Pediatric Cancer Immunotherapy: Opportunities and Challenges

Mary Frances Wedekind1,2 • Nicholas L. Denton2 • Chun-Yu Chen2 • Timothy P. Cripe1,2

Abstract Cancer immunotherapies, widely heralded as transformational for many adult cancer patients, are becoming viable options for selected subsets of pediatric cancer patients. Many therapies are currently being investigated, from immunomodulatory agents to adoptive cell therapy, bispecific T-cell engagers, oncolytic virotherapy, and checkpoint inhibition. One of the most exciting immunotherapies recently FDA approved is the use of CD19 chimeric antigen receptor T cells for pre-B-cell acute lymphoblastic leukemia. With this approval and others, immunotherapy for pediatric cancers is gaining traction. One of the caveats to many of these immunotherapies is the challenge of predictive biomarkers; determining which patients will respond to a given therapy is not yet possible. Much research is being focused on which biomarkers will be predictive and prognostic for these patients. Despite many benefits of immunotherapy, including less long-term side effects, some treatments are fraught with immediate side effects that range from mild to severe, although most are manageable. With few downsides and the potential for disease cures, immunotherapy in the pediatric population has the potential to move to the front-line of therapeutic options.

Key Points

- Immunotherapy is changing the treatment landscape for specific subsets of pediatric cancer patients.
- Several monoclonal antibodies are FDA approved for patients with hematologic malignancies but only one is FDA approved for patients with solid tumors; checkpoint inhibition therapy is FDA approved in very limited subsets of pediatric patients, such as those with melanoma, Hodgkin lymphoma, and biallelic mismatch repair deficiency.
- Chimeric antigen receptor T cell (CAR-T) therapy is FDA approved for some pediatric patients with leukemia but challenges remain in leveraging such technology for patients with solid tumors.
- Issues of importance are the investigation of combinations of immunotherapies, the identification of predictive biomarkers, and specific toxicities of immunotherapies in pediatric patients.

1 Introduction

Pediatric patients are often faced with resistant or recurrent cancers that cannot be cured by chemotherapy, radiation, or surgery. Immunotherapies have become viable therapeutic options for many cancer patients. Some of these new pharmacologic medications are changing the landscape of treatment for pediatric cancers, while the utility of others is
not yet known. Monoclonal antibodies (mAbs), checkpoint inhibitors, bispecific T-cell engagers (BiTEs), and chimeric antigen receptor T cells (CAR-Ts) have been FDA approved for use in children, whereas vaccines and oncolytic virotherapy are still being studied to determine their usefulness for pediatric cancer patients. Here we review the landscape of cancer immunotherapies including efficacy and toxicity for pediatric patients as well as emerging predictive biomarkers that might enable personalized approaches.

2 Cancer Immunotherapy/Tumor Microenvironment

Immunotherapy has been documented as a cancer therapy since the late 1800 s. In 1866, Wilhelm Busch in Germany observed tumor regression in a sarcoma patient after an erysipelas infection. In 1891, orthopedic surgeon Coley demonstrated remission in some patients with inoperable sarcomas by injecting streptococcus organisms and their toxins directly into the blood stream [1–4]. Much has been learned since then about the complexities of the immune system, the tumor microenvironment, and their interactions.

The immune system is a highly complex organization of cells and proteins that cooperate to eliminate infections while maintaining tolerance against self. Innate immunity includes nonspecific proteins like complement as well as cells responsible for the initial attack against a foreign pathogen, while the adaptive system requires further development to acquire more specific engagement of targets as well as memory of the foreign antigen [5].

The interplay between the patient’s immune system and cancer includes immune surveillance, immune cell infiltration, and tumor cytosis. Immunosurveillance, first described by Burnet and Thomas in 1957, occurs when a tumor becomes recognized in the body as ‘foreign’ [6]. Cancer cells release pathogen-associated molecular signals (PAMPs), damage-associated molecular signals (DAMPs), and ‘foreign’ antigens typically resulting from mutations in protein-coding genes, termed neoantigens [7]. These signals are detected by the immune system, leading to a coordinated attack by the innate and adaptive immune system to recognize these tumor-associated antigens. In response, cancers often counteract this immune response by downregulation of surface markers, downregulation of antigen presentation by class I molecules, and immunosuppression mediated by cytokines and small molecules expressed in the solid tumor microenvironment [8]. Over time, cancer cells can evolve to metastasize, express different neoantigens, or express further mechanisms of immunosuppression, thus escaping detection and eradication. These steps are the framework for the model of cancer ‘immunoediting’.

