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Review

Prospective immunotherapies in childhood sarcomas: PD1/PDL1 blockade in combination with tumor vaccines

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Progress has slowed substantially in improving survival rates for pediatric sarcomas, particularly in refractory and metastatic disease. Significant progress has been made in the field of tumor vaccines for such malignancies, which target established tumor antigens. While tumor vaccines have demonstrated safety and improved survival rates, they are inadequate in mediating the regression of established tumor masses and metastases. Programmed cell death ligand 1 (PDL1) is a cell-surface protein induced in a number of adult malignancies. By acting on the corresponding T-cell receptor PD1, PDL1 is able to suppress cytotoxic T-cell–mediated tumor responses. Recent therapeutics blocking this interaction have shown promise in various adult cancers by restoring a functional T-cell response and by directing this response toward an activated, rather than regulatory, T-cell phenotype. We shall discuss the current state of tumor vaccines targeting pediatric sarcomas, review PD1–PDL1 interactions and current therapies targeting these interactions in adult malignancies, and discuss recent studies in which tumor vaccines, combined with PDL1 blockades, produced superior tumor regression compared with the vaccine alone. These studies provide a compelling case for investigation of PDL1 expression and its inhibition in pediatric sarcomas, while continuing to utilize tumor vaccines in tandem to achieve superior clinical outcomes.

INTRODUCTION

Sarcomas are rare malignancies of the bone and soft tissue, including osteosarcoma, rhabdomyosarcoma, nonrhabdomyosarcoma soft tissue sarcoma (STS), and Ewing's sarcoma and collectively account for roughly 10% of childhood cancers (1,2). Although considerable progress on survival rates had been made initially with regard to these malignancies, progress has stagnated in the past 25 years (3). Survival rates remain dismal for those patients who present with advanced metastatic disease, recurrence, or those who are refractory to conventional chemotherapy (4–8). Additionally, short-term toxicities from conventional chemotherapy can often hamper the treatment course itself. Long-term toxicities from chemotherapy can manifest in adult survivors as well, and the risk of secondary malignancies looms after a successful clinical outcome (9). Increased-intensity chemotherapeutic regimens for these refractory cancers have failed to yield improved survival rates (10–12). Therefore, there remains an urgent need for new treatment modalities that offer both fewer side effects and better clinical outcomes.

TUMOR VACCINES IN PEDIATRIC SARCOMAS

One novel therapeutic concept that has been explored is the development of tumor vaccines. Following the discovery of molecular markers on cancer cells which served as targets for T-cell recognition, a wide variety of tumor vaccines were designed with the goal of achieving a more focused and potent antitumor response to these tumor antigens from host T cells (13). Exact methods for achieving such a response have been widely varied, but more recent trials in pediatric sarcomas have relied on host dendritic cells (DCs) for the stimulation of a primary host immune response via the presentation of tumor antigens (14,15). In such tumor vaccines, autologous DCs are harvested and expanded and are then "pulsed" with tumor lysate *ex vivo*, or chemically fused with autologous tumor cells themselves (13,15–20).

There are currently several ongoing tumor vaccine trials for pediatric sarcomas, which are summarized in **Table 1** (13,16). NCT01241162, which is currently recruiting patients, uses a tumor vaccine consisting of autologous DCs pulsed with cancer testis antigen to generate an immune response against Ewing sarcoma, osteosarcoma, rhabdomyosarcoma, and synovial sarcoma. Another trial, NCT00405327, employs tumor lysate-pulsed autologous DCs for the treatment of high-risk solid tumors following stem-cell transplantation. Additionally, NCT00923351 utilizes harvested tumor lysates from pediatric rhabdomyosarcoma and Ewing sarcoma patients, which are then pulsed through autologous DCs with or without recombinant IL-7. In adult and pediatric cancer alike, tumor vaccines have been shown to be consistently safe and effective at slowing tumor growth and improving survival, but only rarely cause regression in established tumors; furthermore, they are not approved for single-agent therapy (13,15,16,21–25). This is likely due to multiple factors that contribute to the phenomenon of immune escape by tumors, which are largely facilitated

