction with a TCR on specifically primed CTLs.

Immune Escape Mechanisms in Cancer

A growing body of evidence indicates that a major obstacle to the success of immunotherapy is represented by the many escape mechanisms utilized by tumor cells to avoid recognition and destruction by the host's immune system [20]. Among them are defects in the HLA class I antigen. Many malignancies have been shown to have absent or downregulated expression of HLA class I antigen components [20–32]. While defects in the HLA class I antigen heavy and beta chains has implications at the tumor cell surface, improper or incomplete TA processing in the cytosol and ER can also lead to immune escape. Issues with TA processing will affect TA peptide loading, and will therefore prevent T cell recognition of tumor cells.

The presence of tumor-infiltrating lymphocytes has been associated with improved prognoses in numerous malignancies, including melanoma [33–36], breast cancer [37–40], lung cancer [41–45], colorectal cancers [46–67], and ovarian cancer [68–70]. Data from large clinical studies demonstrate that a robust infiltration of neoplastic lesions by specific immune cell populations, including, but not limited to, $CD8^+$ cytotoxic T lymphocytes (CTL), Th1 and Th17 $CD4^+$ T cells, natural killer (NK) cells, dendritic cells, and M1 macrophages, constitutes an independent favorable prognostic indicator in several types of cancer [71]. The mechanism by which this effect occurs is currently unknown; however, more recently, increasing attention has been paid to the role of immunosurveillance in the pathogenesis and clinical course of cancer, with particular emphasis on the application of immunotherapy for treatment [72]. The aforementioned studies suggest that the immune system plays a dynamic role in regulating tumors. The immune cell-malignant cell interaction is important in not only preventing malignant cells from colony formation but also in keeping clonal expansion at bay. Thus, the immune system plays a role in controlling tumor progression even if it has failed in preventing tumor formation. This has led to renewed interest in understanding the mechanisms that drive these processes, as well as developing strategies to stimulate the immune system as a way to treat cancer.

Immunotherapeutic Strategies for the Treatment of Chordoma

For decades, the utility of immunotherapy as a treatment for cancers remained a controversial topic. Until recently, aside from the successful use of monoclonal antibodies in certain types of cancers, we have been limited in the number of immune-mediated pathways we could utilize to treat tumors. In the past few years, significant progress has been made in bringing immunotherapeutic strategies back to the forefront of cancer management, and these have garnered considerable attention [73•, 74].

Immune Checkpoint Blockade Highlighting these successes are the recent studies describing the use of immune checkpoint blockade in treating various malignancies [73•]. The immune checkpoint molecules these therapies target are the programmed cell death protein 1 (PD-1) and the cytotoxic T lymphocyte antigen 4 (CTLA4). The binding of PD-1 on T cells with its ligands, PD-L1 and PD-L2, on tumor cells initiates an inhibitory effect that negatively regulates TCR signaling and leads to apoptosis of T cells [75]. Nivolumab and pembrolizumab are examples of monoclonal antibodies directed against PD-1 [76, 77••, 78]. By blocking the interaction of PD-1 with its ligands, these antibodies prevent T cell exhaustion and death, and thereby boost the immune response specifically targeted against tumors. CTLA4 is expressed on helper T cells and has a similar structure to CD28. While CD28 is a costimulatory molecule that binds to the B7 family of molecules on antigen presenting cells and enhances the immune response, CTLA4 is its antagonist and initiates a cascade of inhibitory signaling that ultimately serves as an "off" switch for T cell-based immune attack [79]. As another example of a negative regulator of the immune response, CTLA4 was identified as a target for blockade, and ipilimumab, a CTLA4-specific monoclonal antibody, was the first example of FDA-approved immune checkpoint blockade therapy for cancer [80].

Two recent studies have reported PD-L1 expression in chordoma [81, 82]. In the study by Feng et al., the authors

found that PD-L1 was constitutively expressed in three chordoma cell lines; UCH1, UCH2, and CH22, as measured by Western blot analysis [81]. Interferon-gamma increased PD-L1 expression in UCH1 and UCH2, but not in CH22. All cell lines maintained their brachyury expression. Feng et al. also performed Western blot analysis on nine human chordoma tissue samples, demonstrating variable PD-L1 expression in all samples. Additionally, in a tissue microarray (TMA) of 78 chordoma tissue samples from 56 patients, the authors found that 94.9 % of chordoma tumors expressed PD-L1 by immunohistochemical (IHC) analysis, with variable staining intensity. There was no statistical correlation between PD-L1 expression and age, gender, tumor location, or between primary samples and those that locally recurred. There was, however, a statistically significant correlation between increased PD-L1 expression in metastatic tumor samples as compared to primary specimens. Furthermore, there was a trend toward higher median survival in patients with low PD-L1 expression.