Immunoediting consists of three different phases: elimination, equilibrium, and escape [9]. Elimination involves the innate and adaptive cells identifying the neoantigens, forming tumor-reactive T cells, and destroying cancer cells. Some tumor cells survive the elimination phase and enter the equilibrium stage. During the equilibrium stage, the tumor is held dormant by the adaptive immune system. Finally, tumor cells evolve and evade the immune system, leading to the escape phase with subsequent cancer cell proliferation and/or T-cell exhaustion [9, 10]. The mechanisms behind the tumor cells evading the immune system are numerous and include loss of expression of tumor antigens and down-regulation of human leukocyte antigens (HLA) from tumor surfaces (so-called ‘edited’ tumor), recruitment of immunosuppressive regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSC), or tumor-associated M2-like macrophages, upregulation of inhibitory receptors (i.e., cytotoxic T lymphocyte associated protein 4 [CTLA-4], Programmed death receptor 1 [PD-1]) on T cells, or upregulation of inhibitory ligands (PD-L1) on tumor and/or stromal cells [11–13] (Fig. 1). By targeting this tumor microenvironment, immunotherapies aim to counteract this escape phase and reinvigorate the patient’s immune system to recognize and eliminate cancer cells. As physicians, our ability to leverage this knowledge to develop cancer immunotherapies for children is largely in its infancy.

3 Immunostimulatory Agents

As a broad category, immunostimulatory agents enhance the elimination phase of the immunoediting paradigm (Fig. 2a).

One of the best studied examples of an immunomodulatory agent is liposomal muramyl tripeptide phosphatidylethanolamine (L-MTP-PE), which is a synthetic analog of a bacterial cell wall component that induces activation of the immune system, particularly macrophages [14–16]. Nucleotide-binding oligomerization domain-containing protein 2 (NOD2) detects L-MTP-PE, activating NF-κB to stimulate the production of interleukin (IL)-1β, IL-6, and tissue necrosis factor (TNF)-α, which stimulate macrophages and monocytes [17–19]. Initially L-MTP-PE was studied in canine bone and soft tissue sarcomas and demonstrated a median overall survival (OS) of 222 days in the L-MTP-PE-treated group compared with 77 days in the control group [5]. In 1993, a cooperative group clinical trial INT0133 (http://www.clinicaltrials.gov, NCT00631631) analyzed whether, in addition to chemotherapy with methotrexate, doxorubicin, cisplatin,
L-MTP-PE and/or ifosfamide would improve outcomes. The study enrolled 662 patients and found improvement of the 6-year OS from 70 to 78% \((p = 0.03)\) with addition of L-MTP-PE; the hazard ratio was 0.71 (95% CI 0.52–0.96).

Also, in a separate analysis of 91 patients with metastasis, there was no statistically significant survival difference between the groups \((p = 0.27)\) [20–23]. These results have led to conflicting decisions about L-MTP-PE, with approval only in the UK, Turkey, Spain, Israel, and Mexico for patients aged 2–30 years with newly diagnosed non-metastatic osteosarcoma [22].

Cytokines have also been tested as immunotherapies in pediatric cancers. The IFN family of molecules bind to IFN receptors with the type I IFNs, IFN-\(\alpha\) and IFN-\(\beta\), increasing antigen presentation to T cells. Type I IFNs have been approved for many adult cancers including IFN-\(\alpha2a\) for stage II melanoma, hairy cell leukemia, chronic myeloid leukemia, and AIDS-related Kaposi's sarcoma, and IFN-\(\alpha2b\) for hairy cell leukemia, malignant melanoma, and AIDS-related Kaposi's sarcoma. Numerous clinical trials have investigated both IFN-\(\alpha2a\) and IFN-\(\alpha2b\). IFN-\(\alpha2a\) was shown to be feasible in children with resected high-risk melanoma [26]. IFN-\(\alpha\) has been investigated in osteosarcoma as monotherapy [27] and in combination with chemotherapy [28]. These studies showed IFN-\(\alpha\) caused improvement in metastatic-free survival and sarcoma-free survival compared with surgery alone; however, no differences were found in disease-free survival compared with chemotherapy [27, 28].

EURAMOS-1 investigated the addition of PEGylated IFN-\(\alpha2b\) to standard chemotherapy compared with standard chemotherapy and found no statistical differences between the two groups (3-year event-free survival (EFS) 80 vs 77%, respectively) [29].

Thus, there is a continued need for more pediatric studies to determine the usefulness of IFN-\(\alpha\).