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Table 1. Current clinical trials for pediatric cancer involving tumor vaccines

Tumor vaccine trials for pediatric sarcomas					
Trial number	Regimen	Phase	Eligible tumor types	Status	Ages eligible for study
NCT01241162	Autologous CTA-specific DC vaccine preceded by decitabine	I	NB, ES, osteogenic sarcoma, RMS, SS	Recruiting	1 y to 17 y
NCT00405327	Tumor lysate pulsed DC vaccine following autologous stem cell transplant	II	Sarcoma, NB, Wilm's tumor	Active, not recruiting	up to 30 y
NCT00923351	Tumor lysate pulsed autologous DCs with autologous lymphocytes with or without recombinant IL-7	I/II	NB, sarcoma, ERMS, ARMS, PPNET	Active, not recruiting	19 mo to 35 y
NCT01803152	Tumor lysate pulsed autologous DCs plus imiquimod with and without gemcitabine	I	Sarcoma, STS, bone sarcoma	Recruiting	1 y and older
NCT00001566	DCs pulsed with peptides derived from tumor-specific translocations in combination with autologous T-cell transplant	II	ES, RMS	Completed	5 y to 35 y
NCT00001564	Peptide pulsed APCs plus rhIL-2 with or without autologous T-cell transplantation	II	ES, RMS	Completed	up to 30 y

APC, antigen-presenting cell; ARMS, alveolar rhabdomyosarcoma; CTA, cancer testis antigen; DC, dendritic cell; ERMS, emryonal rhabdomyosarcoma; ES, Ewing sarcoma; NB, neuroblastoma; OS, osteosarcoma; PPNET, peripheral primitive neuroectodermal tumor; rhIL-2, recombinant human interleukin 2; RMS, rhabdomyosarcoma; SS, synovial sarcoma; STS, soft tissue sarcoma.

by suppression of the cytotoxic T-cell response against the tumor (13,17). This suppression can be directly mediated by the tumor itself, or by subpopulations of cells, including myeloid-derived suppressor cells and T regulatory cells (Tregs) which, following their induction by proinflammatory cytokines within the tumor microenvironment, suppress robust immune responses via either antigen-specific or nonspecific manners. Novel means of circumventing this phenomenon of immune escape, either through new mechanisms or further refinement of existing tumor vaccines, are of paramount importance for the improvement of therapeutic options in pediatric tumors.

TARGETING THE PD1/PDL1 PATHWAY AND ITS APPLICABILITY TO PEDIATRIC CANCERS

The PD1/PDL1 pathway has recently emerged as a central component of immune regulation that is also utilized by cancer cells to evade host immune response (**Figure 1**). Under normal conditions, PD1 (also known as BH7) is expressed on activated CD8+ T cells, and its interaction with PDL1 on host tissues leads to the inhibition of T-cell receptor (TCR) signaling via SHP1/2, limits the interactions between T cells and DCs, and ultimately leads to T-cell inactivation and apoptosis (26–30). Additionally, PD1/PDL1 signaling has been shown to convert Th1 cells into FOXP3+ Tregs, thereby preventing clonal T-cell expansion (31). PDL1 can be constitutively expressed or induced by localized inflammatory stimuli, such as interferons (27). This pathway is critical for preventing autoimmunity by maintaining self-tolerance to protect host tissues from any protracted immune response (27).