The other study looking at PD-L1 expression in chordoma had less robust findings. Mathios et al. also found PD-L1 and PD-L2 expression in three chordoma cell lines; UCH1, UCH2, JHC7, by flow cytometry [82]. However, they demonstrated expression in less than 5 % of cells, though overall expression by mean fluorescence intensity was augmented with interferon-gamma in all three cell lines. The authors also examined ten chordoma tissue samples from patients using IHC analysis. They noted tumor-infiltrating lymphocytes in six samples, and chose to examine these tumor microenvironments for PD-1 and PD-L1 expression. Three of these six samples had lymphocytes expressing PD-1, and four out of six had PD-L1 expression, though none had robust expression. These data suggest a more conservative expression profile of immune checkpoint molecules in human chordoma tumors.

Clinical trials utilizing monoclonal antibodies directed against the immune checkpoint molecules PD-1 and CTLA4 have been successful in a variety of tumors, as previously described. Though the two early studies highlighted in the above paragraphs have the drawbacks of small sample sizes and limited tissue analysis in what is known to be a heterogeneous tumor, they provide insight into the possible use of immune checkpoint blockade therapies in chordoma patients. It is possible that PD-1/PD-L1/PD-L2 and CTLA4/B7 interactions play a role in chordoma pathogenesis. These pathways represent mechanisms for negative regulation of the T cell priming and effector phases, respectively, serving as a stopgap for unchecked immune responses and thus allowing tumors to escape T cell interactions [83]. Immunotherapy focused on checkpoint blockade interferes with these negative regulators of T cells in order to drive an antitumor immune response.

Chimeric Antigen Receptors Another immunotherapeutic strategy recently garnering significant interest is the use of chimeric antigen receptors (CARs), or artificially engineered TCRs specific to a tumor antigen. Briefly, CARs are fusions of single-chain variable fragments from monoclonal antibodies with the intracytoplasmic and transmembrane domains of the CD3-ζ molecule. In a process known as adoptive cell transfer, T cells are isolated from a patient, and CTLs are modified to express the TA-specific CAR via viral vector transduction. The result is CAR-modified T cells with TCRs that are unique and specific to the tumor cells they intend to target, leading to tumor cell destruction when reintroduced into the patients [84]. Early pre-clinical and phase I clinical studies utilizing adoptive cell transfer of CAR-modified T cells in various malignancies show promise that this strategy could soon become another option in cancer therapy [85]. This is of particular interest since our group has preliminary data reporting HLA class I expression defects in human chordomas (unpublished data). Immune checkpoint blockade relies on the interaction between a TCR and HLA class I antigen; however, the use of CARs does not. This can serve as a viable immunotherapeutic option for patients with chordomas with HLA class I dysregulation.

Chondroitin sulfate proteoglycan 4 (CSPG4), also known as high molecular weight-melanoma associated antigen (HMW-MAA), is a cell surface molecule involved in multiple signaling pathways important for tumor cell growth, survival, migration, and metastasis [86]. A study from our group has shown that chordomas overexpress CSPG4 [87]. Furthermore, CSPG4 expression in chordoma was correlated with an increased risk of metastasis and mortality [88•]. Our collaborators, utilizing anti-CSPG4 monoclonal antibodies, have recently designed and validated second-generation CARs with specificity for CSPG4 [89]. This anti-CSPG4 CAR could be used for retroviral vector transduction of the CAR into peripheral blood T lymphocytes in order to generate anti-CSPG4 CAR-modified T cells. Analysis of cytolysis after co-culturing these CAR-modified T cells with chordoma cell lines would provide insight into the efficacy of anti-CSPG4 immunotherapy, specifically CAR-based strategies, in the treatment of human chordoma. If successful, these in vitro results could lead to the use of anti-CSPG4 CAR-modified T cells in vivo animal studies, and ultimately adoptive cell transfer phase I clinical trials in human patients with chordoma.