IL-2 is an immunotherapy cytokine that activates T-cell proliferation and facilitates maintenance of natural killer (NK) cells [30]. In the pediatric population, IL-2 is most...
noted for its success in high-risk neuroblastoma when combined with an anti-GD2 monoclonal antibody and granulocyte-macrophage colony-stimulating factor (GM-CSF). Unfortunately, many other studies utilizing IL-2 have shown no antitumor effects [31, 32]. Schwinger et al. investigated high-dose IL-2 in patients with heavily pretreated solid tumors after resection of primary and/or metastatic lesions in an attempt to maintain remission. Whether or not IL-2 played any role in the five (neuroblastoma, n = 3; osteosarcoma, n = 2) out of twelve patients who did not experience relapse is unknown [33]. However, all patients experienced significant toxicities and another high-dose IL-2 trial reported 1–2% treatment-related deaths [5]. Due to significant adverse events, it is unlikely that high-dose IL-2 will be used alone but it may be useful in lower doses to augment other immunotherapies.

4 Antibody and Antibody-Like Therapy

Antibody therapy has been used in many pediatric cancer types and has shown much promise. mAbs are engineered to attach to a specific tumor surface antigen with subsequent engagement and activation of NK cells and macrophages via Fc-receptor binding. Once activated, these cells release cytotoxic granules to kill the tumor cell in a process called antibody-dependent cellular cytotoxicity (ADCC). One of the advantages of monoclonal antibody therapy is they are tumor-specific instead of patient-specific, thus can be easily stored in clinics and hospitals without the need for local manufacturing expertise. BiTEs are based on mAb technology, but unlike mAbs, these synthetic molecules connect and activate T cells with the tumor-specific antigen. They consist of two single-chain variable fragments connected by a flexible linker. One side binds to the CD3 receptor of the T cell while the other side binds to the tumor antigen. This results in the activation of T cells and subsequent cytolysis of the tumor [34, 35] (Fig. 2b).

4.1 Monoclonal Antibody (mAb) Therapy for Pediatric Hematologic Malignancies

Rituximab, a CD20 targeting mAb, was the first mAb approved for clinical use in 1997 for adults. Its use is now approved for non-Hodgkin lymphoma (NHL) and chronic lymphocytic leukemia. In the pediatric NHL population, the addition of rituximab to standard chemotherapy increased the 1-year EFS from 81.5 to 94.2%, thus proving its value in pediatric NHL [36]. In 2011, brentuximab vedotin, an anti-CD30 mAb drug conjugate (ADC), was approved by the FDA for relapsed or refractory Hodgkin lymphoma (HL) and anaplastic large-cell lymphoma. A higher overall response rate was seen in patients with relapsed/refractory HL who received brentuximab vedotin compared with vinorelbine (76 vs 50%, respectively) [37]. Currently there is a phase III Children’s Oncology Group (COG) study combining brentuximab vedotin with gemcitabine for relapsed HL (NCT01780662).

In 2000, the FDA approved an anti-CD33 ADC, gemtuzumab ozogamicin, for acute myelogenous leukemia (AML) in adults. The drug was discontinued in 2010 due to concerns for hepatic veno-occlusive disease and a lack of statistically significant clinical benefit in an adult phase III trial [38]. Anti-CD33 mAb interest was renewed with promising data in pediatric AML, but now is primarily utilized as BiTE therapy [39]. Lastly, anti-CD22 mAbs have been utilized in adult and pediatric B-cell acute lymphoblastic leukemia (ALL) with success [40–42].

BiTE therapy has shown much promise in the treatment of pediatric hematologic malignancies. The CD19/anti-CD3 BiTE, blinatumomab, was FDA approved in 2017 for the treatment of relapsed or refractory B-cell ALL in the pediatric population after being approved in 2014 for adult patients. OS, remission rates, and EFS were significantly longer or higher in the blinatumomab group compared with standard chemotherapy [43, 44]. Most recently, the FDA has approved blinatumomab for the treatment of minimal residual disease positive B-cell ALL patients.

4.2 mAb Therapy for Pediatric Solid Tumors

The most notable mAb for pediatric solid tumors is the anti-GD2 mAb dinutuximab, which is FDA approved for neuroblastoma. The pivotal study was performed by COG, which found an improved 2-year EFS of 64% compared with 44% with retinoic acid alone when given in combination with IL-2 and granulocyte monocye colony stimulating factor [45]. Currently, dinutuximab is being used in high-risk neuroblastoma in combination with chemotherapy and radiation therapy [35]. A humanized 14.18 GD2 disialoganglioside mAb conjugated to IL-2 has also shown activity in a COG phase II trial in pediatric relapsed/refractory neuroblastoma [46]. Another anti-GD2 mAb is also being investigated, a humanized GD2 antibody, Hu3F8, in high risk neuroblastoma and other GD2-positive pediatric cancers (NCT01419834).