However, induction of the PD1/PDL1 pathway has recently been shown in a number of adult malignancies as a means by which tumors suppress the host immune response. PDL1 expression has previously been documented in a number of adult tumors, including melanoma, renal, esophageal, breast, and lung carcinomas (26,32–36). Early animal data showed that tumor expression of PDL1 would inhibit the activity of infiltrating tumor-reactive T cells (26,37–39), and further studies demonstrated a correlation between expression of PDL1 and adverse outcomes in adult malignancies (40–42). Unfortunately, data regarding expression of PDL1 in pediatric cancers is minimal, and what does exist is derived from either animal models or study samples involving predominantly adult patient samples, with only a modest proportion of pediatric primary tumor samples. To date, there exist no published studies regarding expression patterns of PDL1 in Ewing sarcoma or rhabdomyosarcoma. Soft tissue sarcomas have been shown to express PDL1 (with corresponding expression of PD1 on tumor infiltrating lymphocytes), and this expression has been correlated with reduced survival (42). In a recent study, subsets of both adult and pediatric osteosarcoma samples were shown to express high levels of PDL1, with high levels of expression trending with decreased overall survival, although it did not quite achieve statistical significance (43). Another recent study demonstrated that PDL1 (along with PD1-positive CD8+ T cells) was upregulated in metastatic but not primary osteosarcoma, and inhibition of PD1 in a mouse model was able to decrease tumor burden and improve survival (44). PDL1 has also been shown to be expressed in adult samples of various hematologic malignancies, including acute myeloid leukemia, acute lymphoblastic leukemia, anaplastic large cell lymphoma, Hodgkin lymphoma, and diffuse large B-cell lymphoma (45–49).

Novel therapeutic agents that inhibit the PD1/PDL1 axis have shown promise in adult malignancies. Monoclonal antibodies (mAbs) against PDL1 and PD1 alike have produced therapeutic responses in significant subsets of patients with melanoma, renal, and lung cancer (50–53). Pembrolizumab (Keytruda, Merck, Kenilworth, NJ), formerly lambrolizumab (MK3475), became the first FDA-approved anti-PD1 mAb in September 2014 based on tumor responses and durability of the responses seen in early clinical trials. It is approved for use in patients with advanced melanoma who have progressed