Tumor Antigen Vaccines A final strategy that might soon offer another opportunity to take advantage of the interaction of the immune system and cancer is the advent of successful tumor antigen vaccination. Patients with tumors known to overexpress a certain antigen can be vaccinated with low-

dose inactivated antigen in order to drive an antigen-specific T cell response. While these vaccines have been notoriously difficult to create and have historically yielded only modest effect in early clinical trials, newer vaccines are showing early signs of success. For example, preliminary results from an open-label phase I clinical trial vaccinating chordoma patients with heat-killed recombinant yeast modified to express the brachyury protein suggest that certain patients have an immune response to brachyury [90, 91•]. While these initial results only represent a small cohort of seven chordoma patients, the study showed that patients who were vaccinated with this yeast-brachyury vaccine were able to mount both CD4 and CD8 responses with increased intracellular expression of pro-inflammatory and immune stimulating cytokines IFN-γ, TNF, and IL-2, as well as the perforin and granzyme marker CD107a. It is important to note that these vaccines would only have a clinical benefit in patients with tumors expressing HLA class I antigen; however, as will be detailed in the next section, HLA class I antigen expression can be modulated with radiation, which may provide the opportunity for dual therapy utilizing tumor antigen vaccination and radiation in order to provide a synergistic effect.

Effect of Radiation on the Immunogenicity of Chordoma

Experiments performed in vitro and in animal models in collaboration with researchers at the National Cancer Institute have shown that low-dose irradiation can upregulate HLA class I APM component expression and can enhance the cell surface expression of calreticulin [92•]. These phenotypic changes increase the immunogenicity of tumor cells and their recognition by cognate T cells.

In light of these new findings, it is possible that radiation therapy could serve multiple roles in the treatment of chordoma. While radiation therapy is becoming part of the standard of care clinically, it may also provide immunogenic modulation of tumors that enhance antigen processing and presentation, allowing for improved T cell effect. At the Stephan L. Harris Center for Chordoma Care at MGH, en bloc surgical resection with neoadjuvant and adjuvant proton beam radiation therapy has been shown in a phase II clinical trial to provide durable local control [10, 11•]. If radiation was found to increase the immunogenicity of chordoma tumors, it would further justify more widespread and aggressive use of radiation in the treatment of chordoma patients.

Conclusion

Chordoma, a rare primary malignancy of the axial skeleton, is a locally aggressive and highly recurrent tumor associated with significant morbidity and mortality. Surgery has remained the mainstay of treatment for the past few decades largely due to ineffective cytotoxic chemotherapy and the lack of first-line small molecule-based targeted therapeutics. Radiation therapy for chordoma is controversial and is not yet widely accepted as standard of care, but newer data suggests it may have a beneficial role in providing better local control of tumors. With the resurgence of interest in immunotherapeutics for cancer, the studies presented in this review make early breakthroughs in our understanding of the role of the immune system in chordoma pathogenesis at a pivotal time.

Tumor-infiltrating lymphocytes have been shown to portend an improved prognosis in patients with a number of malignancies. Taken together, these findings support the possibility that patients mount an immune response against their tumor, and that these intratumoral lymphocytes impose selective pressure on malignant cells, leading to the clonal expansion of cells with the ability to evade immunosurveillance through defective tumor antigen presentation. These immune mechanisms involved in chordoma pathogenesis are critical for prognostication, tumor phenotyping, directing appropriate immunotherapy to the right patient cohorts, optimally selecting patients for various treatment regimens, and stimulating further research into the interplay between the host immune system and chordoma.

In patients with an intact HLA class I antigen complex, many immunotherapeutic strategies are potential options. For example, checkpoint blockade therapies, such as the monoclonal antibodies directed against PD-1 and CTLA4 mentioned above, CAR-based T cell therapy, and tumor antigen vaccines, could be utilized to treat patients. However, in patients with defects in HLA class I antigen components, many immunotherapy options are not possible. In this subset of patients, an argument can be made for more aggressive treatment paradigms, such as en bloc surgical resection with neoadjuvant and adjuvant proton radiation therapy. This therapeutic modality has been shown in a phase II clinical trial to provide effective local control which is durable for at least 5 years [10]. With the advent of newer CAR-based immunotherapies, tumors with defects in HLA class I antigen processing and presentation may still be able to be targeted by T cells if tumor-specific cell surface molecules, such as CSPG4, are identified. The HLA class I antigen complex is utilized to present normally intracellular tumor antigen peptides on the cell surface for immune surveillance by T cells. However, molecules that are routinely transported to the cell surface by the ER-Golgi apparatus do not require intact HLA machinery. With adoptive cell transfer utilizing CARs, a patient's T cells can be engineered artificially to recognize specific tumor cell surface markers, and can then be reintroduced into the patient for cytotoxic effect.

The studies presented in this review represent the early stages of understanding the role of the immune system in chordoma progression and treatment. Before applying

immunotherapeutic strategies to patients, a mechanistic understanding of how the host immune system interacts with the tumor and how tumors attempt to escape immune attack is necessary. We hope these data will stimulate further studies investigating the efficacy of immunotherapeutics in chordoma and their mechanism of action.

Compliance with Ethical Standards

Conflict of Interest Shalin S. Patel and Joseph H. Schwab declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance

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