Antibodies directed to other pediatric solid tumor targets have had less success. A phase II COG study of trastuzumab, a human epidermal growth factor receptor 2 (HER2) mAb, failed to show efficacy in osteosarcoma [47]. Another phase II COG study utilizing cixutumumab, an insulin-like growth factor 1 (IGF-1) mAb, combined with standard chemotherapy in pediatric solid tumors, showed no objective responses [48, 49]. Because GD2 is also expressed in other cancers besides neuroblastoma [50], there are numerous trials investigating anti-GD2 mAbs.
alone or in combination with other immunotherapies in solid tumors expressing GD2 (NCT02100930, NCT01857934, NCT01419834, NCT02502786, NCT01662804). BiTEs for pediatric solid tumors are only beginning to be explored, an example of which is a phase I study utilizing anti-GD2 BiTE in neuroblastoma and osteosarcoma (NCT02173093).

5 Adoptive Therapy

Adoptive cell therapies comprise a variety of strategies that use a patient’s cytolytic immune cells manipulated ex vivo and re-introduced to elicit an anti-tumor response [7]. There are numerous strategies that are being used in many different cancer types, including CAR-T therapy, NK cell, and tumor-infiltrating lymphocytes (TIL) therapy (Fig. 3b).

5.1 Chimeric Antigen Receptor T Cell (CAR-T) Therapy in Pediatric Hematologic Malignancies

Tumors have the ability to evade the immune system by decreasing expression of their HLA molecules and/or tumor antigens. A therapeutic option to overcome this challenge includes CAR-Ts that are engineered to engage a specific antigen without the need of HLA presentation of tumor neoantigens. A chimeric antigen receptor is composed of an extracellular domain with an antigen-binding domain derived from a monoclonal antibody specific for a tumor surface antigen, a spacer domain, a transmembrane domain, and an intracellular signal-transducing chain of the T-cell receptor [51, 52]. The process includes harvesting autologous T cells from the patient, ex vivo expansion with proliferative cytokines, transduction of cells with an engineered T-cell receptor, and reinfusion of selected T cell into the patient [34].

The most exciting and impressive immunotherapy results have come from the CD19 CAR-T therapy for pre-B-cell ALL. Adult studies first showed profound reduction in tumor burden in a majority of patients with chemorefractory B-cell ALL [53, 54]. Soon after, pediatric studies confirmed efficacy in childhood B-cell ALL [55, 56]. In the first phase I trial, two children with refractory, heavily pretreated B-cell ALL achieved complete remissions; however, one relapsed with CD19-negative disease [57]. The full study from Children’s Hospital of Philadelphia included 25 children, with the majority being post-allogeneic stem-cell transplant, who received CD19 CAR-T therapy with a complete response in 90% of the patients [56]. From these studies, CD19 CAR-T therapy (Kymriah) was FDA approved in 2017 [35]. As illustrated by patients who experienced relapse due to antigen escape, more CAR-T therapy targets are needed for hematologic malignancies. A novel CD-22 CAR-T has been developed and shown promising preclinical data in pediatric CD19+ and CD19− B-cell ALL [58, 59]. Other CAR-T targets being investigated in the laboratory setting include CD30 [60], thymic stromal lymphopoietin receptor [61], and CD123 [62].

5.2 CAR-T Therapy in Pediatric Solid Tumors

Pediatric patients with solid tumors have experienced less efficacy of CAR-T therapy compared with those with...
hematologic malignancies; however, some promising results are emerging. In a phase I study using GD2 CAR-T cells in refractory neuroblastoma, 27% with active disease eventually achieved complete response with two patients achieving durable remission of > 60 months [63]. HER2 CAR-T cells have also been utilized in some solid tumors; however, during a phase I trial, an adult patient died unexpectedly from immune-mediated toxicity [64]. Concerns were raised that the HER2 CAR-T cell recognized low levels of HER2 on the lung and heart, a theory that has largely been debunked [64]. Recently, a phase II trial of a HER2 CAR-T in osteosarcoma (NCT00924287) showed no dose-limiting toxicities, suggesting safety in pediatric patients, and some patients experienced stable disease [65]. In patients with glioblastoma, CAR-Ts are under study against interleukin-13 receptor alpha (IL-13Rα) and epidermal growth factor receptor variant III, which are not expressed on normal CNS cells [66, 67]. Other preclinical models of solid tumors utilizing CAR-T directed against IL11-Rα and HER2, or CAR-T against IGF1-R and tyrosine-kinase-like orphan receptor 1 showed suppressed tumor growth and prolonged animal survival [34, 68].