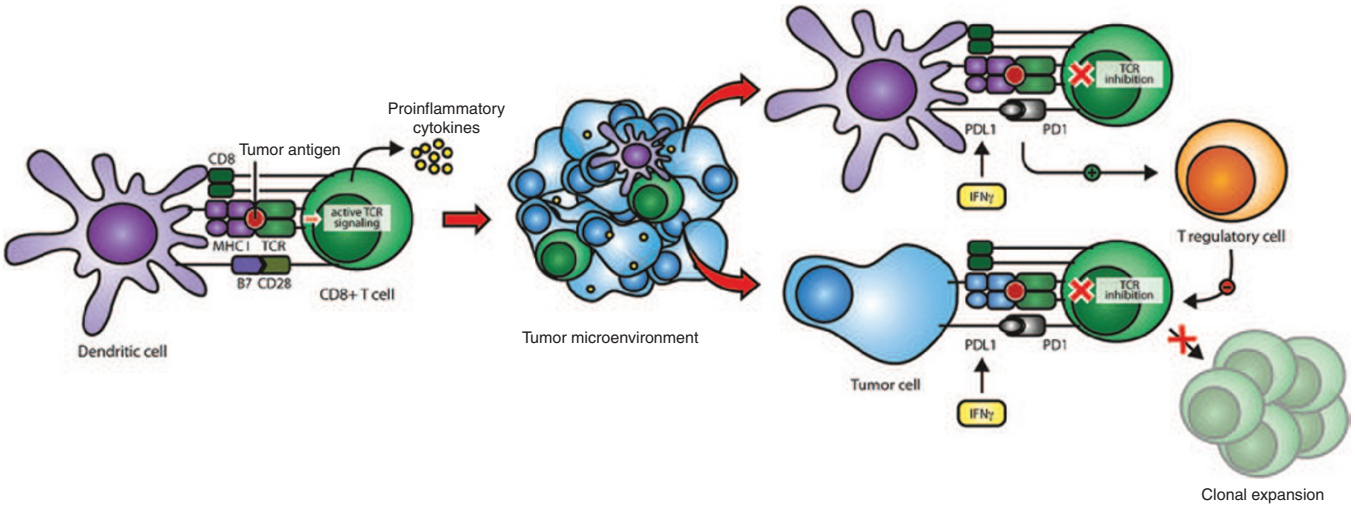

Figure 1. Initial activation of CD8+ T cells occurs when dendritic cells (DCs) present tumor antigen in context of major histocompatibility class I (MHC I) to the T-cell receptor (TCR). In addition to antigen recognition, a co-stimulatory signal resulting from the B7-CD28 interaction is also necessary for activation. T-cell activation results in upregulation of PD1 on the cell surface and the secretion of proinflammatory cytokines, such as interferon gamma (IFNγ), into the tumor microenvironment. IFNγ induces the upregulation of PDL1 on DCs and tumor cells. The PD1/PDL1 interaction inhibits TCR signaling and leads to an increase in T regulatory (Treg) cells, which inhibit CD8+ T-cell activity and prevent clonal expansion. This adaptive immune resistance allows the tumor cells to evade a T-cell-mediated antitumor response.

on ipilimumab and BRAF-directed therapy in patients who carry a BRAF mutation (54,55). Nivolumab (BMS-936558, Bristol-Meyers-Squibb, New York, NY), a fully humanized IgG4 anti-PD1 mAb, was initially shown in phase I clinical trials to induce objective responses in 18–28% of patients with melanoma, renal cell carcinoma (RCC), and non–small-cell lung carcinoma (NSCLC) (51). Subsequent phase II trials in metastatic RCC recapitulated objective responses in a subset (22–26%) of patients, as well as dose-dependent increases in overall survival (56). It is now FDA approved as monotherapy for treatment of unresectable or metastatic melanoma, and disease progression following treatment with ipilimumab and BRAF-directed therapy in patients with tumors possessing the BRAF mutation (57,58). Additionally, nivolumab has recently been FDA approved for combination therapy against NSCLC which has progressed after platinum-based chemotherapy, following the results of a recent phase III clinical trial which demonstrated superior osteosarcoma compared to standard of care (59). Pidilizumab (CT-011, CureTech, Yavne, Israel) is an anti-PD1 mAb which has been studied in phase I trials in a small sample of patients with diffuse large B-cell lymphoma, with 33% of patients demonstrating increased overall survival (60). A recent phase II clinical trial in which pidilizumab was administered to diffuse large B-cell lymphoma patients following autologous stem cell transplant showed increased duration of progression-free survival (61). Additionally, in studies involving nivolumab, pembrolizumab, and pidilizumab, many of the responders continued to demonstrate sustained regression even after the treatment was discontinued (50–61). Anti-PDL1 mAbs have shown similar efficacy in phase I clinical trials as well. One such mAb, BMS-936559, showed objective response rates in a small subset (10–17%) of melanoma, RCC, ovarian cancer, and NSCLC patients, with sustainable, durable tumor regression for over 1 year in nearly half of the responders (52). MPDL3280A, another anti-PDL1 mAb, induced objective responses in roughly 25% of melanoma, NSCLC, RCC, and metastatic bladder cancer patients (62,63). Multiple phase III clinical trials are now either recruiting or ongoing involving MPDL3280A, and it was recently granted breakthrough status by the FDA for treatment of NSCLC. Furthermore, clinical trials are currently being planned exploring the use of nivolumab, pembrolizumab, and pidilizumab in pediatric tumors. While many of these trials are not yet recruiting and therefore have yet to demonstrate therapeutic efficacy, they plan to explore the use of these agents against osteosarcoma, Ewing sarcoma, RMS, malignant gliomas, and soft tissue sarcoma (**Table 2**).