5.3 NK Cell-Based Therapy

NK cells are lymphocytes in the innate immune system that are unlike T and B cells in that they can recognize a target without engaging specific antigens. Utilizing NK cells for the destruction of tumor cells was first performed by Kiessling et al. in mice with leukemia and has now been verified in preclinical and clinical trials [69]. AML patients have experienced the most success, with these studies confirming that haploidentical NK cells could be expanded in vivo and induce remissions [70, 71]. A pilot study of ten children with AML utilized haploidentical donor NK cells combined with IL-2 and showed remission in all patients 2 years after the treatment [72]. There are a few clinical trials ongoing utilizing NK cell therapy for pediatric hematologic malignancies (NCT02763475, NCT03068819).

In the adult population, there have been some successes in patients with solid tumors utilizing NK cell therapy [73, 74]. In a pilot study of pediatric patients with refractory solid tumors, haploidentical stem cell transplant led to 50% survival at 14 months with haploidentical NK cell infusion resulting in complete and partial responses [75]. In another study, a pediatric patient with rhabdomyosarcoma experienced resolution of lung metastases following NK cell therapy [76]. There are also numerous ongoing clinical trials for pediatric solid tumors utilizing NK cell therapy (NCT01807468, NCT03420963, NCT02573896, NCT02650648, NCT02100891).

6 Oncolytic Virotherapy

Oncolytic viruses that are engineered to selectively infect and destroy cancer cells are being tested in preclinical and clinical trials for pediatric cancer. Oncolytic viral infection not only directly kills tumor cells, but also releases PAMPs and DAMPs resulting from so-called ‘immunogenic cell death,’ leading to adaptive immune responses [77] (Fig. 3a). Numerous studies have shown that intratumoral injection of talimogene laherparepvec (Imlygic or T-VEC), a herpes simplex virus type 1-derived oncolytic virus expressing GM-CSF, has caused benefit on both injected and non-injected lesions (abscopal effect) in preclinical and clinical trials [78, 79], leading to FDA approval in adult patients with melanoma.

Preclinical data have shown the benefit of oncolytic virotherapy in numerous pediatric tumor models [80–83]. In a phase I dose escalation study, a genetically modified herpes simplex virus designed to only replicate in cancer cells was utilized intratumorally in nine pediatric patients with relapsed/refractory, non-CNS solid tumors. This study suggested that intratumoral virotherapy was safe in the pediatric population, but no objective responses were seen [84]. A COG phase I study of reovirus in children with relapsed or refractory extracranial solid tumors demonstrated safety but there were no responses seen in any of the patients [85, 86]. Finally, another phase I trial in children using a modified vaccinia virus in patients with extracranial solid tumors showed safety, but again no responses were seen [87]. It should be noted that doses were not escalated to a maximum tolerated dose, suggesting that higher doses may be needed. In addition, their lack of toxicities suggest they can likely be safely combined with other cancer therapeutics including other immunotherapies, a strategy that has been successful in animal models [88–90]. Currently, there are three ongoing studies of oncolytic viruses in pediatric brain tumor patients using attenuated versions of herpes simplex type 1 (NCT02457845), polio virus (NCT03043391), and measles virus (NCT02962167).

7 Checkpoint Inhibitors

The anti-tumor effect of immunotherapies is not only dependent on the quantity of the immune cells present, but also the quality and function of these cells. Past endeavors have been focused on “pushing the gas pedal” by supplying the tumor microenvironment with a higher number of immune cells. Recently, researchers have recognized the importance of “taking the foot off the brake” by reducing the immunosuppressive tumor microenvironment to enhance antitumor immunity [91]. Most prominently,
studies have shown that immunogenic tumors can escape immune surveillance by dampening the immune response via checkpoint ligands [92]. There have been many successes in the adult population using T-cell checkpoint inhibition, including metastatic melanoma [93], non-small-cell lung cancer (NSCLC) [94], HL [95], bladder cancer [96], and head and neck cancer [97].

CTLA-4 is an immune checkpoint that functions to prevent autoimmunity in Tregs and memory T cells [5, 98, 99]. CTLA-4 is expressed on the surface of T cells and binds to CD80/86 on dendritic cells (DCs), leading to deactivation of the T cell [93, 100–103]. CTLA-4 signaling is utilized by some tumor types to evade T-cell antitumor immunity [104]. Blockade of CTLA-4 signaling is FDA approved for adult and pediatric melanoma, but preclinical data also suggest other solid tumors have high expression of CTLA-4 as well [105–107]. A recent phase I study (NCT01445379) of pediatric patients with melanoma and other solid tumors treated with CTLA-4 blockade revealed increased cytotoxic T lymphocyte activation without increased infiltration of Tregs; however, there were no observable antitumor responses [108].