While PD1/PDL1 blockade provides durable therapeutic responses in a subset of patients (many of whom have proven refractory to conventional chemotherapy), the response rate to therapy is still present in only a minority of patients. Therefore, identification of factors that predict a positive response to anti-PD1/PDL1 therapy is a rapidly growing area of interest. While early studies involving patients treated with a nivolumab showed that PDL1 expression in tumor tissues had the strongest association with therapeutic response to treatment (64), PDL1 expression is a dynamic phenomenon which is regulated by inflammatory cytokines within the tumor microenvironment (65,66). This induction of PDL1 via localized inflammation, termed adaptive immune resistance, requires the initial presence of tumor-infiltrating CD8+ T cells (26,39). These T cells recognize tumor antigens via TCRs, which triggers their initial activation, expression of PD1, and the production of local interferons as part of their initial antitumor response. These interferons then induce the expression of PDL1 in tumor cells, leading to the suppression of this immune response (67,68).

Table 2. Anti-PD1 monoclonal antibody trials for pediatric cancers

Trial number	Drug	Phase		Status	Ages eligible for study
NCT02304458	Nivolumab, ipilimumab	I/II	Childhood solid neoplasm, recurrent childhood RMS, recurrent ES/PPNET, recurrent NB, recurrent OS	Not yet recruiting	12 mo and older
NCT02301039	Pembrolizumab	II	STS, bone sarcoma	Not yet recruiting	12 y and older
NCT01176461	Nivolumab alone or in combination with peptide vaccine	I	Melanoma	Active, not recruiting	16 y and older
NCT01176474	Nivolumab or nivolumab and ipilimumab with peptide vaccine	I	Melanoma	Recruiting	16 y and older
NCT01952769	Pidilizumab	I/II	Malignant glioma	Recruiting	3 y to 90 y
NCT02332668	Pembrolizumab	I/II	Melanoma, lymphoma, solid tumor	Not yet recruiting	6 mo to 17 y

APC, antigen-presenting cell; ARMS, alveolar rhabdomyosarcoma; CTA, cancer testis antigen; DC, dendritic cell; ERMS, emryonal rhabdomyosarcoma; ES, Ewing sarcoma; NB, neuroblastoma; OS, osteosarcoma; PPNET, peripheral primitive neuroectodermal tumor; rhIL-2, recombinant human interleukin 2; RMS, rhabdomyosarcoma; SS, synovial sarcoma; STS, soft tissue sarcoma.

Recent work has demonstrated that tumor-infiltrating CD8+ T cells do indeed appear to be a prerequisite for an effective response to PD1/PDL1 inhibition. Tumeh *et al.* (69) recently showed that metastatic melanoma patients who responded to PD1 blockade with pembrolizumab had higher pretreatment levels of tumor-infiltrating CD8+ T cells expressing PD1, as well as higher PDL1 expression in the tumors themselves. Additionally, they were able to demonstrate that these T cells, in addition to functionally producing interferon, had a more clonal TCR population, implying specific targeting of the tumor itself. The fact that these clonal restrictions on tumor-infiltrating T cells, as well as the PD1/PDL1 expression patterns were present in the responder patients even prior to treatment with pembrolizumab, demonstrates the necessity for these preexisting immunologic conditions prior to successful therapeutic inhibition of PD1/PDL1. Additionally, the patients who responded to treatment were found to have further post-treatment proliferation of clonally expanded, TCR-restricted CD8+ T cells, and this proliferation was noted to correlate with reduction in tumor size.