PD-1 is expressed on chronically activated T cells, B cells, DCs, and macrophages. PD-1 signaling limits the inflammatory immune response to prevent autoimmunity [109, 110]. PD-1 interacts with PD-L1 expressed on numerous cancer types and PD-L2 expressed on macrophages and DCs [5, 111, 112]. There have been a number of studies detailing the expression of PD-L1 and PD-1 on numerous pediatric cancer subtypes. Most of these studies show conflicting data on expression levels with Majzner et al. finding only 9% of 451 pediatric tumors demonstrating >1% expression while Geoerger et al. showed 33% of patients had expression [113–118]. In the first phase I study of a PD-1 antibody in children, the Sarcoma Alliance for Research through Collaboration investigated single therapy PD-1 antibody in advanced soft tissue and bone sarcomas. Side effects were similar to the adult studies, but there were no antitumor effects noted in any tumor types except undifferentiated pleomorphic sarcoma (40% with objective response) [119]. Confiming these results, the KEYNOTE-051 study demonstrated tolerance of PD-1 therapy at adult doses, but no objective responses [118]. In March 2017, the FDA approved the anti-PD1 antibody, pembrolizumab, for the treatment of both adults and children with refractory classic HL or those who relapsed after three or more prior treatments. The KEYNOTE-087 trial included 210 adult patients with classical HL and demonstrated an overall response rate of 69% with complete remission of 22% and partial remission rate of 47% in the pembrolizumab group [120]. Efficacy in the pediatric population was extrapolated from the results in adults with safety demonstrated in the aforementioned KEYNOTE-051 study [121]. With the exception of HL, for which anti-PD1 therapy is FDA approved, single therapy checkpoint inhibition has been disappointing in pediatric clinical trials.

Lymphocyte activation gene 3 (LAG-3) and T-cell immunoglobulin and mucin domain containing 3 (TIM-3) are two immune checkpoint proteins that have gained recent interest in cancer therapy. LAG-3 is expressed on activated T cells and binds to major histocompatibility complex II (MHC II), which then causes CD8+ T-cell exhaustion and CD4+ T-cell down-regulation; this results in tumor evasion from the antitumor immune response [122, 123]. TIM-3 is expressed on activated T helper cells and TILs, which causes T-cell inhibition or apoptosis when TIM-3 binds galactin 9 or other unknown ligands [124]. Preclinical data of patients with colon carcinoma treated with PD-1/TIM-3 dual inhibition demonstrated reactivation of TILs and increased numbers of tumor regressions [125]. LAG-3 inhibition is currently in clinical trials for adult solid tumors and hematologic cancers (NCT01968109, NCT02061761) but has not yet progressed to pediatric trials.

One of the hypotheses to explain the differences in response rates between certain adult cancers compared with pediatric cancers is the mutational load or lack thereof. Checkpoint inhibitors permit stimulation of T-cell-mediated antitumor responses to neoantigens presented by tumor cells via the MHC [35] (Fig. 3c). Thus, the higher the number of neoantigens, the higher the probability of successful therapy with checkpoint inhibitors. A high mutational load in the tumor leads to more neoantigens and a more immunogenic tumor [126, 127]. The success of checkpoint inhibitors in the treatment of melanoma and NSCLC appears to be due to the high mutational load of both of these cancer types [96, 128–132]. In contrast, pediatric cancers in general do not have high rates of mutations [133]. The one exception involves pediatric patients with biallelic mismatch repair deficiency (bMMRD). This diagnosis leads to numerous childhood cancers and it is associated with a high mutational rate, even higher than adult cancers [134]. The FDA has approved the PD-1 antibody pembrolizumab for the treatment of mismatch repair deficiency tumors in patients 12 years and older.

8 Combination Therapies

Despite the promising developments in immunotherapy for adult oncology, fewer successes have been achieved in the pediatric setting [135, 136]. This result may in part be due to the significantly lower mutational load in pediatric cancers, which limits the number of neoantigens for
immunotherapies to target. Preclinical data also demonstrate that tumors can quickly develop resistance to immunotherapy if treatment is limited to a single approach [112, 137, 138]. Therefore, combinations of multiple immunotherapeutics may be required to overcome these challenges in pediatric cancer immunotherapy.

While both PD-1 and CTLA-4 act as immune checkpoint proteins, they function on different stages of the immune response. PD-1 signaling primarily regulates CTL proliferation while CTLA-4 has a unique role inhibiting memory T-cell activity [78, 91]. Thus, combination therapy may enhance the response. PD-1/CTLA-4 signaling blockade combination therapy against adult metastatic melanoma resulted in 30% of patients experiencing a > 80% decrease in tumor volume [139, 140]. Combination therapy in preclinical metastatic osteosarcoma models resulted in 50% of treated mice experiencing complete protection from metastasis and T-cell memory against tumor rechallenge [138]. Currently, there is an ongoing trial testing PD-1/CTLA-4 signaling blockade combination therapy against recurrent/refractory pediatric cancers (NCT02304458) [34].