This evidence of the need for a preexisting antitumor T-cell response for optimal effectiveness of PD1/PDL1 inhibition provides the pediatric oncology community with an opportunity for augmenting the potential effectiveness of existing experimental tumor vaccines via simultaneous inhibition of PD1/PDL1. While study of PDL1 expression patterns in pediatric tumors and clinical trials involving PD1/PDL1 inhibition is a logical extension of the highly promising work from adult cancers, synergizing techniques for the treatment of pediatric tumors could improve response rates. Indeed, pediatric tumors which are more immunogenic and possess inherent tumor-infiltrating T cells, such as osteosarcoma, may be the best candidates for PD1/PDL1 inhibition. This may be due to the fact that osteosarcomas are genetically diverse malignancies with multiple chromosomal abnormalities and hyperdiploidy (70), which may lend itself to greater antigenic variety increasing the likelihood of a T-cell response. Early attempts at immunotherapy against osteosarcoma (using interleukin and interferon administration to treat both local and metastatic disease, as well as granulocyte-macrophage colony-stimulating factor in metastatic disease) were based on this principle of augmenting existing immune responses to improve patient outcomes (16). Recent studies demonstrating the importance of CD8+ T-cell responses in better patient outcomes (71,72) have lead to additional immunotherapies which focus on selective augmentation of the T-cell response through other mechanisms, including selective expansion of T cells present in the tumor microenvironment, and even genetically engineered T cells with chimeric antigen receptors specific for a given tumor (13). While focused study of less genetically and immunogenically diverse, more primitive tumors such as Ewing's sarcoma and rhabdomyosarcoma may demonstrate less robust PDL1 expression and PD1-positive CD8+ T-cell infiltration of the tumor, inducing such activity with either dendritic cell (DC) or T-cell-based tumor vaccines and subsequently combining PD1/PDL1 inhibition may yield superior clinical responses.

Combining PD1/PDL1 inhibition with DC-based vaccines that augment T-cell response to increase the likelihood of initial antitumor activity is already in the early stages of being studied. A recent study by Fu *et al.* (73) showed that an interferon-inducing cancer vaccine did indeed upregulate PDL1 expression in tumor tissue, as well as increasing the amount of tumor-infiltrating CD8+ T-cells. Moreover, coadministration of a PD1-blocking mAb caused a complete regression of established tumors in a mouse model of melanoma. Several other preclinical mouse models combining various tumor vaccines with anti-PD1/PDL1 antibodies have also demonstrated similarly enhanced CD8+ T-cell activity against a variety of solid tumors, including mouse models of sarcoma, pancreatic ductal adenocarcinoma, ovarian, breast, colon, and hepatocellular carcinoma (74–78). Given the wide variety of subtypes of tumor vaccines used in these studies (DNA vaccines, DC vaccines, peptide vaccines), it may be that any or all of them may prove useful for potential clinical trials.

Additionally, PD1/PDL1 interactions between DCs and T cells lead to increases in Tregs, as well as concomitant decreases in CD8+ T-cell activity. Inhibition of DC-based PD1/PDL1 interactions in this context would itself directly contribute to