Another approach to improving immune checkpoint inhibition therapy is to increase the infiltration of TILs. Metastatic lesions of osteosarcoma have also been shown to have higher TIL infiltration in addition to higher PD-L1 expression compared with primary tumors, suggesting that metastatic osteosarcoma patients would benefit from TIL activation combined with PD-1 inhibition therapies [112, 141–143]. Other pediatric cancers can also overcome their poor immunogenic potential by combining immune checkpoint inhibition with TIL-activating therapies like chemotherapy, cancer vaccines, or T-cell-based therapy [144, 145]. Chemotherapy agents that induce immunogenic cell death [92] and therefore might potentiate immunotherapies include taxanes, cyclophosphamide, and platinum analogs [100, 146]. Nivolumab and platinum-based chemotherapy for advanced NSCLC showed 2-year OS of 62% [147]. Currently, there is a trial of nivolumab with cyclophosphamide in recurrent pediatric cancers (NCT02813135).

Cancer vaccines work by stimulating T cells with tumor neoantigens, thus leading to a greater anti-tumor response [148–151]. Cancer vaccines alone have been shown to activate antigen-specific T cells; however, these T cells eventually become dampened by the suppressive tumor microenvironment [91]. These T cells have been shown to have an increased expression of PD-1, thus suggesting a role for PD-1 blockade [150, 151]. Preclinical murine models of prostate, neuroblastoma, and pancreatic cancers have demonstrated increased immunogenicity by increasing TILs with subsequent greater anti-tumor effect when combining CLTA-4 blockade with cancer vaccines [152–154]. Metastatic melanoma and osteosarcoma models in particular are vulnerable to cancer vaccine and immune checkpoint combination therapy [155].

Lastly, immune checkpoint inhibition can enhance T-cell therapies such as CAR-T and BiTE therapies, which like other TILs are prone to exhaustion [156–158]. PD-1 expression can be increased by Treg infiltration, immunosuppressive cytokine signaling, loss of neoantigen expression, and genomic instability; ultimately this results in T-cell therapy exhaustion and tumor recurrence [156, 159–163]. Preclinical studies on hepatocellular and prostate cancers found that PD-1 blockade could lead to increased anti-tumor responses and T-cell proliferation [157, 158]. There are ongoing clinical trials combining immune checkpoint inhibition, cancer vaccine, and T-cell therapy (NCT02070406 and NCT02775292).

9 Challenges: Limited Biomarkers

A critical challenge that must be overcome is the identification of biomarker(s) to identify patients who would benefit from immunotherapy. Ideally, we should identify prognostic biomarkers, so that patients can be placed in appropriate individualized risk-stratified treatment groups, and predictive biomarkers, so that response can be monitored. In melanoma and NSCLC, there has been an association with high PD-L1 expression and poor prognosis [164]. There are many reports suggesting the PD-L1 expression is correlated with response to PD-1 antibody in adult patients [164–168]; however, there are others that have found some patients will respond without elevated levels of PD-L1 [140, 169–171]. Other studies have also shown that PD-L1 expression is heterogeneous within a tumor and also amongst metastatic lesions [112, 172]. Preliminary results demonstrated that patients whose NSCLC tumors express PD-L1 and IFNγ have better outcomes compared with those expressing only PD-L1, but there are not yet data in other cancer types [92].

Another potential biomarker is the ‘hot versus cold’ tumor delineation, in which hot tumors have abundant T cells whereas cold tumors lack such infiltrative cells. TILs have been associated with better patient survival in numerous cancer types [173–181]. One study showed that increasing the amount of TILs increased the response to PD-L1 therapy and demonstrated that PD-L1+ tumors with low levels of TILs were unresponsive to PD-L1 therapy [182]. Unfortunately, these observations have just been associations and have not been validated as true biomarkers.

A biomarker that has shown a positive correlation with response is the mutational burden of the tumor [94, 130, 167, 183, 184]. Somatic mutations lead to higher
rate of neoantigens, which alert the immune system to the
tumor as something foreign. One study found that patients
with tumors of mismatch repair deficiency (MMRD)
showed a response rate of 40% with the PD-L1 inhibitor
pembrolizumab compared with a response rate of 0% in
those tumors without MMRD [132]. In the pediatric pop-
ulation, bMMRDs have been found to have mutations of
> 250 per megabase and show response to checkpoint
inhibition [134]. A high frequency of nonsynonymous
mutational burden, tumor antigens, and mutations in DNA
repair pathways were strongly associated with therapeutic
benefit after CLTA-4 and PD-1 blockade. Two studies
suggest a mutational threshold of approximately 100
mutations per exome would be needed to show clinical
benefit after CLTA-4 and PD-1 blockade. Two studies
suggest a mutational threshold of approximately 100
mutations per exome would be needed to show clinical
response to checkpoint inhibition [128, 132]; however, this
number has not been validated.

Besides biomarkers, it is critical that adequate
immunotherapy targets are identified as well for CAR-T,
mAb, and BiTE therapies. An immunotherapy target must
be highly expressed on the tumor tissue and poorly
expressed on normal tissue to provide a sufficient therapeu-
tic window [185]. Optimal targets are exceedingly rare,
as most targets expressed on tumors are also expressed on
vital normal human tissues. It is also necessary that the
target is presented on the surface of the cell for mAb
therapy and CAR-T. Of the 75 National Cancer Institute
consensus high value targets, two-thirds are internal anti-
gens [7]. Efforts are underway to identify and validate
adequate targets for selection.

10 Challenges: Toxicity

In general, immunotherapies are thought to exhibit fewer
long-term toxicities than chemotherapy and radiation, a
significant appeal in pediatrics. The immunomodulatory
agents, cytokines and L-MTP-PE, are generally well tol-
erated. There are potentials for chills, fever, headaches,
myalgias, and fatigue, especially during the first infusion.
For L-MTP-PE, the reaction to the medicine will decrease
in intensity with subsequent doses [22].

mAbs are also well tolerated. Acute infusion reactions
are fairly common, but easily managed with antipyretics,
antihistamines, and/or corticosteroids. mAbs will deplete
the body of all cells that express its directed target, even if
they are normal. For example, rituximab will cause B-cell
depletion and humoral immunosuppression. Depending on
the target, this could lead to higher risk for certain infec-
tions [186].

In adoptive T-cell transfer, toxicity poses a serious
concern. Due to T-cell therapy using targets that are
expressed on normal tissues, there is the potential that these
normal tissues (even if expression is low) will be targeted
and destroyed. The results of this attack can be life
threatening. Thus, there are limitations on the choice of
certain targets that can be used [187, 188]. Another toxicity
associated with adoptive T-cell therapy is cytokine release,
which can also be severe and fatal. Cytokine release syn-
drome occurs when an overwhelming amount of immune
cells are activated leading to large amounts of inflamma-
tory cytokines being released through the body, resulting in
organ dysfunction and death [189].

With checkpoint inhibition, adverse drug events (ADEs)
were mild to moderate and affected 70% (any Common
Terminology Criteria for Adverse Events [CTCAE] grade)
of patients treated with ipilimumab [105, 190]. In the
pediatric population, incidence of grade 3–4 ADE was 27%
with most common being pancreatitis, pneumonitis, colitis,
and hepatitis when treated with ipilimumab [147]. The
toxicity profile of PD-1/PD-L1 blockade was less severe
than ipilimumab with grade 3–4 ADE incidence of 7–14%
[191]. Toxicity of anti-PD1 is immune related, including
pneumonitis, colitis, hepatitis, hypophysitis, thyroiditis,
and dermatitis [105, 108, 192–195]. Most of these side
effects responded to early and aggressive high-dose ster-
oids with minimal long-term effects [193]. Interestingly,
ADEs were associated with tumor responses and favorable
outcomes, likely due to the fact that these patients have a
more active immune system [105, 108, 192–194]. Another
interesting phenomenon that has been seen with checkpoint
inhibition and oncolytic virotherapy is so-called pseudo-
progression, which occurs when the tumor lesion increases
in size during therapy as part of the antitumor immune
response. This phenomenon has led to a modified response
assessment to allow for this immune-related response
[196].

11 Conclusion

Although the ultimate contribution of immunotherapies to
the outcome of pediatric cancer patients is uncertain, the
landscape of therapy in the near future is likely to be quite
different from traditional surgery, radiation, and
chemotherapy. A variety of immunotherapies hold signif-
icant promise for children with cancer, both in terms of
improving survival outcomes and reducing late effects. As
we continue to increase our understanding of cancer cells,
the immune system, and the tumor microenvironment, we
are likely to devise novel ways in which to decrease
immunosuppressive factors, interrupt pathways used for
immune evasion, and identify useful biomarkers for treat-
ment stratification and monitoring. We are optimistic that
the incorporation of immunotherapies into treatment regi-
mens will enable increased patient survival and quality of
life for children with cancer.
Compliance with Ethical Standards

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Conflict of interest Mary Frances Wedekind, Nick Denton, Chun-Yu Chen, and Timothy Cripe declare that they have no conflicts of interest that might be relevant to the contents of this manuscript.

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