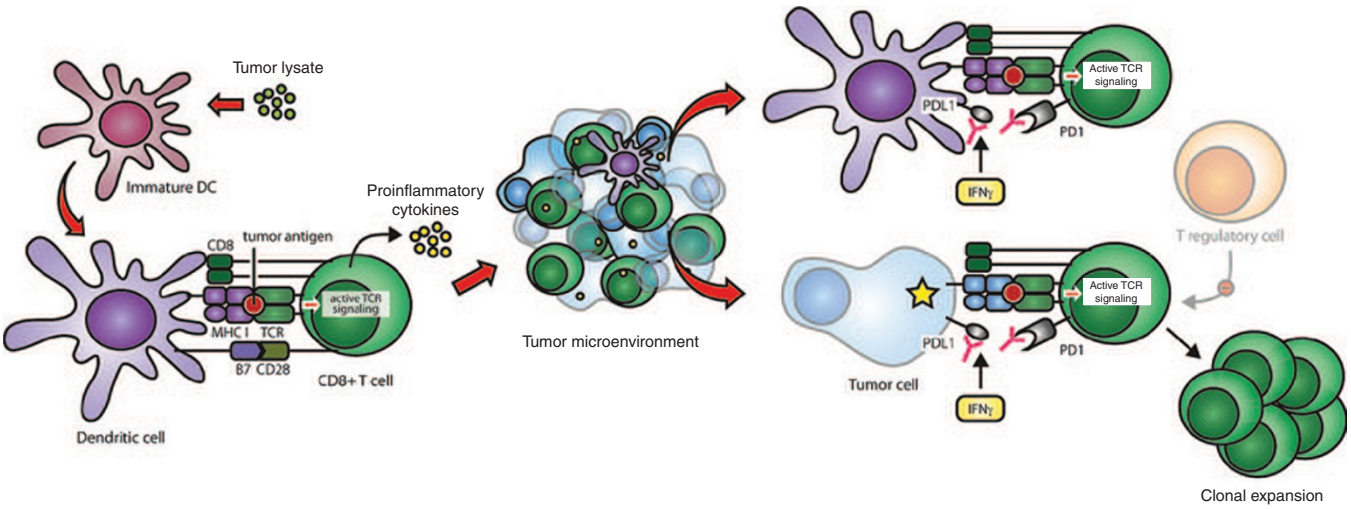

Figure 2. Tumor-pulsed DCs activate CD8+ T cells against tumor-specific antigens and increase the number of CD8+ T cells. Inhibition of the PD1/PDL1 interaction between T cells and tumor cells through the use of monoclonal antibodies increases cytotoxic T-cell antitumor response. Furthermore, blockade of this interaction between T cells and DCs reduces Treg activity and enhances CD8+ T-cell clonal expansion. Since response to PD1/PDL1 blockade requires the presence of CD8+ T cells, vaccine-induced DCs can be utilized to increase the number of CD8+ T cells and maximize antitumor activity.

the decreased Treg activity and enhanced CD8 clonal expansion needed for antitumor activity (79–81). Given how the majority of experimental pediatric sarcoma vaccines rely on tumor-pulsed DCs, we have the opportunity to enhance the potential effectiveness of these agents from multiple angles: triggering an inflammatory tumor microenvironment in which the DC primes and activates CD8+ T cells against a tumor, inhibiting the PD1/PDL1 interaction between the tumor and the CD8+ T cells to perpetuate their antitumor activity, and inhibiting the PD1/PDL1 interaction between the DCs and T cells to decrease Treg activity and enhance the clonal expansion of these tumor-specific CD8+ T cells (**Figure 2**). Preclinical studies recapitulating the successes of adult cancers are necessary prior to the use of tumor vaccines in combination with PD1/PDL1 inhibitors in clinical trials for pediatric malignancies, and there may be challenges in making such therapeutics widely available and equally or more cost-effective than conventional medical therapy (for example, many tumor vaccines require individual tumor samples pulsed with harvested DCs, which would only be available at specialized centers). However, if such studies are successful, they could yield new treatment options for aggressive pediatric tumors and potentially improve their otherwise bleak prognosis.

CONCLUSIONS

The value of anti-PD1/PDL1 therapy in cancer has been shown in multiple adult malignancies, and the further study of this system and its therapeutic inhibition in pediatric sarcomas and other pediatric solid tumors is a logical extension of this body of work. Providing additional opportunity, however, is the mounting evidence that a preexisting immune response to tumors is both necessary and sufficient for a therapeutic response to PD1/PDL1 blockade. The growing number of preclinical trials demonstrating that PD1/PDL1 inhibition, combined with cancer vaccines which generate directed T-cell responses against tumors, greatly enhances tumor regression and clinical responses provides a means to further utilize existing pediatric cancer vaccines to enhance their effectiveness. By instigating a directed T-cell immune response against pediatric malignancies with personalized cancer vaccines, and by then sustaining that response via inhibition of the adaptive immune resistance of PDL1 induction caused by this T-cell response, we have the potential to create novel, personalized therapeutic options and improve the clinical outcomes of some of the most aggressive and deadly pediatric tumors.

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